CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol.11(4)
2019

Technical University of Cluj Napoca
U.T.Press Publishing House

Carpathian Journal of Food Science and Technology

Print : ISSN 2066-6845
Online : ISSN 2344-5459
ISSN-L 2066-6845

Vol. 11, Nr.(4) 2019

UNIVERSITATEA TEHNICĂ DIN CLUJ-NAPOCA

Technical University of Cluj Napoca
U.T.Press Publishing House

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY
ISSN-L 2066 -6845

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

Technical University of Cluj Napoca, Romania
U.T. Press Publishing House

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CONTENT

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal home page:**http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

QUALITY CHARACTERISTICS OF BISCUIT PREPARED FROM WHEAT AND MILK THISTLE SEEDS (*SILYBUM MARIANUM* (L) GAERTN) FLOUR

Amina Menasra[1*] and Djamel Fahloul[1]

[1]*Laboratory of Food Science (LSA), Department of Food Technology, Institute of Veterinary Sciences and Agronomic Sciences, University Hadj Lakhdar -Batna 1- (UHB1), Algeria.*
[*]*menasraamina@gmail.com*

https://doi.org/10.34302/crpjfst/2019.11.4.1

Article history:
Received:
9 February 2019
Accepted:
28 September 2019

Keywords:
Biscuit;
Enrichment;
Milk thistle;
Properties.

ABSTRACT

Milk thistle (Silybum marianum) is a rich source of ingredients, such as minerals, aminoacids, fatty acids and phytochemicals exhibiting nutraceutical effects on human health. In this work, roasted milk thistle seeds flour was replace wheat flour to improve biscuit quality. The influence of 10% addition of milk thistle seeds flour on biscuit quality was studied by analyzing the physicochemicals and sensory properties in order to further exploit the functionality of milk thistle seeds in bakery products. Comparing control and enriched biscuits, we see major differences in terms of quality. Enriched biscuit has a smaller values of weight (6.63g), of spread ratio (6.38) and of density (0.49 g/cm^3), with a bigger volume (13.48 cm^3), a dark crust (L* : 64.43), a slight milk thistle seeds taste, and with an important nutritional intake (ash : 2.33%, starch : 60.69%, gluten : 28.23%). Finally, Milk thistle seeds flour can be used for biscuit enrichment.

1.Introduction

Bakery products are one of the most profitable segments in supermarket retailing. Biscuits are convenient food products, becoming very popular among both rural and urban populations of worldwide. Some of the reasons for such wide popularity are low cost among other processed foods, varied taste, easy availability and longer shelf life (Dayakar Rao and Bhargavi, 2017).

Over the years, a number of studies have been reported to improve nutritive value of biscuits by incorporating bean, sesame seeds, chickpea, barley, cowpea, lupine, soy protein and corn fiber (Serrem, 2010; Hyun-Jung et *al*., 2014).

Milk thistle seeds are better known as having medicinal benefits. Milk thistle is used internally in the treatment of liver and gall bladder diseases, jaundice, cirrhosis, hepatitis and poisoning (Aliyas, 2015). Numerous studies have shown that seeds are rich in proteins, lipids and total carbohydrates (Abu Jadayil et *al*. 1999; Abd Raboh, 2012). Milk thistle seeds can be used as food. It is a great tonic, increases appetite and aids in digestion. Seeds of milk thistle is a good source of minerals. Presences of rich amount of calcium and magnesium are a special feature (Aliyas, 2015). The importance of milk thistle seeds in human nutrition will also increase, as growing number of consumers are looking for products with pro-health properties (Andrzejewska et *al*., 2015). Up to now, information on incorporation of milk thistle seeds flour in bakery products is scarce (Aliyas, 2015). The addition of milk thistle may be a little-known for its properties (Brodowska et *al*., 2014). Apostol et *al*. (2017) was study the properties of the mixture of wheat and milk thistle seeds flours. Moreover, Sadowska (2006) and Shahat et *al*. (2016) were examined the effect of milk thistle adding on

bread quality. Furthermore , Brodowska et *al*. (2014) reported that the levels of silymarin added were selected to be not higher than 10% of medicinal dose.

However, no work has been done on the effect of milk thistle seeds (*Silybum marianum* (l) Gaertn) flour addition on biscuit quality.

The objective of this work was to characterize the mixture of wheat and milk thistle seeds flours through evaluation of physicochemical and sensory properties of biscuit, which are main quality attributes of this mixture for use in bakery. Moreover, differents properties of prepared biscuits were compared using principal component analysis method (PCA).

2. Materials and methods

2.1. Materials

Soft wheat flours (72%) were obtained from Aures Flour Mills Company, Batna city-Algerria. Milk thistle seeds (*Silybum marianum* (l) Gaertn) were collected in Bouhmar region of Batna city, located in northeast of Algeria, during 2017 Autumn season. Hydrogenated vegetable shortening, grained sucrose, salt, Ammonium bicarbonate (NH_4HCO_3), Sodium bicarbonate ($NaHCO_3$) and mineral water were obtained from supermarket. Biscuit ingredient and milk thistle seeds were stored at 4°C until testing. All reagents and chemicals used in the experimental work were of analytical grade and were purchased from Sigma Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Milk thistle seeds flour preparation

Milk thistle seeds were roasted in a convention electric oven (R-5550, Sharp, Osaka, Japan) for 7 minutes at 130±2°C (degree celsius) (Pandey and Awasthi, 2015). The roasted milk thistle seeds were grounded in laboratory mill and sieved (one millimeter), to obtain a fine homogeneous flours. Samples were stored at 4°C until analysis.

2.2.2. Physicochemical and functional poperties of wheat and milk thistle seeds flours

Moisture content was determined according to AACC Method 44 19.01 (AACC, 2000). Ash content was measured according to AACC Method 930.22 using muffle furnace at 450-500°C (AACC, 2000). Flours were characterized for pH according to AACC Method 02-52 (AACC, 2000). Total titratable acidity (TTA) was determined by a Sodium hydroxide
(NaOH) titration according to AOAC (2005). Total starch content was measured by polarimetric method (Korus et *al*., 2015). Color of flour was determined according to Francis (1998).

Bulk density was determined as described by Chinma et *al*. (2009). Water and oil absorption capacities were measured as determined by Zouari et *al*. (2016). Emulsifying and foaming capacity were defined as described by Elkhalifa and Bernhardt (2010). Swelling capacity was measured as determined by Mateos-Aparicio et *al*. (2010).

2.2.3. Biscuit preparation

Control and enriched biscuit with 10% supplementation level of milk thistle seeds flour were prepared according to AACC Method 10.50 with slight modeifications (AACC, 2000). The recipe contains 80.0±2 g standard wheat flour, 35.0±2 g (gram) grained sucrose, 20.0±2 g hydrogenated vegetable shortening, 1.0±2 g Sodium chloride (NaCl), 0.4±2 g Ammonium bicarbonate (NH_4HCO_3), 0.8±2 g Sodium bicarbonate (N_aHCO_3) and 17.6±2 mL (milliliter) of water. Biscuits dough was sheeted and cut into circular shapes using a cutter (Reddy et *al*., 2005).

2.2.4. Biscuit baking

Control and enriched biscuits were baked in an electrical baking oven (Teba High-01 Inox) under convection conditions at 180°C for 15±2 minutes (Sakin et *al*., 2007).

2.2.5. Physicochemical properties of biscuits

Control and enriched biscuits with 10% supplementation level of milk thistle seeds flour were analyzed for moisture and ash contents according to AACC Methods 44-19 and 40-70, by gravimetric method at 105 °C and incineration at 550 °C, respectively (AACC, 2000). pH determination was carried by mixing 5g of biscuit sample with 100 ml of freshly boiled distilled water (cooled to a temperature of 40±5°C). The mixture was left for 1h (hour), with regular mixing every 15 minutes. Afterward, pH was measured using a pH-meter Schott CG 843 with a combined electrode BlueLine 11 (Schott Geräte GmbH, Mainz, Germany) (Budryn et *al*., 2013). Total titratable acidity was determined as described by Rizzello et *al*. (2010). Starch content was measured by polarimetric method (Korus et *al*., 2015). Gluten was estimated by a Sodium hydroxide (NaOH) titration according to AACC Method 38-10 (AACC, 2000). Measurement of upper surface color of biscuits was carried out using a colorimeter (CR-10, Konica Minolta Sensing Inc., Osaka, Japan). Results were expressed using the CIELab system. The following parameters were determined: leightness (L* = 0 black, L* = 100 white), redness (a* < 0: green, a* > 0: red), yellowness (b* < 0: blue, b* > 0: yellow). Total color change (ΔE), was calculated from the following equation taking dough color as a reference, denoted by L_0^*, a_0^* and b_0^*:

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2\ ]^{1/2} \quad (1)$$

hue angle (h°) and chroma (c*) of biscuits were determined. Three measurements per cookie were taken and reported as average (Sakin-Yilmazer et *al*. 2013). Results were used to calculate the browning index (BI value) from equation (2):

$$BI = \frac{\left[100 \times (\frac{a+1.79L}{5.645L + a - 3.012b}) - 0{,}31\right]}{0{,}17} \quad (2)$$

Whiteness index (WI) values of biscuits were measured as described by Zucco et *al*. (2011) as follows:

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^2 + b^2} \quad (3)$$

2.2.6. Physicals dimensions of biscuits (baking quality)

Diameter (width) and thickness of biscuits were determined according to AACC Method 10-53 (AACC, 2000). Spread ratio was calculated according to Youssef and Mousa (2012) using the following equation:

$$\text{Spread ratio} = \frac{\text{diameter}}{\text{thickness}} \quad (4)$$

Weight loss (WL) during baking was measured using the following equation:

$$WL = \frac{(W_{dough} - W_{biscuit})}{W_{dough}} \times 100 \quad (5)$$

Where, W is biscuits weight (g) (Agrahar-Murugkar et *al*., 2015). Volume (V) in cubic centimeter (cm^3) was calculated as a function of radius (r) (Serrem, 2010):

$$V\ (cm^3) = r^2 \times \text{thickness} \times 3.14 \quad (6)$$

Biscuits density was determined and expressed as g per cm^3 (Serrem, 2010; Sozer et *al*., 2014):

$$D\ (\frac{g}{cm^3}) = \frac{\text{mass}}{\text{Volume}} \quad (7)$$

2.2.7. Sensory evaluation of biscuits

Sensory analysis of biscuits was carried out by 10 non-trained panelists from Food Sciences Laboratory in Food Technology Department. Samples presentation to panelists was at random and one at a time. Panelists were given enough water to rinse their mouths between each serve (Galla et *al*., 2007). Sensory evaluation was carried out on control and enriched biscuits.

Evaluated attributes were appearance, color, texture, taste, odor and overall acceptability. For each sample, panelists scored their liking of characteristics using nine point hedonic scale (Agrahar-Murugkar et *al*., 2015). Panelists scored for different properties with a maximum score of 9 for like extremely, 8-like very good, 7-like good, 6-like moderately, 5-neither like nor dislike, 4-dislike moderately, 3- dislike fairly, 2-dislike very much and 1 for dislike extremely (Galla et *al*., 2007).

2.2.8. Statistical analysis

The experimental data collected in triplicate was analyzed for significant differences with the help of analysis of variance (ANOVA) conducted using SPSS 25.0 software (SPSS Inc., Chicago, IL, USA) (Barak et *al*., 2014; Mogol and Gökmen, 2014). Furthermore, a principal component analysis (PCA), which is a multivariate approach designed for multicorrelated data, was performed to visualize possible relationships within data matrix. To decide the number of principal components (PCs), the eigenvalues of the correlation matrix, indicating the percentage of variability explained by each component, were tabulated and a scree plot was constructed (Aponte et *al*., 2014). Principal Component Analysis (PCA) of biscuits properties was completed by statistical software, STATISTICA version 10.0 (StatSoft, France) (Onacik-Gür et *al*., 2015).

3.Results and discussions

3.1. Physicochemical and funtcional properties of flours

Physicochemical and functional properties of wheat and milk thistle seeds flours are presented in Table 1. Moisture content of flours was found to lie in the acceptable limits (0-13%). Values lie within the limits that enable safe storage (Yusuf et *al*., 2008).The level of moisture content of wheat flour (9.48%) is close to that obtained amount (10.23%) by Oppong et *al*. (2015) of soft wheat flour. The moisture content of milk thistle seeds flour was the lowest (7.53%) and was significantly ($P \leq 0.05$) different from wheat flour (Table 1). Abu Jadayil et *al*. (1999) found that milk thistle seeds contained 5.8% of moisture. Claculated moisture content by Awad-Allah (2013) of pine nut meal is 7.9%.

Wheat flour had low ash content (0.99%). This value is close to that reported (1%) by Oppong et *al*. (2015). Milk thisle flour contained 3.1% of ash content. Abu Jadayil et *al*. (1999) found that milk thistle seeds contains 4.8% of ash.

Wheat flour had the highest carbohydrates content (75.76%). This result is close to that found (77.9%) by Ragaee et *al*. (2006). Milk thistle seeds flour contained 51.4% of total carbohydrates. Abu Jadayil et *al*. (1999) found a low values (24.3% and 10%) of carbohydrate in milk thistle and sesame seeds flours, respectively.

Table 1. Physicochemical and functional properties of wheat and milk thistle seeds flours.

Parameters	Wheat flour	Thistle milk seeds flour
Moisture (%)	9.48 ± 0.0057^{aA}	7.53 ± 0.0058^{aB}
Dry matter (%)	90.52 ± 0.0057^{abcA}	92.47 ± 0.0058^{aB}
Ash (%)	0.99 ± 0.011^{abcA}	3.1 ± 0.057^{aB}
Organic matter (%)	99.01 ± 0.011^{abcA}	96.9 ± 0.057^{aB}
Total starch (%)	75.76 ± 0.0057^{bcA}	51.4 ± 0.1^{aB}
Color	99.6 ± 0.058^{cA}	72.8 ± 0.1^{aB}
Bulk density (g/cm^3)	0.72 ± 0.008^{abcA}	0.08 ± 0.01^{aB}
Water absorption capacity (g/g)	1.50 ± 0.002^{aA}	1.01 ± 0.01^{aB}
Oil absorption capacity (g/g)	1.03 ± 0.03^{aAB}	0.99 ± 0.0057^{aAB}
Emulsion capacity (%)	62.2 ± 0.3^{abcA}	42.69 ± 0.015^{aB}

Foam capacity (%)	13.53±0.035^{bA}	4.4±0.4^{aB}
Swelling capacity (%)	7.50±0.4^{aA}	6.73±0.0057^{aB}

Values followed by different letters (lower-case letters in the same column, and upper-case letters in the same line) are significantly different at 5% level of significance ($\alpha<0.05$).

Color value of wheat flour (99.63) is higher than milk thistle seeds flour (72.8). Milk thistle seeds flour was darker than wheat flour (WF). Eke- Ejiofor et *al*. (2014) reported that jackfruit seeds flour had a color equal 74.79%. Flour extracted under perfect conditions is pure white in color and it is an important criterion for flour quality (Eke- Ejiofor et *al*., 2014).

Bulk density of wheat flour (0.72 g/cm^3) is close to that reported by Baljeet et *al*. (2014) (0.70 g/cm^3). Obtained bulk density (0.08 g/cm^3) of milk thistle seeds flour was lower than wheat flour. The low bulk density of flour could be attributed to the relatively lower protein content (gluten-free) (Korus et *al*., 2015; Oppong et *al*., 2015).

Wheat flour had a higher water absorption capacity (1.50 g/g) than milk thistle seeds flour (1.01 g/g). Water absorption capacity of wheat flour is similar to that reported (1.50 g/g) by Oppong et *al*. (2015). Awad-Allah (2013) found water absorption capacity of pine nut meal equal 1.4 g/g. Flour with high water absorption capacity would be useful in foods such as bakery products which require hydration to improve handling features (Oppong et *al*. (2015).

Wheat flour had a higher oil absorption capacity (1.03 g/g) compared to milk thisle seeds flour (0.99 g/g). Oil absorption capacity of wheat flour is similar to that found (1 g/g) by Oppong et *al*. (2015). Awad-Allah (2013) reported oil absorption capacity of pine nut and walnut meals equal 1.3 and 1.2 ml/g, respectively. Flour with high oil absorption capacity could be useful in food formulation where oil holding capacity is needed such as sausage and bakery products (Oppong et *al*. (2015).

Emulsion capacity of wheat flour (62.2%) is lower than found (42.77%) by Zouari et *al*. (2016). Milk thistle flour had a lower emulsion capacity (42.69%). Awad-Allah (2013) mentioned emulsion capacity of pine nut meal equal 63.6 ml/g.

Wheat flour had a higher foam capacity (13.53%). This result is close to that found (13.19%) by Zouari et *al*. (2016). Foam capacity (FC) of milk thistle seeds flour is 4.4%. The higher value of foam propertie might be due to the high starch content (Awad-Allah, 2013). Foam capacity is important for flour used in many leavening food products such as baked goods, cakes and biscuits (Elkhalifa and Bernhardt, 2010).

Wheat flour had the higher value of swelling capacity (7.50 ml/g). This result is similar to that found by Oppong et *al*. (2015). Swelling power of milk thistle seeds flour is 6.73%. Flour with high swelling capacity could be useful in food systems where swelling is required (Oppong et *al*., 2015).

3.2. Physical dimensions of biscuits (baking quality)

Results of various physical dimensions of biscuits are shown in Table 2. Control biscuit had a higher values of weight (7.17g) and weight loss (15.25%) than enriched biscuit with milk thistle seeds flour. Serrem (2010) found a reduction in weight of enriched biscuit with soy flour. The reduction of total solids in dough results biscuit has lower weight (Serrem, 2010). Control biscuit had the highest spread ratio (7.24). When more free water exists, internal dough viscosity becomes lower and thus spread factor of baked cookies increased (Hyun-Jung et *al*., 2014). Difference in spread ratio depends on source of flours substitution. Cookies having higher spread ratio are considered the most desirable (Hyun-Jung et *al*., 2014). It can be seen that biscuits density seemed to be affected by milk thistle flour addition (0.49 g/cm^3). Density is considered the best index of sensory texture of biscuits (Mamat and Hill. 2014).

Table 2. Physicochemical properties of biscuits.

Parameters	CB	BTMSF
Weight (g)	7.17 ± 0.02^{dA}	6.63 ± 0.152^{dB}
Weight loss (%)	15.25 ± 0.042^{fA}	14.11 ± 0.32^{gB}
Diameter (mm)	48.03 ± 0.1527^{iAB}	47.86 ± 0.152^{jAB}
Thickness (mm)	6.63 ± 0.1527^{dA}	7.5 ± 0.2^{eB}
Volume (cm^3)	12.015 ± 0.353^{eA}	13.48 ± 0.342^{fB}
Spread ratio	7.24 ± 0.142^{dA}	6.38 ± 0.178^{dB}
Density (g/cm^3)	0.59 ± 0.0157^{cA}	0.49 ± 0.02^{cB}
Moisture (%)	4.76 ± 0.057^{oA}	4.43 ± 0.115^{pB}
Dry matter (%)	95.24 ± 0.057^{bA}	95.57 ± 0.115^{bB}
Ash (%)	1.25 ± 0.1^{pA}	2.33 ± 0.20^{qB}
Organic matter (%)	98.75 ± 0.1^{dA}	97.67 ± 0.02^{dB}
pH	6.61 ± 0.02^{aA}	6.38 ± 0.02^{aB}
TTA (%)	0.25 ± 0.01^{kA}	0.33 ± 0.0152^{lB}
Starch (%)	66.78 ± 0.01^{gA}	60.69 ± 0.01^{hB}
Dry gluten (%)	32.33 ± 0.02^{lA}	28.23 ± 0.152^{mB}
L^*	74.033 ± 0.57^{hA}	64.43 ± 0.30^{iB}
c^*	36.87 ± 0.095^{nA}	30.096 ± 0.072^{oB}
h°	85.76 ± 0.152^{mA}	84.76 ± 0.152^{nB}
ΔE	82.71 ± 0.554^{qA}	71.11 ± 0.25^{rB}
BI	248.99 ± 0.702^{jA}	254.66 ± 0.553^{kB}
WI	54.89 ± 0.264^{A}	53.40 ± 0.27^{B}

TTA: total titratable acidity, L^* : leightness, c^* : chromaticity, h° : hue angle, ΔE : total color change, BI : browning index, WI : whiteness index, CB: control biscuit, BTMSF: enriched biscuit with milk thistle seeds flour. Values followed by different letters (lower-case letters in the same column, and upper-case letters in the same line) are significantly different at 5% level of significance ($\alpha < 0.05$).

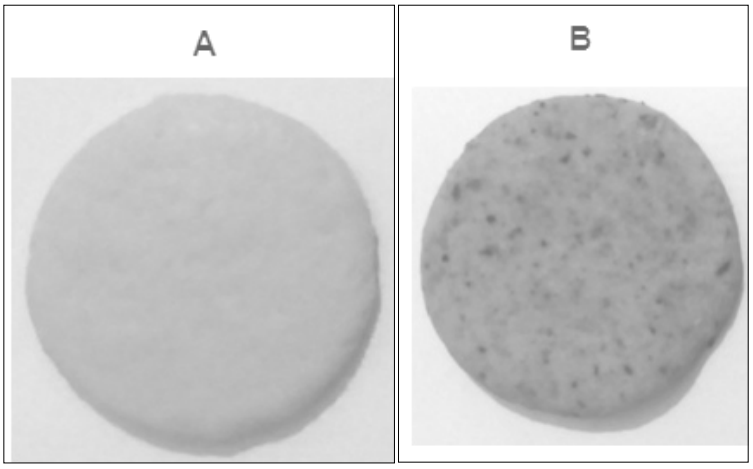


Figure 1. Control biscuit (A) and enriched with milk thistle seeds flour (B).

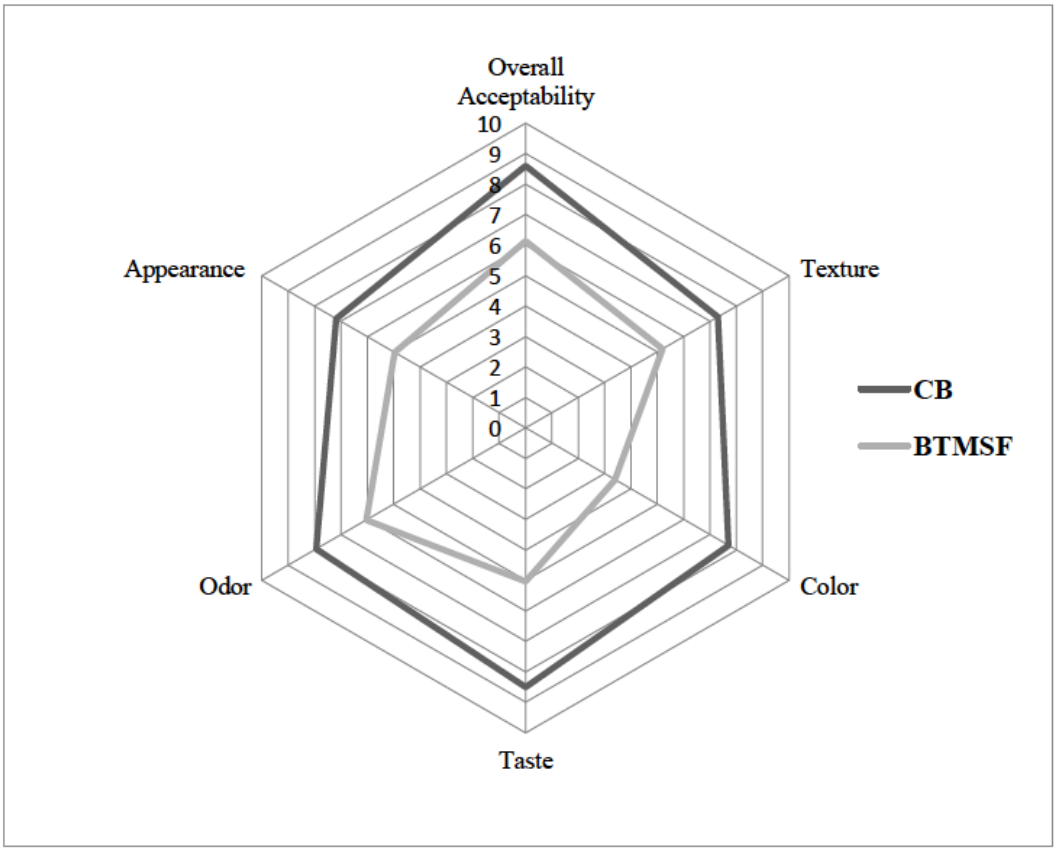


Figure 2. Spider diagram of sensory evaluation of biscuits.
CB: control biscuit, BMTSF : enriched biscuit with milk thistle seeds flour. Scores are based on a 9-point hedonic scale with 1, dislike extremely; 5, neither like nor dislike; and 9, like extremely; number of panelists (n)=10.

Brodowska et *al*. (2014) reported that added doses of 0.16 g, 0.33 g and 0.56 g/100 g of milk thistle fruit did not influence physical properties of wheat dough of bread. Also, the levels of silymarin added were selected to be not higher than 10% of medicinal dose (Brodowska et *al*., 2014).

3.3. Physicochemical properties of biscuits

Table 2 shows physicochemical properties of control and enriched biscuits. Eenriched biscuit with milk thistle seeds flour had lower moisture content (4.43%) than control biscuit (4.76%). This is due to lowest moisture content of milk thistle seeds flour compared to wheat flour (Table 1). The reduction of total solids in dough results biscuit has lower weight (Serrem, 2010).

Enriched biscuit with milk thistle seeds flour contain a higher value of ash (2.33%). Difference in ash content between control and enriched biscuits is certainly due to flour quality. Serrem (2010) found an increase in ash content of enriched biscuit with soy flour. Ash content of biscuit could be raised if milk thistle seeds flour is incorporated. This finding showed that addition of milk thistle flour in cereal products enhanced their nutritional values.

Found pH values are very close (Table 2). This confirms acidity results which also showed a slight difference. pH decreased and total titratable acidity was slightly increased in enriched biscuit with milk thistle seeds flour. Rizzello et *al*. (2010) reported a decrease in pH with an increase in total titratable acidity of

prepared bread with fermented wheat germ flour.

Enriched biscuit with milk thistle flour had a lower starch content (Table 2). This decrease in starch content could be explained by the low carbohydrate content of flour. Similarly, Serrem (2010) found a decrease in starch content of enriched biscuit with soy flour.

Low value of dry gluten content was found in enriched biscuit with milk thistle seeds flour compared to control biscuit. This effect is a self-evident result from the substitution of wheat flour with milk thistle flour. Similarly, Hallén et *al*. (2004) found a decrease in gluten content of enriched biscuit with cowpea flour.

Cookies color was generated mainly during baking process from Maillard reaction between reducing sugars and protein (Hyun-Jung et *al*., 2014). Substituting wheat flour with milk thistle seeds flour in biscuit formulation affects significantly surface color of biscuit. Biscuit crust became brown with milk thistle flour addition (Figure 1). Thus, enriched biscuit had a low value of leightness (L^* : 64.43) compared to control biscuit. Similarly, Sozer et *al*. (2014) mentioned that biscuit became darker with bran flour adding. Color difference (ΔE^* : 71.11) decreased but chromaticity (c^* : 30.096) and hue angle (h° : 84.76) values increased when milk thistle seeds flour added to biscuit. These results are similar to thoses reported by Hegazy et *al*. (2014) for enriched biscuits with chestnut flour. Enriched biscuit had a higher value of browning index (BI : 254.66) compared to control biscuit. Acrylamide formation and concentration during biscuit baking was generally reported to have correlation with brown surface formation (Sakin-Yilmazer et *al*., 2013). Thermal degradation of originally colourless complex polyphenols to coloured phenols during baking increases browning index (BI) of enriched biscuit. The increase in brown index of enriched biscuit with milk thistle seeds flour may be ascribed to polyphenoloxidases activity as a result of biscuit baking (Jimoh et *al*., 2009). Whiteness index decreased in enriched biscuit with milk thistle seeds flour (WI : 53.40). Similarly, Zucco et *al*. (2011) observed a reduction in whiteness index values of cookies as the level of pulse flour substitution increased. Also, Ostermann-Porcel et *al*. (2017) found a reduction of whiteness index (WI) of enriched cookies with okara flour. The higher the whiteness index the whiter the sample (Zucco et *al*., 2011).

3.4. Sensory characteristics

The effect of adding of milk thistle seeds flour to wheat flour on sensory properties of biscuit was evaluated and presented in Figure 2. Control biscuit had the highest ratings for all tested sensory parameters. Results indicated that, adding natural milk thistle seeds flour to biscuit led to decrease of sensory scores of color, appearance and taste. In the same time odor, texture and overall acceptance of enriched biscuit with milk thistle seeds flour are not more affected significantly. Hegazy et *al*. (2014) reported similar results for enriched biscuit with natural chestnut (*Castanea sativa* Mill.) flour. Most of panelists reported that biscuit becam brown with milk thistle flour addition in biscuit. Moreover, they mentioned that enriched biscuit gives a sense to the presence of remains of thin tissue in tongue. This may be related to the presence of some seeds coat of the milk thistle. These results are in agreement with Abd Raboh (2012). Hyun-Jung et al. (2014) reported that taste is important characteristic in determining cookies acceptability. Also, Heinio et *al*. (2016) mentioned that raw materials and ingredients are the key factor for flavour formation of of cereal foods.

Brodowska et *al*. (2014) reported that added doses of 0.16g, 0.336g and 0.56 g/100 g of milk thistle fruit did not influence sensory properties of wheat dough of bread.

3.5. Principal component analysis (PCA) of biscuits properties

Relationships between biscuits properties were obtained using factorial principal component analysis (PCA). Original data set was renormalized by an autoscaling transformation (data not shown) and different parameters were analyzed by a multivariate approach (Popovic et

al., 2013). The two first principal components (PCs) were sufficient to explain the maximum variation in all original data. Figure 3 shows plots of loadings (Figure 3A) and scores (Figure 3B) obtained from PCs, where the first two principal components (PC1 and PC2) accounted for 96.32% of the total variance of data. In particular, PC1 explained 92.88% of the variation of the data, while PC2 explained 3.44% (Aponte et *al*., 2014). For Figure 3A, in the unit circle, parameters (moisture (M), dry matter (DM), ash (A), organic matter (OM), pH, total titratable acidity (TTA), starch (S), dry gluten (DG), weight (W), weight loss (WL), thickness (Thk), spread ratio (SR), volume (V), density (Ds), total color change (ΔE), leightness (L^*), hue angle (h°), chromaticity (c*), browning index (BI), wheitness index (WI), overall acceptance (Ov Acc), texture (Txt), color (Col), taste (Tst), Odor (Od) and appearance (App)) are well presented in the first axis than diameter (D) parameter in second axis. Figure 3A divided the previous parameters very well in the following way:

- Axis 1, for PC1:
  - Thickness (Thk), total titratable acidity (TTA), volume (V), dry matter (DM) and ash (A) are strongly negatively correlated with moisture (M), organic matter (OM), pH, starch (S), dry gluten (G), weight (W), weight loss (WL), spread ratio (SR), density (Ds), total color change (ΔE), leightness (L^*), hue angle (h°), chromaticity (c*), browning index (BI), wheitness index (WI), overall acceptance (Ov Acc), taste (Tst), texture (Txt), color (Col) and appearance (app). These variables contribute strongly to the formation of axis 1 (Popovic et *al*., 2013) ;
  - Thickness (Thk), total titratable acidity (TTA), volume (V), dry matter (DM) and ash (A) have a great effect on PC1 than moisture (M), organic matter (OM), pH, starch (S), dry gluten (G), weight (W), weight loss (WL), spread ratio (SR), density (Ds), total color change (ΔE), leightness (L^*), hue angle (h°), chromaticity (c*), browning index (BI), wheitness index (WI), overall acceptance (Ov Acc), taste (Tst), texture (Txt), color (Col) and appearance (app), because they were positively correlated by PC1 and any increase in these variables produces an increase in PC1. On the other hand, moisture (M), organic matter (OM), pH, starch (S), dry gluten (G), weight (W), weight loss (WL), spread ratio (SR), density (Ds), total color change (ΔE), leightness (L^*), hue angle (h°), chromaticity (c*), browning index (BI), wheitness index (WI), overall acceptance (Ov Acc), taste (Tst), texture (Txt), color (Col) and appearance (app) were negatively correlated by PC1 (Popovic et *al*., 2013) ;
  - Overall acceptance (Ov Acc), spread ratio (SR) and wheitness index (WI) parameters are positioned closely due to the positive correlations among them (Popovic et *al*., 2013);
  - Also, color (Col) and total color change (ΔE) parameters are positioned closely due to the significant positive correlations among them (Popovic et *al*., 2013);
  - Moreover, weight (W) and weight loss (WL) parameters are positioned closely due to the positive correlations among them (Popovic et *al*., 2013);
  - Opposite direction of moisture (M), ash (A) and total titratable acidity (TTA) on one side and dry matter (DM), organic matter (OM) ad pH on another side, indicates that moisture (M), ash (A) and total titratable acidity (TTA) are the major contributors of dry matter (DM), organic matter (OM) and pH, respectively (Popovic et *al*., 2013).
- Axis 2, for PC2:
  - Diameter (D) variable contributes slightly to the formation of axis 2 (Popovic et *al*., 2013);
  - Diameter (D) has a little small effect on PC2 because it was negatively correlated by PC2, and any increase in this variable produces a decrease in PC2 (Popovic et *al*., 2013).

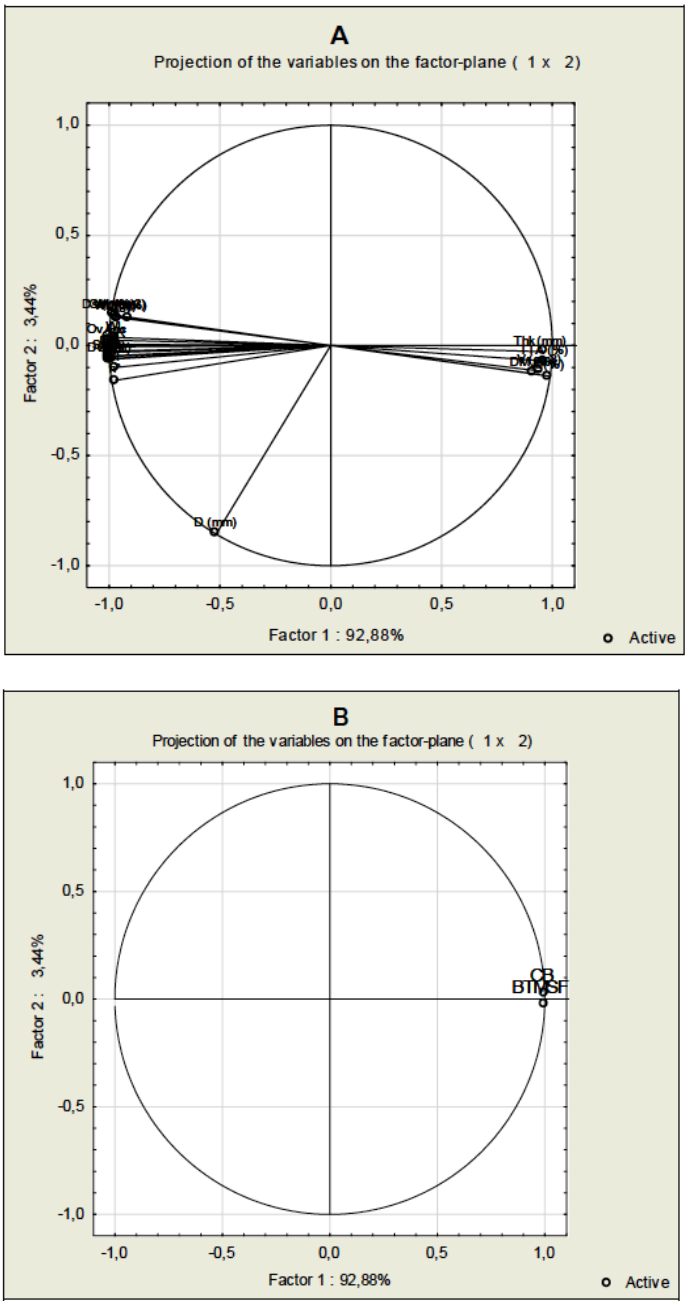


Figure 3. Graph of loading plot of physicochemical and sensory properties (A) and scores plot (B) for biscuits.

M: moisture, DM: dry matter, A: ash; OM: organic matter, TTA: total titratable acidity, S: starch, DG: Dry gluten, W: weight, WL: weight loss, d: diameter, Thk: thickness, V: volume, SR: spread ratio, D: density, L^* : leightness, h° : hue angle, c^* : chromaticity, ΔE : total color change, BI : browning index, WI : whiteness index, CB: control biscuit, BTMSF: enriched biscuit with milk thistle seeds flour.

For Figure 3B, scores distribution allowed for clustering of the samples into two groups (control biscuit (CB) and enriched biscuit with milk thsitle seeds flour (BMTSF)). The difference between these groups is based on PC1 (total titratable acidity (TTA), dry matter (DM), thickness (Thk), ash (A) and volume (V)) (Popovic et *al*., 2013). All two groups were positively negatvely scored on PC1 (Popovic et *al*., 2013). Control biscuit (CB) showed positive

score on PC2 but enriched biscuit with milk thsitle seeds flour (BMTSF) was entirely located in the negative part. In particular, enriched biscuit wih milk thistle seeds flour proved to be strongly characterized by Thickness (Thk), total titratable acidity (TTA), volume (V), dry matter (DM) and ash (A). So, thickness (Thk), total titratable acidity (TTA), volume (V), dry matter (DM) and ash (A) are higher in enriched biscuit with milk thistle seeds flour (BMTSF) than control biscuit (CB).

Results of principal component analysis (PCA) revealed the influence of milk thistle seeds flour addition on physicochemical and sensory properties of biscuit :

- ✓ Dry matter is heigher in enriched biscuit because milk thistle seeds flour had lower moisture content than wheat flour (Table 1). The reduction of total solids in dough results biscuit has lower weight (Serrem, 2010).
- ✓ Enriched biscuit with milk thistle seeds flour contains high value of ash and a low value of organic matter. Hegazy et *al*. (2014) and Serrem (2010) found an increase in ash content of enriched biscuits with chestnut *(Castanea sativa* Mill.) and soy flours, respectively. Ash content of biscuit could be raised if milk thistle seeds flour is incorporated. This finding showed that addition of milk thistle seeds flour in cereal products enhanced their nutritional values ;
- ✓ pH decreased and total titratable acidity increased sleightly in enriched biscuit with milk thistle seeds flour. Rizzello et *al*. (2010) reported a decrease in pH with an increase in total titratable acidity of prepared bread with fermented wheat germ flour ;
- ✓ Enriched biscuit with milk thistle seeds flour had lower starch content. This decrease in starch content could be explained by the low carbohydrate content of flour. Similarly, Serrem (2010) found a decrease in starch content of enriched biscuit with soy flour ;
- ✓ Dry gluten content decreased significantly with milk thistle seeds flour addition in biscuit (Table 2). This effect is a self-evident result from the substitution of wheat flour with milk thistle seeds flour, reducing the amount of gluten in the composite flour. Hallén et *al*. (2004) found a decrease in gluten content of enriched biscuit with cowpea flour ;
- ✓ It was observed that biscuit made with milk thistle seeds flour had the highest thickness and volume and the least weight, diameter and spread ratio than control biscuit. Difference in spread ratio depends on source of flours substitution. Cookies having higher spread ratio are considered the most desirable (Hyun-Jung et *al*., 2014) ;
- ✓ It can be seen that biscuit density seemed to be affected by milk thistle seeds flour addition. Density is considered the best index of sensory texture of biscuits (Mamat and Hill. 2014) ;
- ✓ Substituting wheat flour with milk thistle seeds flour in biscuit formulation significantly affects surface color of biscuit. Biscuit crust became darker with milk thistle seeds flour addition (Figure 1). Enriched biscuit leightness (L^*) decreased compared to control biscuit. Similarly, Sozer et *al*. (2014) mentioned that biscuit became darker with bran flour adding ;
- ✓ Chromaticity and hue angle values increased but color difference (ΔE^*) decreased when milk thistle seeds flour was added to biscuit. These results are similar to those found by Hegazy et *al*. (2014) for enriched biscuit with chestnut flour ;
- ✓ It was observed a general increasing trend in browning index (BI) value of enriched biscuit with milk thistle seeds flour. This increase in browning index was due to acrylamide formation and concentration. Acrylamide concentration was generally reported to have correlation with surface color formation (Sakin-Yilmazer et *al*., 2013). Also, thermal degradation of

originally colourless complex polyphenols to coloured phenols during biscuit baking increases biscuits browning index (BI). The increase in browning index of enriched biscuit with milk thistle seeds flour may be ascribed to polyphenoloxidases activity as a result of biscuit baking (Jimoh et *al*., 2009) ;

- ✓ As the milk thistle seeds flour added to biscuit, a reduction of whiteness index values was observed. Similarly, Ostermann-Porcel et *al*. (2017) found that as the okara content increased in cookies formulation, a reduction of whiteness index (WI) values was observed ;
- ✓ Biscuits made with wheat had the highest ratings for all tested sensory parameters. Results indicated that, adding of natural milk thistle seeds flour to biscuit led to decrease of sensory scores of color, appearance and taste. In the same time odor, texture and overall acceptance of enriched biscuit with milk thistle seeds flour are not more affected significantly. Hegazy et *al*. (2014) reported similar results for enriched biscuit with natural chestnut (*Castanea sativa* Mill.) flour.
- ✓ In addition, milk thistle seeds flour addition may provide additional flavor of milk thistle seeds to cookies which could change consumer's acceptability Abd Raboh (2012).

4. Conclusions

The results of this study suggest that milk thistle seeds flour may be blended with wheat flour at levels of 10% to obtain enriched biscuit. Compared to control biscuit, we see major differences in terms of quality of enriched biscuit (increase in volume (13.48 cm^3) with a decrease in weight (6.63 g), in density (0.49 g/cm^3) and in spread ratio (6.38)). Also, as milk thistle seeds flour added, biscuit color becam brown (L*: 64.43). Moreover, the characterization performed in this study proved that the milk thistle seeds flour is a valuable source of minerals (3.1%), starch (51.4%) and it could be used as a natural enrichment of gluten-free bakery product.

5. References

Abd Raboh, F. F. (2012). Chemical studies on milk thistle seeds as a novel source of human food. Egypt: Tanta University, PhD thesis.

Abu Jadayil, S., Tukan, S. K. and Takruri, H. R. (1999). Bioavailability of iron from four different local food plants in Jordan. *Plant Foods for Human Nutrition*, 54(4), 285-294.

Agrahar-Murugkar, D., Gulati, P., Kotwaliwale, N. and Gupta, C. (2015). Evaluation of nutritional, textural and particle size characteristics of dough and biscuits made from composite flours containing sprouted and malted ingredients. *Journal of Food Science and Technology,* 52(8), 5129-5137.

Aliyas, I. M. (2015). Wild Milk Thistle Unique Fatty Plant. *International Journal of Science and Research,* 6(1), 1227-1229.

American Association of Cereal Chemistry (A.A.C.C.). (2000). Official Methods of Analysis. 9th ed. U.S.A.: St. Paul., Minnesota.

Andrzejewska, J., Martinelli, T. and Sadowska, K. (2015). Silybum marianum: non-medical exploitation of the species : review article. *Annals of Applied Biology*, 167, 285-297.

Aponte, M., Boscaino, F., Sorrentino, A., Coppola, R. M. P. and Romano, A. (2014). Effects of fermentation and rye flour on microstructure and volatile compounds of chestnut flour based sourdoughs. *LWT - Food Science and Technology,* 58, 387-395.

Apostol, L., Iorga, S., Mosoiu, C., Racovita, R. C. and Niculae, O. M. (2017). The effects of partially defatted milk thistle (*silybum marianum*) seed flour on wheat flour. *Journal of International Scientific Publications, Agriculture and Food*, 5, 74-84.

Association of Official Analytical Chemists (A.O.A.C.). (2000). Official Methods of Analysis. 17th ed. U.S.A.: Arlington, Virginia.

Awad-Allah, M. A. A. (2013). Evaluation of selected nuts and their proteins functional properties. *Journal of Applied Sciences Research,* 9(1), 885-896.

Baljeet, S. Y., Ritika, B.Y. and Reena, K. (2014). Effect of incorporation of carrot pomace powder and germinated chickpea flour on the quality characteristics of biscuits. *International Food Research Journal,* 21(1), 217-222.

Barak, S., Mudgil, D. and Khatkar, B. S. (2014). Effect of flour particle size and damaged starch on the quality of cookies. *Journal of Food Science* and *Technology,* 51(7), 1342-1348.

Brodowska, M., Guzek, D. and Wierzbicka, A. (2014). Modern technological solutions used in the production of bakery products with high biological value. *Advances in Science and Technology Research Journal*, 8(22), 83-92.

Budryn, G., Żyżelewicz, D., Nebesny, E., Oracz, J. and Krysiak, W. (2013). Influence of addition of green tea and green coffee extracts on the properties of fine yeast pastry fried products. *Food Research International*, 50, 149-160.

Chinma, C. E., Adewuyi O. and Abu J. O. (2009). Effect of germination on the chemical, functional and pasting properties of flour from brown and yellow varieties of tigernut (*Cyperus esculentus*). *Food Research International,* 42, 1004-1009.

Dayakar, R. B. and Bhargavi, G. (2017). Technology Involved in Quality of Biscuits: Influence of Factors and Impact on Processing – A Critical Review. *International Journal of Pure & Applied Bioscience,* 5(4), 532-542.

Eke-Ejiofor, J., Beleya, E. A. and Onyenorah, N. I. (2014). The effect of processing methods on the functional and compositional properties of jackfruit seeds flour. *International Journal of Nutrition and Food Sciences*, 3(3), 166-173.

Elkhalifa, A. O. and Bernhardt, R. (2010). Influence of grain germination on functional properties of sorghum flour. *Food Chemistry,* 121, 387-392.

Francis, F. (1998). Colour analysis in: food S.S. (Nielsen ed.). Aspen Publishers, Gaithersburg. U.S.A.: 559-612.

Galla, N. R., Pamidighantam, P. R., Karakala, B., Gurusiddaiah, R. M. and Akula, S. (2007). Nutritional, textural and sensory quality of biscuits supplemented with spinach (*Spinacia oleracea* L.). *International Journal of Gastronomy and Food Science,* 7, 20-26.

Hallén, E., Ibanoglu, S. and Ainsworth, P. (2004). Effect of fermented/germinated cowpea flour addition on the rheological and baking properties of wheat flour. *Journal of Food Engineering,* 63, 177-184.

Hegazy, N. A., Kamil, M. M., Hussein, A. M. S. and Bareh, G. F. (2014). Chemical and technological properties of improved biscuit by chestnut flour. *International Journal of Food and Nutritional Sciences*, 3(6), 7-15.

Heinio, R. L., Noort, M. W. J., Katina, K., Alam, S. A., Sozer, N., de Kock, H. L., Hersleth, M. and Poutanen, K. (2016). Sensory characteristics of wholegrain and bran-rich cereal foods -A review. *Trends in Food Science and Technology*, 47, 25-38.

Hyun-Jung, C., Ahra, C. and Seung-Taik, L. (2014). Utilization of germinated and heat-moisture treated brown rices in sugar-snap cookies. *LWT - Food Science and Technology,* 57, 260-266.

Jimoh, K. O., Olurin, T. O. and Aina, J. O. (2009). Effect of drying methods on the rheological characteristics and colour of yam flours. *African Journal of Biotechnology,* 8(10), 2325-2328.

Korus, J., Witczak, M., Rafał, Z. and Juszczak, L. (2015). The influence of acorn flour on rheological properties of gluten‐free dough and physical characteristics of the bread. *European Food Research* and *Technology,* 240, 1135-1143.

Mamat, H. and Hill, S. E. (2014). Effect of fat types on the structural and textural properties of dough and semi-sweet biscuit. *Journal of Food Science and Technology*,

51(9), 1998-2005.

Mateos-Aparicio, I., Redondo-Cuenca, A. and Villanueva-Suárez, M. J. (2010). Isolation and characterisation of cell wall polysaccharides from legume by-products: Okara (soymilk residue), pea pod and broad bean pod. *Food Chemistry*, 122, 339-345.

Mogol, B. A. and Gökmen, V. (2014). Mitigation of acrylamide and hydroxymethylfurfural in biscuits using combined partial conventional baking and vacuum post-baking process: Preliminary study at the lab scale. Innovative *Food Science and Emerging Technologies,* 26, 265-270.

Onacik-Gür, S. Ż. A. and Jaroszewska, A. (2015). Effect of high-oleic sunflower oil and other pro-health ingredients on physical and sensory properties of biscuits. *CyTA–Journal of Food,* 13(4), 621-628.

Oppong, D., Eric, A., Samuel, O. K., Eric, B. and Patrick, S. (2015). Proximate composition and some functional properties of soft wheat flour. *International Journal of Innovative Research in Science, Engineering and Technology,* 4(2), 2347-6710.

Ostermann-Porcel, M. V., Quiroga-Panelo, N., Rinaldoni, A. N. and Campderrós, M. E. (2017). Incorporation of okara into gluten-free cookies with high quality and nutritional value. *Journal of Food Quality*, 2017, 1-8.

Pandey, H. and Awasthi, P. (2015). Effect of processing techniques on nutritional compositio and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seeds flour. *J*ournal of *Food Science and Technology,* 52(2), 1054-1060.

Popovic, B. M. D., Stajner, D. R., Zdero, O. S. and Galic, Z. (2013). Antioxidant characterization of oak extracts combining spectrophotometric assays and chemometrics. *The Scientific World Journal*, 2013, 1-8.

Ragaee, S., Abdel-Aal, E. S. M. and Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry,* 98, 32-38.

Reddy, V., Urooj, A. and Kumar, A. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chemistry,* 90, 317-321.

Rizzello, C. G., Nionelli, L., Coda, R., Di, C. R. and Gobbetti, M. (2010). Use of sourdough fermented wheat germ for enhancing the nutritional, texture and sensory characteristics of the white bread. *European Food Research* and *Technology*, 230, 645-654.

Sadowska, K. (2006). Owoce ostropestu plamistego jako prozdrowotny dodatek do pieczywa. *Żywność. Nauka. Technologia. Jakość*, 47(2), 290-296.

Shahat, M. S., Hussein, A. S. and Hady, E. A. (2016). Preparation of bread supplemented with milk thistle flour and its effect on acute hepatic damage caused by carbon tetrachloride in rats. *Middle East Journal of Applied Sciences,* 6(3), 531-540.

Sakin, M., Kaymak-Ertekin, F. and Ilicali, C. (2007). Modeling the moisture transfer during baking of white cake. *Journal of Food Engineering,* 80, 822-831.

Sakin-Yilmazer, M., Kemerli, T., Isleroglu, H., Ozdestan, O., Guven, G., Uren, A. and Kaymak-Ertekin, F. (2013). Baking kinetics of muffins in convection and steam assisted hybrid ovens (baking kinetics of muffin. . .). *Journal of Food Engineering*, 119, 483-489.

Serrem, C. A. (2010). Development of soy fortified sorghum and bread wheat biscuits as a supplementary food to combat protein energy malnutrition in young children. Department of Food Science. Faculty of Natural and Agricultural Sciences. University of Pretoria, South Africa : pp 78, p 54, 55, 89-91.

Sozer, N., Cicerelli, L., Heiniö, R. L. and Poutanen, K. (2014). Effect of wheat bran addition on in vitro starch digestibility, physicomechanical and sensory properties of biscuits. *Journal of Cereal Science*, 60, 105-113.

Youssef, H. M. K. E. and Mousa, R. M. A.

(2012). Nutritional Assessment of Wheat Biscuits and Fortified Wheat Biscuits with Citrus Peels Powders. *Food and Public Health*, 2(1), 55-60.

Yusuf, A. A., Ayedun, H. and Sanni, L. O. (2008). Chemical composition and functional properties of raw and roasted Nigerian benniseed (Sesamum indicum) and bambara groundnut (Vigna subterranean). *Food Chemistry*, 111, 27-282.

Zouari, R., Besbes, S., Ellouze-Chaabouni, S. and Dhouha, G. (2016). Cookies from composite wheat–sesame peels flours: dough quality and effect of Bacillus subtilis SPB1 biosurfactant addition. *Food Chemistry*, 194, 758-769.

Zucco, F., Borsuk, Y. and Arntfield, S. D. (2011). Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. *LWT - Food Science and Technology,* 44, 2070-2076.

Acknowledgment

Food Science Laboratory technicians are thanked for their assistance throughout the preparation of this work.


CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal home page:**http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECT OF PROCESSING ON BETA CAROTENE, ASCORBIC ACID AND CHLOROPHYLL RETENTION OF SPINACH AND MINT

Pinki Saini[1]*, Priyanka Singh[1], Anchal Singh[1] and Urvashi Srivastava[1]

Centre of Food Technology, Science Faculty Campus University of Allahabad Prayagraj, 211002, UP, India

**pspinki55@gmail.com*

https://doi.org/10.34302/2019.11.4.2

Article history:
Received:
9 February 2019
Accepted:
28 September 2019

Keywords:
Chlorophyll;
Leafy vegetables;
Ascorbic acid.

ABSTRACT

Spinach and mint were dehydrated in a cabinet and microwave drier and were subsequently studied for rehydration characteristics. Dehydration varied from 10.20:1.0 to 21.55:1.0, while rehydration ratio varied from 1.0:3.57 to 1.0:5.02 for the two green leafy vegetables. The bulk density of dried vegetables varied from 53.38 to 120.40, whereas the angle of repose was in the range of 40.8 to 56.6. Colour values L, a, b and ΔE were reduced with blanching and drying. Retention of chlorophyll, β carotene and ascorbic acid in dehydrated products varied from 42.54 to 55.45%, 32.08 to 51.96% and 25.58 to 45.85% respectively. On rehydration the ascorbic acid retention was further reduced to 4.52 to 15.80%.

1.Introduction

Green leafy vegetables have unique place among vegetables because of there color, flavor and health benefit. They boost coronary health since they are surprisingly low in fat and high in dietary fibre. The beneficial nutrients present in leafy vegetables include folic acid, vitamin C, potassium and magnesium. They also play host to a wide variety of phytochemicals, such as lutein, beta-cryptoxanthin, zeaxanthin, and beta-carotene.

Drying is a method of food preservation that works by removing water from the food, which prevents the growth of microorganisms and decay. By drying process, it restrict the wastage of over produced leaves, preserve the macro and micro nutrients of leaves and make available leaves after the season. Due to high production and high nutritional value, but short storage life due to high moisture content it is necessary to remove moisture by drying and increase the shelf life of spinach and mint leaves. Dehydrated vegetables are simple to use and have a longer shelf life than fresh vegetables (Chauhan and Sharma 1993, Gupta et al., 2013). Kuppuswamy and Rao (1970) reported that to obtain high-quality dehydrated vegetables, the drying process should allow effective retention of color, flavor, texture, taste and nutritive value. Mint and spinach are rich in iron, b-carotene, ascorbic acid and chlorophyll. The current study was undertaken to assess the effect of processing on the physicochemical properties of mint and spinach.

2.Material and methods

2.1.Raw materials

Spinach and mint used for this investigation has been purchased from the local market of Allahabad, Uttar Pradesh, India.

2.2.Drying

Stalks of spinach, mint were removed, washed and chopped. Chopped stalks were blanched in water (95/2min) followed by dip in potassium metabisulfite solution (5g/L) for 1 min. Blanched vegetables were dehydrated in a cabinet drier consisting of 0.4 ×0.84 0×.95m stainless steel inner chamber ,5 kW heater and a fan to circulate air inside the chamber at 65±3C for 5 h. The blanched vegetables were also subjected to microwave drying at 640W for 6 min and 10 min respectively for spinach and mint. Mass of the material was noted at every stage. Experiments were carried out in duplicate.

2.3. Rehydration

Rehydration was carried out by steeping the dried samples (5g) in water (120ml) at 65C for 40 min for hot water rehydration (HWR) and at 27C for 2h for cold water rehydration (CWR).

2.4.Dehydration and Rehydration Ratio

Dehydration and rehydration ratio was calculated by dividing the weight of the fresh or rehydrated sample by the weight of the dried sample.

2.5.Bulk and True density, Angle of repose

Dried sample of know weight was placed into a measuring cylinder, and its volume was noted to compute bulk density. A weighed amount of dehydrated sample was added to a measuring cylinder containing a known volume of toluene and its rise in volume per unit mass gave true density. The angle between the horizontal axis and slope of heap of the dried sample was measured with a protractor to obtain the angle of repose.

2.6.Color Measurement

Visual color was measured using Hunter colorimeter (X-rite) in term of L (lightness), a (redness), and b (yellowness and blueness). The values were used to compute $\tan^{-1}(b/a)$ and $\Delta E=\sqrt{L^2+a^2+b^2}$

2.7.Moisture

Moisture content of spinach and mint was determined by using the hot air oven drying method at 110C for 2 h (AOAC 1990).

2.8.β-carotene

A sample (2 gm) was extracted with acetone and then transferred to a separating funnel containing 10 to 15 ml of petroleum ether (Rangana 1986).

2.9.Ascorbic acid

The sample was extracted with (3%) metaphosphoric acid and titrated against standardized 2, 6 dichlorophenol indophenol dye till the appearance of light pink color which persisted for 15 sec (Ranganna 1986).

2.10.Chlorophyll

A sample (10) was mixed with magnesium carbonate (0.1g) and extracted with acetone in a pestle mortar using purified sand until the residue become colorless **(**Ranganna 1986). Volume of extracted was made to 50ml and this was taken in a separating funnel along with petroleum ether (50ml) water was transferred from the sides of the separating funnel until the water layer was of all fat soluble pigment s . the water layer was drained off, and the ether layer was washed 5-10 times with distilled water and anhydrous sodium sulphate (3gm) is put on filter paper in funnel and pass the extracted through it and was diluted by petroleum ether to 100ml in volumetric flask, and this OD was measured at 660.0 and 642.5 nm using a UV visible spectrophotometer (Thermoscientific Pvt. Ltd.).

2.11.Statistical Analysis

The mean and SD of moisture content, bulk density, angle of repose, colour values, chlorophyll content, β-carotene and ascorbic acid were calculated. One way analysis of variance and least significant difference were employed to check the significant effect ($P<0.05$) of dehydration, rehydration, blanching and drying on spinach and mint (Cochran and Cox, 1957).

3.Results and Discussion

3.1. Dehydration and Rehydration of Green Leafy Vegetables

Moisture content in fresh mint and spinach was 86.16% and 93.91% on w.b., respectively. Mint contained more solids than spinach. The weight of fresh green leafy vegetable was reduced from 100.0 to 88.95 and 67.32 g on blanching for mint and spinach, respectively (Table 1). Higher weight loss due to blanching was observed in spinach followed by and mint. Loss of weight during blanching was due to loss of solids and water. Statistical analysis revealed that moisture content decreased ($P < 0.05$) on blanching in all the three vegetables. Spinach and mint were then dried in a cabinet drier and microwave oven. Studies showed that the moisture content of the cabinet dried product was lower than microwave dried products. The weight of the product was also lower in the cabinet drier, which indicated higher solid loss during cabinet drying. Negi and Roy (2000) had reported that sun drying of leafy vegetables seemed to present a disadvantage of more solid losses during drying. The dehydration ratio for cabinet-dried mint and spinach were 11.24:1.0 and 21.55:1.0 respectively (Table 2). The dehydration ratio in microwave drying was lower than that of cabinet drying. Low dehydration ratio implied that yield was more in microwave dried products. Statistical analysis showed that the dehydration ratios of microwave dried mint and spinach were ($P < 0.05$) lower than those of sun dried. Gupta and Nath (1984) have also reported higher dehydration ratio in sun-dried samples than that of cabinet-dried samples. Previous studies showed that dehydration ratio was 22:1 in cabinet-dried spinach (Lal 1944).

Dried samples were rehydrated in cold and hot water. Weight of the cabinet and microwave dried mint was increased to 21.18 and 17.87 g in CWR and to 24.45 and 20.60 g in HWR, respectively, per 100-g fresh green leafy vegetable. Similarly weight of the cabinet and microwave dried spinach was also increased to 18.24 and 21.55 g in CWR and to 24.50 and 22.44 g in HWR, respectively, per 100-g fresh green leafy vegetable (Table 1). Moisture content of the rehydrated vegetables varied between 75.15 and 80.75%. The rehydration ratio in cabinet-dried mint was the highest both in cold and hot water (Table 2). Rehydration ratio in microwave dried mint was the lowest both in cold and hot water. However, cabinet dried spinach samples showed rehydration ratio in the range of 1.0:3.64–1.0:4.35. Statistical analysis showed ($P < 0.05$) different rehydration ratios. Bajaj *et al.* (1993) gave different blanching treatments to get better quality dried fenugreek product for which rehydration ratio ranged from 1.0:5.9 to 1.0:7.2. The effect of blanching, pricking and drying air temperature on the rehydration ratio of peas dehydrated in fluidized bed dryer showed that unpricked samples had a rehydration ratio less than 1.0:3.0, while pricked samples had a rehydration ratio greater than 1.0:3.2 (Narain and Kanawade, 1993). Thus, the rehydration ratio of spinach, mint and mustard was lower than dried fenugreek but was higher than peas.

Table 1. Effect of dehydration and rehydration on yield and moisture content of mint and spinach (n=3)

Processing condition	Mint		Spinach	
	Yield g/100g raw material	Moisture Content (%)	Yield g/100g raw material	Moisture Content (%)
Fresh	100	86.16±0.99[a]	100	93.91±1.19[a]
Blanched	88.95	84.79±0.09[b]	67.32	91.94±1.08[b]
Cabinet dried	8.5	6.43±0.35[g]	4.64	4.57±0.07[g]
Microwave dried	9.8	8.46±0.15[f]	5.20	5.14±0. 13[f]
CWR				

Cabinet dried	21.18	76.22±0.35^{e}	18.24	75.21±0.98^{e}
Microwave dried	17.87	80.55±1.25^{c}	21.55	80.75±0.22^{c}
HWR				
Cabinet dried	24.45	76..20±1.54^{e}	24.50	75.15±1.45^{e}
Microwave dried	20.60	78.5±21.48^{d}	22.44	79.48±1.20^{d}

CWR: Cold Water Rehydration; **HWR**: Hot Water Rehydration
Means in a column, within processing condition, not followed by a common letter are significantly different at $P < 0.05$

Table 2. Dehydration and rehydration ratios, bulk density and angle of repose of cabinet dried and microwave dried mint and spinach (n=3)

Sample	**Dehydration ratio**	**Rehydation ratio**		**Bulk density (Kg/m^3)**	**Angle of repose (º)**
		Cold water	Hot water		
Cabinet dried mint	11.24:1.0^{c}	1.0:4.23	1.0:5.02	53.380±.091^{d}	50.5±2.87^{b}
Cabinet dried spinach	21.55:1.0^{a}	1.0:3.64	1.0:4.35	102.38±0.07^{c}	40.8±2.53^{d}
Microwave dried mint	10.20:1.0^{d}	1.0:3.57	1.0:4.12	105.15±0.06^{b}	56.6±1.24^{a}
Microwave dried spinach	19.23:1.0^{b}	1.0:3.84	1.0:4.49	120.40±0.02^{a}	43.2±2.44^{c}

Means in a column, within processing condition, not followed by a common letter are significantly different at $P < 0.05$
Means without superscript are non significant

3.2.Bulk Density and True Density

Microwave dried samples had more bulk density than cabinet-dried samples (Table 2). Microwave dried spinach had the highest bulk density (120.40 kg/m3), whereas mint had bulk density in the range of 53.380 – 105.15 kg/m3. Cabinet-dried spinach also had higher bulk density of 102.38 kg/m3 than mint. Statistical analysis revealed that dried spinach had ($P < 0.05$) higher bulk density than mint. The bulk density of fresh spinach was 224 kg/m3, and that of cabbage was 449 kg/m3 (Mohsenin 1970). The bulk density of rapeseed was observed to be 585.1–612.1 kg/m3 at three different moisture contents (Sedat *et al.* 2004). Results show that products produced in the current study were fluffy, having low bulk density.

3.3. Angle of Repose

Angle of repose was the highest for microwave dried mint (56.6°) and the lowest for cabinet-dried spinach (40.8°) (Table 2). The cabinet-dried mint and spinach both had lower angle of repose than the microwave dried one. Difference in angle of repose would be due to the difference in the surface properties of the dried vegetables. Statistical analysis showed ($P < 0.05$) different values of angle of repose of dehydrated leafy vegetables. The angle of repose was reported to be 39.7° at 3.46% moisture content for wheat grains (Fowler and Wyatt 1960). Results show that the angle of repose values were near to those of wheat grains.

3.4. Colour

There was decrease in greenness after blanching and drying (Table 3). In the case of mint, a value changed from an initial value of -6.17 in fresh sample to -6.07 after blanching and to -2.58 after cabinet drying. It indicated a decrease in greenness with blanching and drying. In fresh mint, L value was 32.53, which decreased to 28.81 after blanching, 17.51 after cabinet drying and 25.26 after microwave drying. Thus, lightness (L value) also decreased in fresh samples and was least in cabinet-dried samples. ΔE value for fresh mint was 31.84, which decreased to 25.16 after blanching. After

cabinet drying and microwave drying of mint, ΔE value was 18.35 and 20.59, respectively. Therefore, ΔE value was the maximum for fresh samples, and it decreased after blanching and drying. For fresh mint, tan^{-1} b/a value was -0.995, and after blanching, it was -0.835. After cabinet drying and microwave drying, tan^{-1} b/a value was -1.038 and -1.189, respectively. Similar results were observed in the case of spinach. Statistical analysis did not show significant difference in color. Color degradation kinetics of spinach, mustard leaves and mixed puree was studied in respect to both visual green color (-a value) and total color (L x [-a] x b) (Ahmed *et al.* 2002). It was observed that during thermal processing, apart from Hunter -a value, both L and b values also decreased with time at a given temperature. Rocha *et al*. (1993) studied the effects of pretreatments and drying conditions on drying rate and on the chlorophyll and color retention (L, a, b value) of air-dried basil. L and b values decreased with the increase in drying temperature. So, the results of the current study are in accordance with Ahmed *et al.* (2002) and Rocha *et al.* (1993).

Table 3. Color values of mint and spinach at different drying stage (n=3)

Processing condition	***L***	***a***	***b***	***Tan-1 b/a***	$\Delta E=\sqrt{L^2+a^2+b^2}$
Mint					
Fresh	32.53± 0.17^{b}	-6.17±0.05^{b}	13.02±0.24^{a}	-0.995± 0.33^{c}	31.84±1.17^{b}
Blanched	28.81± 1.30^{c}	-6.07±0.76^{b}	10.62±0.22^{c}	-0.835 ±1.25^{a}	25.16±2.05^{d}
Cabinet dried	17.51± 0.68^{g}	-2.58±0.31^{e}	7.83±0.39^{e}	-1.038± 0.35^{bc}	18.35±0.05^{f}
Microwave dried	25.26± 3.43^{d}	-2.53±0.27^{e}	6.23±0.33^{f}	-1.189± 0.21^{ab}	20.59±1.15^{e}
Spinach					
Fresh	34.91±0.87^{a}	-6.04±0.12^{b}	11.81±0.31^{b}	-0.850± 0.16^{a}	35.25±0.85^{a}
Blanched	24.28±1.15^{e}	-7.14±0.31^{a}	9.16±0.30^{d}	-0.815± 0.24^{a}	28.83±2.50^{c}
Cabinet dried	22.33±2.32^{f}	-3.13±0.12^{d}	6.44±0.18^{f}	-1.105± 0.09^{b}	18.17±0.54^{f}
Microwave dried	24.10±0.79^{e}	-4.75±0.80^{c}	8.10±0.44^{e}	-1.275± 0.58^{a}	20.75±0.38^{e}

L, lightness /darkness; **a**, redness +greenness; **b**, yellowness and blueness
Means in a column, within processing condition, not followed by a common letter are significantly different at $P < 0.05$

3.5. Chlorophyll

Chlorophyll content in fresh mint and spinach was 181.92 and 100.24 mg/100 g raw material on w.b., respectively. Chlorophyll content was higher in mint (1,426.18 mg/100 g raw material, d.b.) than spinach (Table 4). Fresh spinach contained 1,310 mg chlorophyll/100 g raw material (d.b.) (Negi and Roy 2000). The chlorophyll retention after blanching was higher in spinach followed by mint. The chlorophyll retention was the maximum in microwave drying as compared to cabinet drying. Chlorophyll content was 1,085.24 and 1,095.23 mg/100 g raw material (d.b.) in cabinet and microwave dried spinach, respectively. Retention of chlorophyll after dehydration was found to be in the range of 42.54 – 55.45%. Retention of chlorophyll in kachi, dhantu and honagone upon dehydration was in the range of 52–71% (Madhura and Majumdar 2001). Chlorophyll content was compared to greenness (-a value) determined by Hunter colorimeter. As the chlorophyll content decreased after blanching and drying, similarly, greenness (-a value) also decreased after blanching and drying in spinach and mint.

Table 4. Effect of blanching and drying on chlorophyll, beta carotene and ascorbic acid content per 100gm raw material (d.b) of mint and spinach

Processing condition	Chlorophyll mg/100 g RM(d.b)	Overall retention (%)	β- carotene mg/100g RM(d.b)	Overall retention (%)	Ascorbic acid mg/100g (d.b)	Overall retention (%)
Mint						
Fresh	1,426.18±7.56^{a}	100	35.40± 0.03^{a}	100	285.33± 2.84^{a}	100
Blanched	1,010.83±10.52^{b}	55.21	27.21± 1.91^{b}	68.10	173.35± 2.19^{b}	48.98
Microwave dried	822.52± 1.38^{c}	48.81	17.18± 1.05^{c}	51.96	108.09± 0.19^{c}	37.54
Cabinet dried	742.33± 1.18^{d}	42.54	10.61± 1.75^{f}	42.15	86.67± 0.20^{d}	25.58
CWR						
Microwave dried	718.15± 2.84^{e}	40.85	16.34± 0.94^{d}	48.73	25.28± 2.35^{e}	9.20
Cabinet dried	692.20± 1.12^{f}	39.45	10.02± 2.24^{g}	40.06	17.44± 0.7^{g}	6.32
HWR						
Microwave dried	685.72± 1.89^{g}	38.82	14.29± 2.27^{e}	45.88	18.84± 0.08^{f}	5.37
Cabinet dried	635.85± 2.20^{h}	33.52	6.83± 0.25^{h}	38.05	12.43± 0.17^{h}	4.52
Spinach						
Fresh	1,385.80± 1.32^{a}	100	38.55± 0.65^{a}	100	135.89± 6.42^{a}	100
Blanched	1,243.14± 4.52^{b}	57.58	35.67± 1.04^{b}	58.15	103.09± 0.20^{b}	51.73
Microwave dried	1,095.23±2.48^{c}	55.45	23.33± 2.15^{c}	50.16	91.67± 0.15^{c}	45.85
Cabinet dried	1,085.24±1.65^{d}	50.56	11.0 4± 1.80^{g}	32.08	65.25± 0.10^{d}	32.58
CWR						
Microwave dried	1,066.36±1.85^{e}	48.75	22.33± 0.08^{d}	45.81	35.57± 1.41^{e}	15.80
Cabinet dried	1,045.56±2.21^{f}	44.85	14.09± 2.25^{f}	30.67	20.60± 0.58^{f}	9.21
HWR						
Microwave dried	1,042.38±1.29^{g}	46.56	17.48± 1.25^{e}	41.33	19.16± 0.21^{g}	5.87
Cabinet dried	975.78± 3.75^{h}	42.89	9.55± 0.45^{h}	22.51	17.48± 1.25^{h}	8.98

CWR: Cold Water Rehydration; **HWR**: Hot Water Rehydration; **RM**: Raw material; **d.b**,: Dry Basis
Means in a column, within processing condition, not followed by a common letter are significantly different at $P < 0.05$

In CWR, loss of chlorophyll in dried samples was less as compared to HWR. After the CWR process, chlorophyll content in cabinet dried mint and spinach was 692.20 and 1.45.56 mg/100 g raw material (d.b.), respectively, while for microwave dried mint and spinach, the chlorophyll content was 718.15 and 1,066.36 mg/100 g raw material (d.b.). There was an increased loss of chlorophyll upon rehydration in hot water. Chlorophyll content after HWR process in cabinet-dried mint and spinach was 635.85 and 975.78 mg/100 g raw material (d.b.), whereas in microwave dried mint and spinach, it was 685.72 and 1042.38 mg/100 g raw material (d.b.), respectively. Overall retention of chlorophyll in CWR and HWR varied between 39.45–48.75 and 33.52 – 46.56%, respectively. Statistical analysis of chlorophyll content of mustard at different stages showed a ($P < 0.05$) reduction or loss on blanching and drying.Asimilar trend was also observed in mint and spinach. Schwartz and Lorenzo (1991) observed that chlorophyll is sensitive to heat. Chlorophyll degradation in processed foods and plant tissues has been reviewed by Heaton and Maragoni (1996).

3.6. β-carotene

β-carotene was higher in fresh spinach (38.55 mg/100 g raw material, d.b.) than mint. The β-carotene content of common leafy

vegetables ranged from as low as 0.12 mg/100 g to as high as 5.580 mg/100 g raw material on w.b. (Kowsalya *et al.* 2001). The β-carotene content in fresh mint was found to be 34.49 mg/100 g (d.b.) (Kowsalya *et al*. 2001). After blanching, the β-carotene content was found to be 27.21 mg/100 g raw material (d.b.) in mint and 35.67 mg/100 g raw material (d.b.) in spinach. Overall retention of β-carotene was 68.10% in mint and 58.15% in spinach after blanching.

The β-carotene content in spinach was higher during microwave drying, i.e., 23.33 mg/100 g raw material (d.b.), than cabinet drying (11.04 mg/100 g raw material [d.b.]). In mint the β-carotene content after microwave and cabinet drying was 17.18 and 10.61 mg/100 g raw material (d.b.), respectively. After cabinet drying, the β-carotene retention varied between 32.08 and 42.15%, and after microwave drying, it was 50.16–51.96%. Devadas *et al*. (1978) revealed that sun drying of green leafy vegetables and their subsequent storage for 1 year resulted in 10–60% retention of β-carotene. Carotene is degraded by a free radical oxidation mechanism, and the degree of oxidation depends on drying temperature (Harris and Karmas 1975). Retention of β-carotene after sun drying ranged between 22.26 and 26.08%. Kowsalya *et al.* (2001) reported β-carotene retention after sun drying from 17.7 to 32.4%. The results are in accordance with the range given by earlier studies. In the earliest study by Negi and Roy (2000), β-carotene retention after drying in spinach ranged from 20 to 40%. In the present study, β-carotene retention after drying in spinach varied between 32.08 and 51.96%. So, the results are in accordance with previous studies.

After the CWR process, the β-carotene content in microwave and cabinet-dried mint was 16.34 and 10.02 mg/100 g raw material (d.b.). In microwave and cabinet-dried spinach after the CWR process, the β-carotene content was 22.33 and 14.09 mg/100 g raw material (d.b.). There was considerable effect on β-carotene content after the HWR process. The β-carotene content in microwave and cabinet-dried mint was 14.29 and 6.83 mg/100 g raw material (d.b.) after the HWR process. In microwave and cabinet-dried spinach β-carotene content was 17.48 and 9.55 mg/100 g raw material (d.b.) after the HWR process. After the CWR process, there was not much effect on the β-carotene content of dried samples, but after the HWR process, there was loss of β-carotene from the dried samples. Heating for long times can decrease β-carotene content via reactions like oxidation and isomerization (Speak *et al*. 1988). Statistical analysis indicates that there was significant ($P < 0.05$) reduction in β-carotene content as a result of blanching, drying and rehydration. These results are in agreement with Uadal and Sagar (2008) who have studied the retention of β-carotene in dehydrated amaranth, fenugreek and spinach.

3.7. Ascorbic Acid

Ascorbic acid content in a fresh sample was higher in mint (285.33 mg/100 g raw material [d.b.]) than spinach (Table 4). Ascorbic acid after blanching was higher in mint (173.35 mg/100 g raw material, d.b.) as compared with spinach (103.09 mg/100 g raw material, d.b.). Overall retention of ascorbic acid after blanching was 48.98% in mint and 51.73% in spinach. Badify and Onayemi (1987) reported that ascorbic acid retention was 42–53% in water blanching. The aforementioned results are also in this range.

There was reduction in ascorbic acid content after drying; however, retention was maximum in microwave drying followed by cabinet drying. In cabinet and microwave dried mint, the ascorbic acid content was 86.67 and 108.09 mg/100 g raw material (d.b.), while in cabinet and microwave dried spinach, the ascorbic acid content was 65.25 and 91.67 mg/100 g raw material (d.b.). Retention of ascorbic acid ranged from 32.08 to 42.15 mg/100 g raw material (d.b.) in cabinet dried leafy vegetables, while it was 91.67 to 108.09 mg/100 g raw material (d.b.) in microwave dried leafy vegetables, which corresponds to 25.58 – 32.58% and 37.54 – 45.85%. Lakshmi

and Vimala (2000) reported that retention of ascorbic acid ranged from 15 to 31% in sun-dried leafy vegetables and from 37 to 49% in cabinet-dried leafy vegetables. Thus, in the present study, retention of ascorbic acid is comparable to previous reported values.

Ascorbic acid content in cabinet and microwave dried mint after CWR was 17.44 and 25.28 mg/100 g raw material (d.b.), respectively whereas in spinach, it was 20.60 and 35.57 mg/100 g raw material (d.b.). After rehydration in hot water, there was increased loss in ascorbic acid in microwave dried samples in comparison to cabinet-dried samples. The ascorbic acid content after HWR in cabinet and microwave dried mint was 12.43 and 18.84 mg/100 g raw material (d.b.). Statistical analysis showed a ($P < 0.05$) reduction in ascorbic acid content as a result of leaching and thermal degradation. Overall retention of ascorbic acid in HWR and CWR varied between 12.43–19.16 and 17.44–35.57 mg/100 g raw material (d.b.), respectively. The retention of ascorbic acid in leafy vegetables was in the range of 4.52–15.80%. The losses in ascorbic acid could be attributed to increased activities of ascorbic acid oxidizing enzymes, leaching and destruction (Tapadia *et al*. 1995).

4.Conclusions

Two green leafy vegetables were selected to study the effect of processing on their physical and chemical properties. Dehydration ratio was high in the case of cabinet dried samples as compared to microwave dried samples. Rehydration ratio was in the range between 1.0:3.57 and 1.0:5.02. Bulk density varied between 53.38 and 120.40 kg/m3, and its values were higher in the case of microwave dried samples compared to cabinet-dried samples. Ascorbic acid content was found to be higher in fresh mint (285.33 mg/100 g raw material, d.b.) followed by spinach. The β-carotene content in the fresh sample was found to be higher in spinach (38.55 mg/100 g raw material, d.b.) followed by mint, whereas the chlorophyll content was higher in mint (1,426.18 mg/100 g raw material, d.b.) followed by spinach. Ascorbic acid retention after blanching was in the range of 48.98–51.73%. After drying and rehydration, ascorbic acid retention was found to be in the range of 25.58–45.85 and 4.52–15.80%, respectively. The increased loss of ascorbic acid could be attributed to leaching, oxidation, effect of heat and light. Retention of chlorophyll after drying varied between 42.54 and 55.45% whereas after rehydration, chlorophyll retention was in the range of 33.52–48.75%.

5.References

Ahmed, J., Kaur, A., Shivhare, U. (2002). Color degradation kinetics of spinach, mustard leaves and mixed puree. *Journal of Food Science,* 67(3), 1088–1091.

AOAC. (1990) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed., Assoc. of Official Analytical Chemists, Arlington, VA. 1990.

Badify, G.I.O., Onayemi, O. (1987) Effect of blanching and drying methods on the nutritional and sensory quality of leafy vegetables. *Plant Foods Human Nutrition*, 37, 291–298.

Bajaj, M., Aggarwal, P., Minhas, K.S., Sidhu, J.S. (1993) Effect of blanching treatments on the quality characteristics of dehydrated fenugreek leaves. *Journal of Food Science and Technology,* 30(3), 196–198.

Chauhan, S.K., Sharma, C.R. (1993) Development of instant dehydrated saag. *Beverage and Food World*, 20(4), 25–26.

Cochran, W.G., Cox, G.M. (1957) *Experimental Designs*, John Wiley and Sons, Inc., New York.

Fowler, R.T., Wyatt, F.A. (1960) The effect of moisture content on the angle of repose of granular solids. *Australian Journal of Chemical Engineering,* 1, 5–8.

Gupta, R.G., Nath, N. (1984) Drying of tomatoes. *Journal of Food Science and Technology,* 21(6), 372–376.

Gupta, S., Gowri, B. S., Lakshmi, A. J., & Prakash, J. (2013). Retention of nutrients in green leafy vegetables on dehydration. *Journal of Food Science and*

Technology, *50*(5),918–925. http://doi.org/10.1007/s13197-011-0407-z

Harris, R.S., Karmas, E. (1975) *Nutritional Evaluation of Food Processing*, AVI Publishing Company, Westport, CT.

Heaton, J.W., Maragoni, A.G. (1996) Chlorophyll degradation in processed food and renascent plant tissues. *Trends in Food Science and Technology,* 7, 8–15.

Kowsalya, S., Chandrashekhar, U., Balasasirekha, R. (2001) B-carotene retention in selected green leafy vegetables subjected to dehydration. *Indian Journal of Nutrition and Dietetics,* 38, 374–383.

Kuppuswamy, A., Rao, G. (1970) Dehydration of green peas. *Journal of Food Science and Technology*, 11, 60–63.

Lakshmi, B., Vimala, V. (2000) Nutritive value of dehydrated green leafy vegetables. *Journal of Food Science and Technology*, 37(5), 465–471.

Lal, G. (1944) Dehydration of vegetables. Punjab Fruit J. 8(29), 38–41.

Madhura, C.V., Majumdar, T.K. (2001) Processing effect on colour and vitamins of green leafy vegetables. *Journal of Food Science and Technology,* 38(1), 79–81.

Mohsenin, N.N. (1970) *Physical Properties of Plant and Animal Materials*, Gorden and Breach Science Publishers, New York, NY.

Narain, M., Kanawade, V.L. (1993) Effect of pretreatment and drying air temperature on quality of peas dehydrated in fluidized bed dryer. *Journal of Food Science and Technology*, 30(2), 118–120.

Negi, P.S., Roy, S.K. (2000) Effect of blanching and drying methods on b-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. *Lebensmittel-Wissenschaft & Technologie,* 33, 295–298.

Ranganna, S. (1986) *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, Tata McGraw Hill Publications Company Limited, New Delhi, India.

Rocha, T., Lebert A., Marty-Audouin, C. (1993) Effect of pre-treatments and drying conditions on drying rate and color retention of basil. *Lebensmittel-Wissenschaft & Technologie* 26, 456–463.

Schwartz, S.J., Lorenzo, T.V. (1991) Chlorophyll stability during continuous aseptic processing and storage. *Journal of Food Science,* 56, 1059–1062.

Sedat, C., Jamir, M., Huseyin, O., Ozdeno. (2004) *Physical Properties of Rapeseed*, Department of Agricultural Machinery, Faculty of Agriculture, Selcuk University, Konya, Turkey.

Speak, A.J., Speak, S.S., Schreurs, W.H.P. (1988) Total carotenoid and ß-carotene contents of Thai vegetables and effect of processing. *Food Chemistry,* 27, 245–257.

Tapadia, S.B., Arya, A.B., Rohini Devi, P. (1995) Vitamin-C contents of processed vegetables. *Journal of Food Science and Technology,* 32(6), 513–515.

Uadal, S., Sagar,V.R. (2008). Influence of packaging and storage temperature on the quality of dehydrated selected leafy vegetables. *Journal of Food Science and Technology,* 45, 450–453.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

PRODUCTION OF ANTIHYPERTENSIVE BIOACTIVE PEPTIDES IN FERMENTED FOOD BY LACTIC ACID BACTERIA – A REVIEW

Yuliana Tandi Rubak[1], Lilis Nuraida[1,2], Dyah Iswantini[3], Endang Prangdimurti[1,2]

[1]Food Science Study Program, Department of Food Science and Technology, IPB University (Bogor Agricultural University), Indonesia,
[2]Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, IPB University (Bogor Agricultural University), Indonesia
[3]Tropical Biopharmaca Research Center, and Department of Chemistry, IPB University (Bogor Agricultural University), Indonesia
**lnuraida@gmail.com*

https://doi.org/10.34302/2019.11.4.3

Article history:
Received:
29 April 2019
Accepted:
18 September 2019

Keywords:
ACE;
Bioactive peptides;
Fermented foods,
Hypertension;
Lactic acid bacteria.

ABSTRACT
Antihypertensive bioactive peptides are one of the natural sources that can be used in preventing hypertension. Lactic acid bacteria (LAB) are known to be able to produce antihypertensive bioactive peptides in fermented foods. Angiotensin converting enzyme (ACE) plays a fundamental role in the *Renin-Angiotensin System*, which can increase blood pressure through the inactivation of decapeptide conversion process of Angiotensin I into Angiotensin II. ACE is one of the effective targets for reducing hypertension. ACE inhibitory (ACE-I) peptides had the ability to inhibit ACE by binding to the active site of the ACE enzyme. ACE-I activity were associated with the presence of aromatic and aliphatic amino acids such as Pro, Phe and Tyr on C-terminal and Val and Ile at N-terminal. The formation of ACE-I peptides in fermented foods is associated with proteolytic activity of LAB during fermentation. LAB is able to secrete extracellular proteinases breaking down proteins into simpler molecules. ACE-I peptides are generally short peptides or tripeptide consist of 2 to 20 amino acid residues with a molecular weight range of <5 kDa. The formation of ACE-I peptides in fermented foods is influenced by the LAB strain, substrate and fermentation condition. This review aimed to provide information related to formation of ACE-I peptides by lactic acid bacteria in fermented foods, the mechanism of and the factors influence the formation of ACE-I peptides.

1.Introduction

Lactic Acid Bacteria (LAB**)** are a large group of microorganisms naturally found in the gastrointestinal and urogenital tracts of humans, animals, and various fermented as well as non-fermented foods. The groups of LAB include *Lactobacillus, Streptococcus, Lactococcus, Pediococcus, Streptococcus, Leuconostoc, Oenococcus, Carnobacterium, Weisella and Tetragenococcus,* with the main characteristic of Gram-positive, round or rod, non-spore forming, capable of fermenting carbohydrates and catalase negative. LAB is characterized as facultative anaerobic or microaerophilic and anaerobic bacteria (Axelsson, 2004). LAB has been known to have functional properties conferring health beneficial effect including shortening of diarrhea duration, protecting against enteropathogenic bacterial infections, necrotizing enterocolitis (NEC) and inflammation of the stomach (Culligan *et al*., 2009; Vasiljevic and Shah, 2008), improving

lactose metabolism, decreasing cholesterol, decreasing risk of mutagenicity and carcinogenic and also stimulate the immune system (Kimoto-nira et al., 2007; Lee et al., 2011; Saad et al., 2013).

LAB isolated from breast milk had been demonstrated to be able preventing diarrhea (*L. rhamnosus* strain R23) (Nuraida et al., 2012), and assimilating cholesterol (*Pediococccus pentasaceus)* (Nuraida et al., 2011). One of the functional LAB properties which quite interesting it was their potential to produce antihypertensive peptides in various fermented foods. Antihypertensive peptides had the ability to inhibit Angiotensin Converting Enzyme (ACE).

ACE: peptidyldipeptide hydrolase, EC 3.4.15.1 were metals containing Zinc, located in the endothelial layer of the blood vessels in the lungs that plays an important role in regulating blood pressure (Jung et al., 2006). ACE increases blood pressure throught process of inactivating decapeptide Angiotensin I into Angiotensin II as an active form. The conversion process is carried out through the release of dipeptides at C-terminals from angiotensin I to form angiotensin II is a potent vasoconstrictor (Riordan, 2003), that being able to cause an increase in blood pressure or a very hypertensive compound. ACE will hydrolyze vasoactive bradykinin (Fitzgerald, 2006), stimulate an increase in aldosterone secretion in the adrenal cortex (Cheung et al., 1980) so that it causes vasoconstriction and fluid retention which is one of the causes of hypertension. Efforts to decrease blood pressure in patients with hypertension include non-pharmacologically through lifestyle changes and pharmacologically by administering antihypertensive drugs. Synthetic drugs such as captopryl, ala cepryl, and lisinopryl were widely used for the treatment of hypertensive patients, however the side effects that arise were symptoms of hypersensitivity in the form of hives and symptoms of upper respiratory tract infections such as coughing**.** Antihypertensive peptides in fermented foods produced by LAB is one of the natural sources that potentially used in prevention or treatment of hypertension. The peptides can be isolated from fermented foods, such as fermented fish, pear juice, milk and dairy products. Several researchers had succeeded isolating LAB from fermented foods that have the potential to produce angiotensin converting enzyme inhibitory (ACE-I) peptides. Among different LAB, *Lactobacillus helveticus* has often been used widely as a starter culture in dairy products to produce ACE-I peptides (Chen et al., 2015; Wang et al., 2015). These species has a Generally Recognized as Safe (GRAS) status and reported also has a pharmacological target which promises to reduce blood pressure. Calpis is Japanese-made soft drink made from skim fermented by *L. helveticus* CP 790 and *Saccharomyces cerevisiae* (Nakamura et al., 1995b). Calpis had two ACE-I dipeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) that were similar structure as captopril and enalapril (De Castro and Sato, 2015) which are commonly used for the treatment of hypertension. The purpose of this paper was to provide information on the role of LAB in the formation of ACE-I peptides in fermented foods, the mechanism of its formation, and factors influencing ACE-I peptides formation in fermented food.

2. ACE-I Bioactive Peptides

Bioactive peptide were defined as specific part or fragments of proteins that have a positive impact on body function, which can affect overall health status. The protein fragments have biological activities such as antioxidants, antimicrobial,antithrombotic, antiinflammatory, and antihypertensive (Korhonen, 2009; Choi et al., 2012; de Castro and Sato, 2015; Sanjukta and Rai, 2016). The functional activities of these bioactive peptides were based on the composition and sequence of amino acids. There was a relationship between the size and structure of amino acid peptides and ACE-I activity. ACE-I peptides were generally short peptides or tripeptides with 2-5 amino acid residues with molecular weight ranges <5 kDa (Minervini et al., 2003; Lignitto et al., 2010). Several other studies state that there were 2-20 amino acid

residues (Möller et al., 2008; Phelan and Kerins, 2011; Norris and FitzGerald, 2013)**.** Research conducted by Yamamoto et al. (1994) stated that *Lactobacillus helveticus* CP790 could produce 25 types of short peptides in milk that have ACE-I activity. In traditional fermented fish called "bekasam" Wikandari and Yuanita, (2016) found a short peptide type having ACE-I activity and being resistant to gastrointestinal proteases (pepsin and trypsin). Angiotensin-1-converting enzyme in humans consist of two form;(1) Somatic (sACE) and, (2) germinal/testicular (gACE). The form both encoded by the same gene lacated on cromosome 17 at q23, sACE is a type-I membrane bound protein that consist of a-28 residue C- terminal cytosolic domain, a 22-residue hydrophobic trans membrane domain and 1227-residue extracellular domain that is heavily glycosylated and further divided into a 612- residue N- terminal domain, linked by a 15 residue sequence to a 600-residue C-terminal domain (Zisman, 1998; Riordan, 2003)

The C-terminal domain of is primarily involved in blood pressure regulation, while the N-terminal domain is involved in control of hematopoietic stem cell differentiation and proliferation. Commercial antihypertensive drugs such as Captopril, Lisinopril, and Enalapril had similar mechanism in interacting with the active site of ACE with the domains of C and N, both on sACE or gACE (Riordan, 2003; Sturrocka et al., 2004). Both C- and N-domains containing an active site the sequences His-Glu-XX-His which serves as the zinc binding ligand. These active sites are located within the cleft of the two domain, and are protected by an N-terminal ‘ lid’. This ‘lid’ block access of large polypeptide to the active site. This is thought to explain why small peptide are more effective in inhibition ACE (Gobbetti et al., 2002; Fandiño et al., 2006). The QSAR (Quantitative structure-activity relationship modelling)show that the C-terminal of the peptide had principal importance on ACE inhibitory activity, with hydrophobic C-terminal residue being essential for high potency (Wu et al., 2006b; Wu et al., 2006a)

The active site of ACE has three sub-sites, including S1 (antepenultimate), S1′ (penultimate) and S2 (ultimate) which have different characters to bind three C- terminal amino acids substrates or inhibitors located on two homologous active sites (Brew, 2003). Competitive substrates or inhibitors containing hydrophobic amino acid in C-terminal position were preferred by ACE. To enable interaction between enzymes and inhibitors, the substrate (the inhibitors) must be bound to three sub-sites of the active site of the enzyme with different amino acid sequences (Escudero et al., 2010). Valine-Proline-Proline (Val-Pro-Pro) and Isoleucine-Proline-Proline (Ile-Pro-Pro) were tripeptide produced by *Lactobacillus helveticus* (Nakamura et al., 1995) one of the two well-known antihypertensive peptides had highest ACE-I activity had the same C-terminal sequence. Pro residue at ultimate C-terminal, explaining their high ACE-I activity.

ACE-I activity were associated with the presence of aromatic and aliphatic amino acids such as Pro, Phe and Tyr on C-terminal and Val and Ile at N-terminal (Fuchs et al., 2008; Wijesekara et al., 2011; Jao et al., 2012). Various aromatic AA of ACE-I peptides with in molecular size and activity is presented in Table 1. Hydrophobic amino acids such as Try, Phe, Ala, Ile, Val and Met or positively charged amino acid such as Arg, Lys and Pro at the terminal position peptide show an association with ACE-I activity (Rai et al., 2017). Rodríguez-Figueroa et al. (2012) showed that HPHPHLSFMAIPP peptide fraction had hydrophobic amino acids (Pro) and DDQNPH peptides with histidine residues at C-terminal responsible for high ACE-I activity in fermented milk using *L. lactis* NRRL B-50571.

Daliri et al. (2018) stated that PFNL and FNL peptides had the highest ACE-I activity (IC_{50}: 0.048 and 0.038 mg / mL) of 8 ACE-I peptide found in fermented soy milk using *L. casei* spp. *pseudoplantarum*. The FNL peptide has hydrophobic amino acids (Proline and Phenylalanine) in N-terminal and Leucine in C-terminal.

Bioactive peptides of ACE-I have been classified into 3 groups: (1) true inhibitor type, (2) substrate type, and (3) pro-drug type. IC_{50} values of the true inhibitor is not altered by preincubation with ACE. The substrat type is altered by preincubation with ACE and pro-drug type being converted to true inhibitor type by ACE or gastrointestinal proteases (Fujita et al., 2000). The study Fujita & Yoshikawa, (1999) reported the peptides Leu-Lys-Pro-Asn-Met (IC_{50}:2.4 μM) was hydrolyzed by ACE and 8-fold increased to produce Leu-Lys-Pro (IC_{50}: 0.32 μM)

1

Table 1. **Aromatic AA of ACE-I peptides in MW produced by various LAB in fermented milk**

Aromatic of AA ACE-I peptides	Molecular Weight	ACE-I activity (%) / IC_{50}	LAB producer	References
LI AKAA	<1 kDa	68.21 ± 1.06	*L. casei*	Li et al. (2017)
LHLPLP VRGPFPIIV VLGPVRGPFP	<3 kDa	IC_{50}: < 100 μg protein/mL	*E. faecalis* BCS27	Gútiez et al. (2013)
LQSW PEQSLVYP MFPPQSVLSLSQS LLYQEPVLGP KPAAVRSPAQILQWQV IHAQQK	<3 kDa	IC_{50}: 5 ± 2 μg /mL	*L. plantarum* PU11 and *Lb. lactis* DIBCA2	Nejati et al. (2013)
YQDPRLGPTGELDPA TQPIVAVHNPVIV, PKDLREN LLLAHLL NHRNRMMDHVH	<3 kDa	IC_{50} : 43.52 ± 0.61 mg/L IC_{50} : 7.78 ± 0.29 mg/L IC_{50} : 4.52 ± 0.15 mg/L IC_{50} : 19.60 ± 0.25 mg/L	*Koumiss cultures*	Chen et al. (2010)
LVYPFPG,PIHNSLPQN LVYPFPGPIH	<3 kDa	IC_{50}: 71 μM IC_{50}: 89 μM	*L. jensenii*	Pihlanto et al. (2010)
LVYPFP LPLP	≤3 kDa	IC_{50} : 132 μM IC_{50} :703 μM	*Bifidobacterium bifidum* MF 20/5	Gonzalez-Gonzalez et al.(2013)
QEPVLGPVRGPFPIIV YPSYGL HPHPHLSFMAIPP SLPQNIPPL	<3 kDa	0.041 ± 0.003 μg/mL 0.034 ± 0.002 μg/mL	*Lc. lactis* NRRL B-50572 *Lc. lactis* RRL B-50571	Rodríguez-Figueroa et al. (2012)
LHLPLP LVYPFPGPIPNSLPQNIPP	≤ 3 kDa	IC_{50} : ≤ 5 μM	*E. faecalis* CECT 5727	Quirós et al. (2007)
LVESPPELNTVQ VLESPPELN WGYLAYGLD	≤ 10-kDa	IC_{50}: 0.11 μM IC_{50}: 0.23 μM IC_{50}: 0.10 μM IC_{50}: 0.03 μM IC_{50}: 0.03 μM	*L. casei* and *kombucha cultures*	Elkhtab et al. (2017)

Aromatic of AA ACE-I peptides	Molecular Weight	ACE-I activity (%) / IC_{50}	LAB producer	References
VAPFPEVFGK LVYPFPGPLH FVAPEPFVFGKEK		IC_{50}: 0.75 µM		
DKIHPFAQ,TQTPVVVP, KAVPQ, RPKHPIKH	≤ 3 kDa	IC_{50}: 39 µM - 257 µM	*L. delbrueckii S. thermophilus* *L. paracasei*	Papadimitriou et al. (2007)
EDEVSFSP EVSFSP SFSP RSPFNL SRPFNL ENPFNL PFNL FNL	≤ 7kDa	IC_{50}: 0.571 ± 0.12 mg/mL IC_{50}: 0.133 ± 0.03 mg/mL IC_{50}: 0.262 ± 0.18 mg/mL IC_{50}: 0.811 ± 0.05 mg/mL IC_{50}: 0.131 ± 0.02 mg/mL IC_{50}: 0.287 ± 0.07 mg/mL IC_{50}: 0.048 mg/mL IC_{50}: 0.038 mg/mL	*Pediococcus acidilactici* SDL1414	Daliri et al. (2018)
AFPEHK	10 kDa	33.19 ± 2.768 37.77 ± 10.222	*L. casei* (NK9) *L. fermentum*	Parmar et al. (2017)
LIVTQ LIVT	10 kDa	IC_{50}: 0.087 µM IC_{50}: 0.110 µM	*L. casei* spp. *pseudoplantarum*	Vallabha and Tiku, (2013)
RPKHPIKHQGLPQEVEV LNENLRF FVAPFPEVFGK YQEPVLGPVRGPF YQEPVLGPVRGPFPI YQEPVLGPVRGPFPIIV	<3 kDa	IC_{50}:<10 µg/mL	*Lc. lactis* ssp/ mix culture *lactis–E.faecium*	Torres-Llanez et al. (2011)

3. LAB of fermented foods producing ACE-I peptides.

Fermented foods have been known and consumed for a long time. Fermentation involves microorganisms that can take place spontaneously or by using a culture starter. Lactic acid bacteria are known as bacteria that are involved in many fermented foods such as fermented milk, meat, legumes and vegetables. A fermented milk product are classified into two major groups on the basic on of microorganism: (1) lactic fermentation, (2) lactic-fungal fermentations (Mayo et al., 2010).

ACE-I activity were found in fermented dairy products such as cheeses (Lignitto et al., 2010; Qureshi et al., 2013; Lu et al., 2016), *dahi* - Indian yogurt (Ashar and Chand, 2004), caprina kefir (Quirós et al., 2005), koumiss (Chen et al., 2010), sheep milk yoghurt (Papadimitriou et al.,

2007), fermented camel milk (Moslehishad et al., 2013), fermented goats milk (Minervini et al., 2009). ACE-I activity was also found in *douchi* (Zhang et al., 2006), fermented fish (bekasam, hezhiko, narezushi), fermented oyster and fermented pear juice (Itou et al., 2007; Ankolekar et al., 2012; Wikandari et al., 2011; Wenno et al., 2016).

The occurrence of ACE-I activity in fermented foods has encouraged many researchers to isolate LAB present in fermented foods. Various LAB producing ACE-I peptides from different fermented food is presented in Table 2. Barla et al. (2016) isolated 52 LAB isolates from various traditional Japanese fermented foods (*Kaburazushi, Narezushi, Konkazuke,* and *Ishiru).* Isolate of *Lactobacillus brevis*, *Lactobacillus buchneri* and *Weissella hellenica* that used as starter cultures in soya and milk fermented were show to posses ACE-I activities (IC_{50}:<1mg protein/mL). Similarly, Wikandari et al. (2011) have isolated 150 LAB from *bekasam* (fermented fish). ACE-I activity of *bekasam* was 51.77%-65.75%. Li et al. (2017) reported that among 41 strains of *L. casei* isolated from fermented food of Tibet, Mongolia, Sichuan and Gansu, 22 strains used as culture starter in fermented milk and showed ACE-I activity above 60%. Chen et al. (2015) has isolated 38 LAB groups of *L. helveticus* from traditional fermented milk and revealed that fermented milk produced with 3 strains of *L. helveticus* (IMAU80851, IMAU80852 and IMAU80872) showed ACE-I activity of 75%. Other LAB groups such as *Lactococcus* and probiotic bacteria that used as starters to make cheddar cheese were capable of producing ACE-I peptides (Ong and Shah, 2008). *Leuconostoc* spp, *S. thermophilus*, *Lactococcuc lactis*, *L. helveticus* and *L. delbrueckii* have also been reported to be able producing ACE-I peptides (Kilpi et al., 2007; Gútiez et al., 2013). LAB group *Lactobacillus* and *Lactococcus* were generally used as starter cultures. *Lactobacillus* was known to have both high proteolytic and ACE-I activities, while *Lactococcus* has ability to degradate lactose in milk and produce ACE-I peptides (Kuipers, 2001; Rodríguez-Figueroa et al., 2010).

In vitro screening of lactic acid bacteria for producing ACE-I peptides could be done with two approaches: (A) the enzymatic characteristics of the bacterial proteinases and, (B) The ability of strain to reduce ACE activity (Beltrán-Barrientos et al., 2016). *Lactobacillus helveticus* species, produces abundant intracellular enzymes, including cell-envelope proteinase, endopeptidases, aminopeptidases, and the X-prolyl dipeptidyl aminopeptidase, PepX (Exterkate, 1995).

4. Factors affecting formation of ACE-I peptides in fermented foods

The formation of ACE-I bioactive peptides in fermented foods influenced by several factors. The main factors that are widely observed and reported by researchers include the type of LAB (strain of starter culture), inoculum density, fermentation time, and substrate composition **(**Li et al., 2017; Shi et al., 2016)

4.1. Starter Culture

ACE-I activity produced in a fermentation foods is largely determined by starter culture. Some types of LAB which are isolated from fermented and non-fermented foods produce ACE-I inhibitory with varies activity (Gobbetti et al., 2000; Gútiez et al., 2013; Rodríguez-Figueroa et al., 2010; Kilpi et al., 2007). During the fermentation process, milk protein was hydrolyzed to produce a bioactive peptides by proteolytic enzymes which produced by starter culture. The activity, size and sequences of ACE-I peptides formed are strongly influenced by the type of LAB starter culture. *L. helveticus* is reported to produce ACE inhibitors peptide namely IPP and VPP in various milk product (Pan & Guo, 2010; Yamamoto et al., 1994; Tsai et al., 2008; Chen et al., 2010) with ACE-I activity 9-74.5% (Wang et al., 2015). Other LAB that are known to be able to produce ACE-I activity are *L. jensenii* (Pihlanto et al., 2010), *Lactococcus lactis* (Kuipers, 2001), and *Enterococcus* (Quirós et al., 2007; Hati et al.,

2015). Research by Chen et al. (2015) showed that ACE-I activity of *Lactobacillus* with different activities, i.e. *L. reuteri* of 95.92%, *L. bulgaricus* of 84.61%, *L. rhamnosus* of 82.79% and *L. helveticus* of 78.57%.

Table 2. ACE-I activity of various LAB in different fermented foods

Lactic Acid Bacteria	Fermented foods	ACE-I activity (%) /IC_{50}	References
L. plantarum 417	yogurt of goat milk	90.70 ± 1.27%	Sathya et al. (2017)
L. delbrueckii subsp. *bulgaricus* ACA-DC 87 *S. thermophilus* ACA-DC 835	yogurt of sheep milk	75% 92%	Georgalaki et al. (2017)
L reuteri, *L. bulgaricus,* *L.rhamnosus* *L. helveticus*	yogurt of goat milk	95.92% 84.61% 82.79% 78.57%	Chen et al. (2015)
S. thermophilus CR12 *L. casei* LC01 *L. helveticus* PR4	yogurt of goat milk	82.0%	Minervini et al. (2009)
L. plantarum C2	soybean milk	75.97 ± 1.5 %	Singh and Vij, (2017)
L. casei	soybean milk	IC_{50}: 0.89-4.31mg mL	Bao and Chi, (2016)
L. buchneri *Weisella hellenica*	soybean milk	IC_{50} : 1.33 ± 0.04 mg/mL^{-1} IC_{50} : 1.37 ± 0.02 mg/mL^{-1}	Barla et al. (2016)
L. plantarum B1765	*bekasam* (fish fermented)	68.17 ± 1.32%.	Wikandari et al. (2012)
L. acidophilus	pear juice	≤ 50 %	Ankolekar et al. (2012)
Lactococcus lactis ssp. *lactis* *Enterococcus faecium*	cheese mexican fresco	IC_{50} : 5.2 ± 0.10 µg mL IC_{50}:10.4 ± 0.40 µg mL	Torres-Llanez et al. (2011)
L. helveticus 881315	yogurt of bovine milk	IC_{50}: 16.91 ± 0.25 mg mL^{-1}	Shi et al. (2016)
L. delbrueckii ssp. *bulgaricus* LB340	yogurt of bovine milk	67.71 ± 7.62 mg mL^{-1}	Qian et al. (2011)
L. rhamnosus PTCC 1637	yogurt of camel milk	IC_{50}: 1.45 ± 0.01 mg mL^{-1}	Moslehishad et al. (2013)
L. rhamnosus NS4	yogurt of camel milk	79.66%	Solanki and Hati, (2018)
L. casei	yogurt of bovine milk	68.21 ± 1.06%	Li et al. (2017)

Lactic Acid Bacteria	Fermented foods	ACE-I activity (%) /IC_{50}	References
L. helveticus H9	cow, mare and soybean milk	70.9-74.5%	Wang et al. (2015)
L. casei Shirota and *S. thermophiles*	yogurt of bovine milk (casein)	IC_{50}: 0.14 μg mL^{-1}	Rojas-Ronquillo et al. (2012)
L. helveticus LB10	yogurt of bovine milk	75.46%	Pan and Guo, (2010)
*L. helveticus (*H521, 4/149, 4/135, Hv25) *L. casei* 2465 *L. asidophilus*	yogurt of bovine milk	60-62% 66% 70%	Stefanova et al. (2009)
L. helveticus ND01	yogurt of bovine milk	69.51 ± 2.32 %	Sun et al. (2009)
Lactococcus lactis DIBCA2	yogurt of bovine milk	IC_{50} : 0.22 ± 0.03 mg/mL^{-1}	Nejati et al. (2013)
Bifidobacterium longum	yogurt of bovine milk	66.30 ± 2.43%	Ramchandran and Shah, (2008)
Bifidobacterium bifidum MF 20/5	yogurt of bovine milk	IC_{50}: 132 μM	Gonzalez-Gonzalez et al. (2013)
E. faecalis QA53	yogurt of bovine milk	IC_{50} : 24.3 μg /mL.	Gútiez et al. (2013)
E. faecalis CECT 5727	yogurt of bovine milk	IC_{50}: 28 ± 2 μg mL	Quirós et al. (2005)
E. faecalis	yogurt of bovine milk	IC_{50} : 34-59 μg mL	Muguerza et al. (2006)

4.2.Substrate

Substrates for fermentation affect ACE-I peptides formation by LAB. Wang et al. (2015) conducted a research using 3 types of milks to determine ACE-I inhibitory activity by *L. helveticus* H9. The milks are nonfat cow milk powder, mare milk powder, and nonfat soy milk powder. The results of the study showed that ACE-I activity was found in all three types of milks (above 50%). However, the highest ACE-I activity was found in cow milk (65.1-77.2%), followed by horse milk (61.7%) and soy milk (42.1- 64.9%). The results also showed that two types of tripeptide, VPP and IPP were only found in cow milk not in soy milk or mare milk. The two types of tripeptide were previously have been shown as ACE-I peptides produced by *L. helveticus* (Stefanova et al., 2009; Chen et al., 2014). *L. helveticus* H9 takes a shorter time to produce ACE

inhibitors in cow's milk than horse milk and soy milk. Casein was the largest component in cow's milk (80%) and whey protein (20%). Protein content in horse milk was lower than in cow's milk. ACE-I peptides are formed by bacterial proteinases when milk protein particularly casein, are hydrolyzed into oligopeptides as nitrogen source for bacterial growth.

Bioactive peptides have been derived from casein (αS1-CN, β-CN, κ-CN) (Torres-Llanez et al., 2011; Lu et al., 2016). Fragment of β-CN, 84–86 [β-CN (f84–86) were found in AA sequence of VPP and fragment β-CN(f74–76) and κ-CN(f108–110) in AA sequence of IPP. Soybeans contain high stachyose and raffinose which are limiting factors for LAB growth and to produce ACE-I peptides. However, some

researchers showed ACE-I activity in fermented soy milk with IC_{50}: 0.28-4.34 mg/mL (Donkor et al., 2005; Vallabha and Tiku, 2014; Bao and Chi, 2016). Major component of soybean protein is glycinin (11S globulin) and β-conglycinin (7S globulin) accounted to approximately 40% and 30%, respectively of the total protein (Utsumi et al., 2002). Study by Gibbs et al. (2004) showed that glycinin was the precursor of 95% of the peptides formed by soybean protein hydrolysis. Nowadays milk from various sources (cows, buffaloes, goats, sheep, camels and yaks) has been widely used to produce ACE-I (Ao et al., 2012; Fadda et al., 2010; Papadimitriou et al., 2007; Moslehishad et al.,2013)

Protein in fermented foods are the natural source of antihypertensive peptides. The protein content in milk was divided into two categories: insoluble protein (casein group) and dissolved protein (whey protein), generally found in lactoserum. The casein group consists of several types namely αs1-, αs2-, β-, K- and γ-casein, while whey protein consists of beta-lactoglobulin, alpha-lactalbumin, lactoferrin, immunoglobulin, serum albumin, glycomacro-peptides, enzymes and growth factors. ACE-I activity in fermented cow's milk was reported to reach ≥ 50%, whereas in goat's milk ACE-I activity was 60-85% (Quirós et al., 2005; Minervini et al., 2009). Goat's milk has α-casein content that lower than cow;s milk, and in contrast β-casein was the majority protein content in goat's milk (Jandal, 1996)

4.3. Inoculum Density

The inoculum density of LAB is reported to influence the ability of LAB to produce peptides with ACE-I activity (Wang et al., 2015; Shu et al., 2015; Li et al., 2017). A study to determine the effect of inoculum density on ACE-I activity in cow's milk. The density *L. helveticus* as inoculum of 1×10^6 CFU /mL produced the highest ACE-I activity of 74.97% compared to three other inoculum densities that are 5×10^6 , 1×10^7, and 5×10^7 CFU/mL (Chen et al., 2015). Similar result were reported by Li et al. (2017) using *Lactobacillus casei* which showed that the inoculum density of $1x10^6$ CFU/mL produced higher ACE-I activity (73.50%) in cow's milk than two other inoculum densities of 5×10^6 and 1×10^7 CFU/mL. Wang et al. (2015) using 4 different *L. helvetikus* H9 inoculum densities, i.e. 2×10^6, 5×10^6, 1×0^7, and 2×0^7 CFU/mL also showed that the use of the 5×10^6 inoculum density resulted in the highest ACE-I activity (70.9-74.5%). The density of the inoculum also seems to be influenced by the type of LAB.

The use of high inoculum density does not always correlate with the high ACE-I activity. It is assumed that the use of a high inoculum density causes an increase the growth rate of LAB resulting in rapid accumulation of acids in the medium as cells metabolite. The acid can suppress proteolytic activity to degrade proteins to produce peptides that have the potential as ACE-I peptides. Previous research by (Samona et al. (1996) and Wang et al. (2015) explained that the density of the inoculum used would determine the rate of increase in total acid in the milk fermented by *L. acidophilus* and *Bifidobacterium.*

4.4. Fermentation Time

Each of LAB has the optimum temperature and time range for its growth and for peptides production. Different temperature and time of fermentation were used for ACE-I peptide formation, i.e 37 ^{0}C for 24 hours used by Fuglsang et al. (2003); Moslehishad et al. (2012); Chen et al. (2014) and Li et al. (2017) and temperatures of 30 ^{0}C for 48 hours used by Muguerza et al. (2006); Quiros et al. (2007); Rodríguez-Figueroa et al. (2012). Li et al. (2017) found that incubation at 30 ^{o}C significantly increrased ACE-I activity in fermented milk by *L. casei* compared to two other temperatures i.e. 33 and 40 ^{o}C with the maximum ACE-I activity of 84.84 ±1.23%. Otte et al. (2011) also found that incubation temperature of 37 °C was optimum for the production peptides with ACE-I activity in milk fermented by *Lactococcus lactis.* Another study evaluated the effect of temperature and incubation time of ACE-I activity in milk by *Lactobacillus plantarum* LP69 (Shu et al.,

2015). The temperatures used are 25, 30,35, 40 and 45 ^{0}C.

The incubation time was 0 to 36 hours. The optimum ACE-I activity was obtained at an incubation temperature of 35 °C for 14 hours with ACE-I activity of 81.25%. It is assumed that ACE-I activity increased with the increase of LAB counts. This incubation time was slightly different from previous studies on milk fermented by *Lb. bulgaricus* LB6 (Shu et al., 2015) where the highest ACE-I activity was obtained at 12 hours incubation time.

Pihlanto et al. (2010) also found that the optimum ACE-I activity of milk fermented by *L. acidophilus* ATCC 4356 and *L. jensenii* ATCC 25258 were obtained after 20 hours incubation. Similar study results showed by Gonzalez-Gonzalez et al. (2013) reported that the incubation time required by *Bifidobacterium bifidum* MF 20/5, *L. salivarius* NCIMB 11975, *L. reuteri* NCIMB 11951, *L. casei* YIT 9029, and *L. plantarum* NCIMB for optimum was 24 hours with ACE-I activity above 85%. Those results show that each strain had a different optimum temperature and incubation time to produce peptides with high ACE-I activity. However, Chen et al. (2015) reported that the incubation temperature did not have a significant effect on ACE-I activity. The incubation temperature of 33, 37, and 40 °C did not affect significantly to ACE-I activity in milk fermented by *L. helveticus* IMAU80872.

5. Conclusions and future perspective

Lactic acid bacteria with proteolytic activity have the potential to produce ACE-I bioactive peptides. ACE-I bioactive peptides generally short peptides or tripeptides with a molecular weight of <5 kDa. The formation of bioactive peptides of ACE-I in fermented foods by LAB provides an opportunity to find new LAB strains with the ability to produce bioactive peptides of ACE-I. The selection of specific starter culture is crucial to deliver specific health properties. Substrate and fermentation conditions are other factors that can play important roles for the formation of bioactive peptides of ACE-I. Potential LAB strains that produce bioactive peptides of ACE-I can be applied as starter cultures or as a food component to develop functional food that recently becoming popular for maintaining human health. In vivo studies also confirm that foods fermented by certain strains of LAB are potential to be used as antihypertensive. This open an opportunity to obtain natural antihypertensive from foods that can be used in the treatment or prevention of hypertension.

6. References

Ankolekar, C., Pinto, M., Greene, D., & Shetty, K. (2012). In vitro bioassay based screening of antihyperglycemia and antihypertensive activities of *Lactobacillus acidophilus* fermented pear juice. *Innovative Food Science and Emerging Technologies*, (*13*), 221–230.

Ao, X., Zhang, X., Zhang, X., Shi, L., Zhao, K., Yu, J., … Cai, Y. (2012). Identification of lactic acid bacteria in traditional fermented yak milk and evaluation of their application in fermented milk products. *Journal of Dairy Science*, *95*(3), 1073–1084.

Axelsson L. 1993. Lactid Acid Bacteria : Classification and physiology. In: Salminen S, dan Von Wright A. (Editors.) Lactid Acid Bacteria: Microbiology and Functional Aspects. 2nd Edition, Marcel Dekker, New York, 1–67.

Bao, Z., & Chi, Y. (2016). In Vitro and In Vivo Assessment of Angiotensin-Converting Enzyme (ACE) Inhibitory Activity of Fermented Soybean Milk by *Lactobacillus casei* Strains. *Current Microbiology*, *73*(2), 214–219.

Barla, F., Koyanagi, T., Tokuda, N., Matsui, H., Katayama, T., Kumagai, H., … Enomoto, T. (2016). The γ-aminobutyric acid-producing ability under low pH conditions of lactic acid bacteria isolated from traditional fermented foods of Ishikawa Prefecture, Japan, with a strong ability to produce ACE-inhibitory peptides. *Biotechnology Reports*, (10), 105–110.

Beltrán-Barrientos, L. M., Hernández-Mendoza, A., Torres-Llanez, M. J., González-

Córdova, A. F., & Vallejo-Córdoba, B. (2016). Invited review: Fermented milk as antihypertensive functional food. *Journal of Dairy Science*, *99*(6), 4099–4110.

Brew K. (2003). Structure of human ACE gives new insights into inhibitor binding and design. *Trends in Pharmacological Sciences,* (24), 8

Chaudhary, S. K., Mukherjee, P. K., Maiti, N., De Kumar, A., Bhadra, S., & Saha, B. P. (2013). Evaluation of angiotensin converting enzyme inhibition and anti-oxidant activity of *Piper longum* L. *Indian Journal of Traditional Knowledge*, *12*(3), 478–482.

Chen, H., Hui, Y., Chen, L., Wan, H., Shu, G., & Li, H. (2015). Effect of probiotic *Lactobacillus* strains on antioxidant activity from fermented goat milk. *Carpathian Journal of Food Science and Technology*, *7*(2), 109–114.

Chen, Y., Li, C., Xue, J., Kwok, L., Yang, J., Zhang, H., & Menghe, B. (2015). Characterization of angiotensin-converting enzyme inhibitory activity of fermented milk produced by *Lactobacillus helveticus*. *Journal of Dairy Science*, *98*(8), 5113–5124.

Chen, Y., Liu, W., Xue, J., Yang, J., Chen, X., Shao, Y., … Zhang, H. (2014). Angiotensin-converting enzyme inhibitory activity of *Lactobacillus helveticus* strains from traditional fermented dairy foods and antihypertensive effect of fermented milk of strain H9. *Journal of Dairy Science*, *97*(11), 6680–6692.

Chen, Y., Wang, Z., Chen, X., Liu, Y., Zhang, H., & Sun, T. (2010). Identification of angiotensin I-converting enzyme inhibitory peptides from koumiss, a traditional fermented mare's milk. *Journal of Dairy Science*, *93*(3), 884–892.

Cheung, H., Wang, F., & Ondetti, M. A. (1980). Binding of Peptide Substrates and Inhibitors. *Journal of Biological Chemistry*, *255*(2), 401–405.

Choi, J., Sabikhi, L., Hassan, A., & Anand, S. (2012). Bioactive peptides in dairy products. *International Journal of Dairy Technology*, *65*(1), 1–12.

Culligan, E. P., Hill, C., & Sleator, R. D. (2009). Probiotics and gastrointestinal disease: Successes, problems and future prospects. *Gut Pathogens*, *1*(1), 1–12.

Daliri, E. B. M., Lee, B. H., Kim, J. H., & Oh, D. H. (2018). Novel angiotensin I-converting enzyme inhibitory peptides from soybean protein isolates fermented by *Pediococcus pentosaceus* SDL1409. *Lwt*, (*93*), 88–93.

de Castro, R. J. S., & Sato, H. H. (2015). Biologically active peptides: Processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. *Food Research International*, (*74)*, 185–198.

Donkor, O. N., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2005). Probiotic strains as starter cultures improve angiotensin-converting enzyme inhibitory activity in soy yogurt. *Journal of Food Science*, *70*(8), M375–M381.

Elkhtab, E., El-Alfy, M., Shenana, M., Mohamed, A., & Yousef, A. E. (2017). New potentially antihypertensive peptides liberated in milk during fermentation with selected lactic acid bacteria and kombucha cultures. *Journal of Dairy Science*, *100*(12), 9508–9520.

Escudero, E., Sentandreu, M. A., & Toldrá, F. (2010). Characterization of peptides released by in vitro digestion of pork meat. *Journal of Agricultural and Food Chemistry*, *58*(8), 5160–5165.

Exterkate, F. A. (1995). The lactococcal cell envelope proteinases: Differences, calcium-binding effects and role in cheese ripening. *International Dairy Journal*, *5*(8), 995–1018.

Fadda, S., Vildoza, M. J., & Vignolo, G. (2010). Hydrolysis During Meat Fermentation. *Journal of Muscle Foods*, *21*(ext 116), 545–556.

Fitzgerald, G. F., Murray B.A. (2006). Bioactive peptides and lactic fermentations, *International Journal of Dairy Technology,*

59(2), 118–125.

Fuchs, S., Xiao, H. D., Hubert, C., Michaud, A., Campbell, D. J., Adams, J. W., … Bernstein, K. E. (2008). Angiotensin-converting enzyme C-terminal catalytic domain is the main site of angiotensin I cleavage in vivo. *Hypertension*, *51*(2), 267–274.

Fuglsang, A., Rattray, F. P., Nilsson, D., & Nyborg, N. C. B. (2003). Lactic acid bacteria: Inhibition of angiotensin converting enzyme in vitro and in vivo. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *83*(1), 27–34.

Fujita, H., Yokoyama, K., & Yoshikawa, M. (2000). Classification and Antihypertensive Activity of Angiotensin I-Converting Enzyme Inhibitory Peptides Derived from Food Proteins. *Journal of Food Science*, *65*(4), 564–569.

Fujita, H., & Yoshikawa, M. (1999). LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology*, *44*(1–2), 123–127.

Georgalaki, M., Zoumpopoulou, G., Mavrogonatou, E., Van Driessche, G., Alexandraki, V., Anastasiou, R., … Tsakalidou, E. (2017). Evaluation of the antihypertensive angiotensin-converting enzyme inhibitory (ACE-I) activity and other probiotic properties of lactic acid bacteria isolated from traditional Greek dairy products. *International Dairy Journal*, *75*, 10–21.

Gibbs, B. F., Zougman, A., Masse, R., & Mulligan, C. (2004). Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food, *Food Research International*, 37(2), 123–131.

Gobbetti, M., Ferranti, P., Smacchi, E., Goffredi, F., & Addeo, F. (2000). Production of angiotensin-I-converting-enzyme-inhibitory peptides in fermented milks started by *Lactobacillus delbrueckii* subsp. *bulgaricus* SS1 and *Lactococcus lactis* subsp. *cremoris* FT4. *Applied and Environmental Microbiology*, 66(9), 3898–3904.

Gobbetti, M., Stepaniak, L., M, D. A., A, C., & Di Cagno, R. (2002). Latent Bioactive Peptides in Milk Proteins: Proteolytic Activation and Significance in Dairy Processing, *Critical Reviews in Food Science and Nutrition*, *42*(3), 223–239.

Gonzalez-Gonzalez, C., Gibson, T., & Jauregi, P. (2013). Novel probiotic-fermented milk with angiotensin I-converting enzyme inhibitory peptides produced by *Bifidobacterium bifidum* MF 20/5, *International Journal of Food Microbiology*, *167*(2), 131–137.

Gútiez, L., Gómez-Sala, B., Recio, I., del Campo, R., Cintas, L. M., Herranz, C., & Hernández, P. E. (2013). *Enterococcus faecalis* strains from food, environmental, and clinical origin produce ACE-inhibitory peptides and other bioactive peptides during growth in bovine skim milk, *International Journal of Food Microbiology*, *166*(1), 93–101.

Hati, S., Sreeja, V., Solanki, J., & Prajapati, J. B. (2015). Significance of proteolytic microorganisms on ACE-inhibitory activity and release of bioactive peptides during fermentation of milk*, Indian Journal of Dairy Science, 68*(6), 584–592.

Itou, K., & Akahane, Y. (2004). Antihypertensive effect of narezushi, a fermented mackerel product, on spontaneously hypertensive rats. *Fisheries Science*, (70), 1121–1129.

Jandal, J. M. (1996). Comparative aspects of goat and sheep milk. *Small Ruminant Research*, 22(2), 177–185.

Jao, C. L., Huang, S. L., & Hsu, K. C. (2012). Angiotensin I-converting enzyme inhibitory peptides: Inhibition mode, bioavailability, and antihypertensive effects. *BioMedicine (Netherlands)*, *2*(4), 130–136.

Kilpi, E. E. R., Kahala, M. M., Steele, J. L., Pihlanto, A. M., & Joutsjoki, V. V. (2007). Angiotensin I-converting enzyme inhibitory activity in milk fermented by wild-type and peptidase-deletion derivatives of *Lactobacillus helveticus* CNRZ32.

International Dairy Journal, *17*(8), 976–984.

Kimoto-nira, H., Mizumachi, K., & Nomura, M. (2007). *Lactococcus* sp . as Potential Probiotic Lactic Acid Bacteria. *Japan Agricultural Research Quarterly*, (41), 181–189.

Kuipers, O. P. (2001). Complete DNA sequence of *Lactococcus lactis* adds flavor to genomics. *Genome Research*, 11(5), 673–674.

Lee, H., Yoon, H., Ji, Y., Kim, H., Park, H., Lee, J., … Holzapfel, W. (2011). Microbiology Functional properties of *Lactobacillus* strains isolated from kimchi. *International Journal of Food Microbiology*, 145(1), 155–161.

Li, C., Kwok, L.-Y., Mi, Z., Bala, J., Xue, J., Yang, J., … Chen, Y. (2017). Characterization of the angiotensin-converting enzyme inhibitory activity of fermented milks produced with *Lactobacillus casei*. *Journal of Dairy Science*, 1–13.

Lignitto, L., Cavatorta, V., Balzan, S., Gabai, G., Galaverna, G., Novelli, E., … Segato, S. (2010). Angiotensin-converting enzyme inhibitory activity of water-soluble extracts of Asiago d'allevo cheese. *International Dairy Journal*, *20*(1), 11–17.

López-Fandiño, R., Otte, J., & van Camp, J. (2006). Physiological, chemical and technological aspects of milk-protein-derived peptides with antihypertensive and ACE-inhibitory activity. *International Dairy Journal*, 16(11), 1277–1293.

Lu, Y., Govindasamy-Lucey, S., & Lucey, J. A. (2016). Angiotensin-I-converting enzyme-inhibitory peptides in commercial Wisconsin Cheddar cheeses of different ages. *Journal of Dairy Science*, 99(1), 1-12.

Minervini, F., Algaron, F., Rizzello, C. G., Fox, P. F., Monnet, V., & Gobbetti, M. (2003). Angiotensin I-Converting-Enzyme-Inhibitory and Antibacterial Peptides from *Lactobacillus helveticus* PR4 Proteinase-Hydrolyzed Caseins of Milk from Six Species. *Applied And Environmental Microbiology*. 69(9), 5297–5305.

Minervini, F., Bilancia, M. T., Siragusa, S., Gobbetti, M., & Caponio, F. (2009). Fermented goats' milk produced with selected multiple starters as a potentially functional food. *Food Microbiology*, *26*(6), 559–564.

Möller, N. P., Scholz-Ahrens, K. E., Roos, N., & Schrezenmeir, J. (2008). Bioactive peptides and proteins from foods: Indication for health effects. *European Journal of Nutrition*, 47(4), 171–182.

Moslehishad, M., Ehsani, M. R., Salami, M., Mirdamadi, S., Ezzatpanah, H., Naslaji, A. N., & Moosavi-Movahedi, A. A. (2013). The comparative assessment of ACE-inhibitory and antioxidant activities of peptide fractions obtained from fermented camel and bovine milk by *Lactobacillus rhamnosus* PTCC 1637. *International Dairy Journal*, 29(2), 82–87.

Muguerza, B., Ramos, M., Sánchez, E., Manso, M. A., Miguel, M., Aleixandre, A., … Recio, I. (2006). Antihypertensive activity of milk fermented by *Enterococcus faecalis* strains isolated from raw milk. *International Dairy Journal*, *16*(1), 61–69.

Nakamura, Y., Yamamoto, N., Sakai, K., & Takano, T. (1995). Antihypertensive Effect of Sour Milk and Peptides Isolated from It That are Inhibitors to Angiotensin I-Converting Enzyme. *Journal of Dairy Science*, 78(6), 1253–1257.

Nejati, F., Rizzello, C. G., Di Cagno, R., Sheikh-Zeinoddin, M., Diviccaro, A., Minervini, F., & Gobbetti, M. (2013). Manufacture of a functional fermented milk enriched of Angiotensin-I Converting Enzyme (ACE)-inhibitory peptides and γ-amino butyric acid (GABA). *LWT - Food Science and Technology*, 51(1), 183–189.

Norris, R., & FitzGerald, R. (2013). Antihypertensive peptides from food proteins. *Bioactive Food Peptides in health and Disease.* 3(4), 45–72.

Nuraida, L., Hana, Hartani, A.W., Prangdimurti,E. (2011). In Vitro Evaluation

of Cholesterol Assimilation and Bile Salt Deconjugation by Lactic Acid Bacteria Isolated from Breast Milk. *Jurnal Teknologi dan Industri Pangan,* 22 (1), 46-52.

Nuraida, L., Hana, Hartani, A.W., Prangdimurti,E. (2012). Potentian of Lactobacillus Isolated from Breast Milk to Prevent Diarrheae. *Jurnal Teknologi dan Industri Pangan,* 23 (2), 158-164.

Ong, L., & Shah, N. P. (2008). Release and identification of angiotensin-converting enzyme-inhibitory peptides as influenced by ripening temperatures and probiotic adjuncts in Cheddar cheeses. *LWT - Food Science and Technology*, 41(9), 1555–1566.

Otte, J., Lenhard, T., Flambard, B., & Sørensen, K. I. (2011). Influence of fermentation temperature and autolysis on ACE-inhibitory activity and peptide profiles of milk fermented by selected strains of *Lactobacillus helveticus* and *Lactococcus lactis*. *International Dairy Journal*, 21(4), 229–238.

Pan, D., & Guo, Y. (2010a). Optimization of sour milk fermentation for the production of ACE-inhibitory peptides and purification of a novel peptide from whey protein hydrolysate. *International Dairy Journal*, *20*(7), 472–479.

Pan, D., & Guo, Y. (2010b). Optimization of sour milk fermentation for the production of ACE-inhibitory peptides and purification of a novel peptide from whey protein hydrolysate. *International Dairy Journal*, 20(7), 472–479.

Papadimitriou, C. G., Vafopoulou-Mastrojiannaki, A., Silva, S. V., Gomes, A. M., Malcata, F. X., & Alichanidis, E. (2007). Identification of peptides in traditional and probiotic sheep milk yoghurt with angiotensin I-converting enzyme (ACE)-inhibitory activity. *Food Chemistry*, *105*(2), 647–656.

Parmar, H., Hati, S., & Sakure, A. (2017). In Vitro and In Silico Analysis of Novel ACE-Inhibitory Bioactive Peptides Derived from Fermented Goat Milk. *International Journal of Peptide Research and Therapeutics*, 24(3), 441–453.

Phelan, M., & Kerins, D. (2011). The potential role of milk-derived peptides in cardiovascular disease. *Food and Function*, (*2*), 153–167.

Pihlanto, A., Virtanen, T., & Korhonen, H. (2010). Angiotensin I converting enzyme (ACE) inhibitory activity and antihypertensive effect of fermented milk. *International. Dairy Journal*, 20(1), 3–10.

Qian, B., Xing, M., Cui, L., Deng, Y., Xu, Y., Huang, M., & Zhang, S. (2011). Antioxidant, antihypertensive, and immunomodulatory activities of peptide fractions from fermented skim milk with *Lactobacillus delbrueckii* ssp. *bulgaricus* LB340. *Journal of Dairy Research*, 78(1), 72–79.

Quirós, A., Hernández-Ledesma, B., Ramos, M., Amigo, L., & Recio, I. (2005). Angiotensin-Converting Enzyme Inhibitory Activity of Peptides Derived from Caprine Kefir. *Journal of Dairy Science*, 88(10), 3480–3487.

Quirós, A., Ramos, M., Muguerza, B., Delgado, M. A., Miguel, M., Aleixandre, A., & Recio, I. (2007). Identification of novel antihypertensive peptides in milk fermented with *Enterococcus faecalis*. *International Dairy Journal*, 17(1), 33–41.

Qureshi, T. M., Vegarud, G. E., Abrahamsen, R. K., & Skeie, S. (2013). Angiotensin I-converting enzyme-inhibitory activity of the Norwegian autochthonous cheeses Gamalost and Norvegia after in vitro human gastrointestinal digestion. *Journal of Dairy Science*, 96(2), 838–853.

Rai, A. K., Sanjukta, S., & Jeyaram, K. (2017). Production of angiotensin I converting enzyme inhibitory (ACE-I) peptides during milk fermentation and their role in reducing hypertension. *Critical Reviews in Food Science and Nutrition*, 57(13), 2789–2800.

Ramchandran, L., & Shah, N. P. (2008). Proteolytic profiles and angiotensin-I converting enzyme and α-glucosidase inhibitory activities of selected lactic acid bacteria. *Journal of Food Science*, 73(2),

75–81.

Riordan, J. F. (2003). Protein family review Angiotensin-I-converting enzyme and its relatives. *Genome Biology*, *4*(8), 225.

Rodríguez-Figueroa, J. C., González-Córdova, A. F., Torres-Llanez, M. J., Garcia, H. S., & Vallejo-Cordoba, B. (2012). Novel angiotensin I-converting enzyme inhibitory peptides produced in fermented milk by specific wild *Lactococcus lactis* strains. *Journal of Dairy Science*, 95(10), 5536–5543.

Rodríguez-Figueroa, J. C., Reyes-Díaz, R., González-Córdova, A. F., Troncoso-Rojas, R., Vargas-Arispuro, I., & Vallejo-Cordoba, B. (2010). Angiotensin-converting enzyme inhibitory activity of milk fermented by wild and industrial *Lactococcus lactis* strains. *Journal of Dairy Science*, 93(11), 5032–5038.

Rojas-Ronquillo, R., Cruz-Guerrero, A., Flores-Nájera, A., Rodríguez-Serrano, G., Gómez-Ruiz, L., Reyes-Grajeda, J. P., … García-Garibay, M. (2012). Antithrombotic and angiotensin-converting enzyme inhibitory properties of peptides released from bovine casein by *Lactobacillus casei* Shirota. *International Dairy Journal*, 26(2), 147–154.

S Vallabha, V., & Tiku, P. K. (2013). Antihypertensive peptides derived from soy protein by fermentation. *International Journal of Peptide Research and Therapeutics*, 20(2), 161–168.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M., & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT - Food Science and Technology*, 50(1), 1–16.

Samona, A., Robinson, R. K., & Marakis, S. (1996). Acid production by bifidobacteria and yoghurt bacteria during fermentation and storage of milk. *Food Microbiology*, *13*(4), 275–280.

Sanjukta, S., & Rai, A. K. (2016). Production of bioactive peptides during soybean fermentation and their potential health benefits. *Trends in Food Science and Technology*, (50*)*, 1–10.

Sathya P, Radha K, Sathian CT, Srinivasan C.(2017). Angiotensin Converting Enzyme Inhibitory Activity from Fermented Goat Milk Produced with Different Lactic Acid Bacteria. *International Journal of Currunt Microbiology and Applied Science*, 6(12), 1670-1676

Shi, M., Ahtesh, F., Mathai, M., McAinch, A. J., & Su, X. Q. (2016). Effects of fermentation conditions on the potential anti-hypertensive peptides released from yogurt fermented by *Lactobacillus helveticus* and Flavourzyme®. *International Journal of Food Science and Technology*, *52*(1), 137–145.

Shu, G., Yang, H., Chen, H., Zhang, Q., & Tian, Y. (2015). Effect of incubation time, inoculum size, temperature, pasteurization time, goat milk powder and whey powder on ace inhibitory activity in fermented milk by *L. plantarum* LP69. *Acta Scientiarum Polonorum, Technologia Alimentaria*, *14*(2), 107–116.

Singh, B. P., & Vij, S. (2017). Growth and bioactive peptides production potential of *Lactobacillus plantarum* strain C2 in soy milk: A LC-MS/MS based revelation for peptides biofunctionality. *LWT - Food Science and Technology* (Vol. 86). Elsevier Ltd.

Solanki, D., & Hati, S. (2018). Considering the potential of *Lactobacillus rhamnosus* for producing Angiotensin I-Converting Enzyme (ACE) inhibitory peptides in fermented camel milk (Indian breed). *Food Bioscience*, (*23)*, 16–22.

Stefanova, T., Urshev, Z., Dimitrov, Z., Fatchikova, N., & Minkova, S. (2009). Species affiliation of dairy *Lactobacilli* with angiotensin converting enzyme inhibitory activity. *Biotechnology and Biotechnological Equipment*, *23*(2), 1250–1254.

Sun, T., Zhao, S., Wang, H., Cai, C., Chen, Y., & Zhang, H. (2009). ACE-inhibitory activity and gamma-aminobutyric acid content of fermented skim milk by

Lactobacillus helveticus isolated from Xinjiang koumiss in China. *European Food Research and Technology*, *228*(4), 607–612.

Torres-Llanez, M. J., González-Córdova, A. F., Hernandez-Mendoza, A., Garcia, H. S., & Vallejo-Cordoba, B. (2011). Angiotensin-converting enzyme inhibitory activity in Mexican Fresco cheese. *Journal of Dairy Science*, *94*(8), 3794–3800.

Tsai, J. S., Chen, T. J., Pan, B. S., Gong, S. Da, & Chung, M. Y. (2008). Antihypertensive effect of bioactive peptides produced by protease-facilitated lactic acid fermentation of milk. *Food Chemistry*, *106*(2), 552–558.

Utsumi, S., Maruyama, N., Satoh, R., & Adachi, M. (2002). Structure-function relationships of soybean proteins revealed by using recombinant systems. *Enzyme and Microbial Technology*, *30*(3), 284–288.

Vasiljevic, T., & Shah, N. P. (2008). Probiotics-From Metchnikoff to bioactives. *International Dairy Journal*, *18*(7), 714–728.

Wang, J., Li, C., Xue, J., Yang, J., Zhang, Q., Zhang, H., & Chen, Y. (2015). Fermentation characteristics and angiotensin I-converting enzyme–inhibitory activity of *Lactobacillus helveticus* isolate H9 in cow milk, soy milk, and mare milk. *Journal of Dairy Science*, *98*(6), 3655–3664.

Wenno, M. R., Suprayitno, E., Aulanni'Am, A., & Hardoko. (2016). The physicochemical characteristics and angiotensin converting enzyme (ACE) inhibitory activity of skipjack tuna (katsuwonus pelamis) "bakasang." *Jurnal Teknologi*, *78*(4–2), 119–124.

Wijesekara, I., Qian, Z. J., Ryu, B., Ngo, D. H., & Kim, S. K. (2011). Purification and identification of antihypertensive peptides from seaweed pipefish (Syngnathus schlegeli) muscle protein hydrolysate. *Food Research International*, *44*(3), 703–707.

Wikandari, P. R., Suparmo, Marsono, Y., & Rahayu, E. S. (2011). Potensi Bekasam Bandeng (Chanos chanos) sebagai sumber angiotensin I converting enzyme inhibitor. Pendahuluan metode penelitian. *Biota*, *16*(1), 145–152.

Wikandari, P. R., Suparmo, Marsono, Y., & Rahayu, E. S. (2012). Potensi Bakteri Asam Laktat yang diisolasi dari Bekasam sebagai penghasil angiotensin converting enzyme inhibitor pada fermentasi "Bekasam-Like" Product. *Jurnal Agritech Fakultas Teknologi Pertanian UGM*, *32*(3), 258–264.

Wikandari, P. R., & Yuanita, L. (2016). Angiotensin converting enzyme inhibitor bekasam dengan *Lactobacillus plantarum* B1765 (The Effect of Degradation of Proteolitic Enzyme on Angiotensin Converting Enzyme Inhibitor Activity of Bekasam with *Lactobacillus plantarum* B1765). *Jurnal Agritech Fakultas Teknologi Pertanian UGM*, *36*(2), 170–175.

Wu, J., Aluko, R. E., & Nakai, S. (2006a). Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative structure-activity relationship modeling of peptides containing 4-10 amino acid residues. *QSAR and Combinatorial Science*, *25*(10), 873–880.

Wu, J., Aluko, R. E., & Nakai, S. (2006b). Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative structure-activity relationship study of Di- and tripeptides. *Journal of Agricultural and Food Chemistry*, *54*(3), 732–738.

Yamamoto, N., Akino, A., & Takano, T. (1994). Antihypertensive Effect of the Peptides Derived from Casein by an Extracellular Proteinase from *Lactobacillus helveticus* CP790. *Journal of Dairy Science*, *77*(4), 917–922.

Zhang, J. H., Tatsumi, E., Ding, C. H., & Li, L. Te. (2006). Angiotensin I-converting enzyme inhibitory peptides in douchi, a Chinese traditional fermented soybean

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage, **http,//chimie-biologie.ubm.ro/carpathian_journal/index.html**

EXTRACTION AND CHARACTERISATION OF PECTIN FROM BANANA PEEL

N. S. Rajendran[1] and B.S. Harikumaran Thampi[2]

[1]*Research Scholar in Biochemistry, Department of Life Sciences, University of Calicut, Kerala, India*
[2]*Department of Life Sciences, University of Calicut, Kerala, India*
**brahmajith02@yahoo.co.in*

https://doi.org/10.34302/2019.11.4.4

Article history,
Received,
29 April 2019
Accepted,
18 September 2019

Keywords,
Agriculture waste;
Value-added products;
Pectin.

ABSTRACT

Pectin is extracted from peel of banana (Musa species). Its structural, rheological and textural features are studied. Optimum conditions of extraction upon yield was also examined. There was significant yield under the given set of experimental conditions. Degree of methylation was about 62 while percentage of anhydrouronic acid was slightly above 70%. Galactose, galacturonic acid, rhamnose, mannose and other sugars were found to be present in it. This pectin was found to be more effective in increasing the viscosity of sugar solutions compared with citrus pectin. Also, banana pectin was used to prepare pineapple jam. Pineapple jam made with banana pectin was found to have more shear modulus compared with the pineapple jam made with citrus pectin.

1.Introduction

A vast majority of the people in India depend upon agriculture as their major source of income. Increasing cost of production, lack of deserving price of the products, crop failure due to biotic and abiotic stress, natural calamities etc. are making agriculture in India a loss for the common farmer. Therefore, educated youth do not consider agriculture as a viable employment. To make agriculture more attractive, production of alternate and non-conventional value-added products from the waste materials thrown away in the farm is a solution for this problem.

According to the data published by the Horticulture Statistics Division, Ministry of Agriculture & farmers' Welfare, Govt. of India, India is the largest producer of banana in the world, with an annual production of over 2,91,63,000 metric tonnes in 2016-17 from a total of about 8,58,000 hectare of land area spread all over the country(Pattanayak 2017). In banana cultivation rain, wind and other natural calamities cause loss for the farmer even before harvesting. Pest infestation is another factor for the loss. After proper harvesting, the bulk of the plant body is left in the farm as waste while only the fruit bunch is commonly collected. From that fruits also, peel is thrown away as waste. The overall quantity of waste left over is much more than the economically used parts in banana cultivation.

Pectin is a plant-derived heteropolysaccharide, widely used as a gelling/stabilising agent in food and pharmaceutical industries. Owing to its versatile structure and composition which ultimately determine its applications, thousands of tonnes of this soluble dietary fibre is produced globally every year and used for a variety of purposes(Willats, Knox, and Mikkelsen 2006). Being a component of cell wall of almost every land plant, it can be extracted from different types and parts of plants at different developmental stages. The structure and composition of the extracted pectin depends upon conditions used for extraction, source

plant, part of the plant used for extraction and even developmental stage of the plant part used(Atmodjo, Hao, and Mohnen 2013). Thus, different pectin samples obtained from different plant sources are different in their structural features and therefore have tremendous potentiality in their applications. Since new areas are identified for the application of pectin every day, it is essential to explore the possibility of new sources of this novel biopolymer. The waste materials from the of banana farm is one such source for pectin.

Traditionally pectin is used as gelling/thickening/stabilizing agents in different industries. While pectin with low degree of methylation forms thermo-reversible gels in presence of calcium ions at acidic pH, pectin with high degree of methylation forms thermo-irreversible gels in presence of sugars at acidic pH(Srivastava and Malviya 2011).

One of the oldest uses of pectin is in the manufacture of fruit jams and jellies[5]. With a soluble sugar content of about 60%, pectin is added at acidic pH in order to regulate the flow behaviour, gel strength and other rheological properties of the jams and jellies so as to increase the jam qualities up to the consumer satisfaction(May 1990).

Pectin, being a natural molecule with infinite structural diversity, offers a wide variety of uses and applications to humanity. It was found in a study that intake of pectin along with diet helps in the lowering of blood cholesterol level(Brouns et al. 2012). It was also found to be reducing the rise of blood level after meals. Pectin is thought to bind with cholesterol and bile acids in the alimentary canal thereby preventing their absorption and promoting excretion(Mudgil and Barak 2013). Different types of pectin obtained from kiwi fruits were found to be efficient in promoting beneficial health effects in alimentary canal(Parkar et al. 2010). Hydrolysates obtained from citrus pectin were reported to be beneficial for the survival of probiotic populations(Ho, Lin, and Wu 2017).

The anticancer role of pectin is well established. It is suggested that some fragments formed from chemically or enzymatically modified pectin may bind to galectin-3, a protein associated with development of cancer, thereby preventing cancer(Maxwell et al. 2012). Pectic oligomers, comprising repeating alternate galacturonic acid and rhamnose residues, extracted from tomato were reported to be inhibiting the activity of galectin-3(Kapoor and Dharmesh 2017). A galactan, having a terminal galactose at the non-reducing end of the chain is capable of binding with human recombinant galectin-3(Gunning, Bongaerts, and Morris 2008).

A matrix consisting of multi particulate calcium pectinate is an effective carrier of drugs for the treatment of colon cancer(Wong, Colombo, and Sonvico 2011) as it moves more slowly down the alimentary canal and therefore gets more contact time for the action of drugs. 5-aminosalicyic acid, after being incorporated in to a matrix of chitosan and coated with pectin, could be used for demonstration of controlled drug delivery in simulated gut in in vitro experiments(Ribeiro et al. 2014). Pectin was useful in this environment as it offers resistance against the acidic conditions of the gut.

Specific strategies for the digestion and removal of pectin from the cell wall of biomass is mooted for the efficient production of biofuels from them(Xiao and Anderson 2013). Experiments indicate that pectin based hydrogels could be used for tissue engineering in mammals(Neves et al. 2015). Some experiments indicate that pectin along with calcium carbonate could be used for the preparation of hydrogels, which could be injected in to the body for the delivery of drugs or even cells for implantation by surgery(Moreira et al. 2014). Experiments already have demonstrated that pectin along with polyvinyl pyrrolidone and glycine, may be used for the preparation of hydrogel membranes(Kumar, Mishra, and Banthia 2011), which may then be used for biomedical applications.

Films prepared using pectin, polyvinyl alcohol and chitosan exhibited good antimicrobial activity and other structural and functional properties thereby offering a potential for use in the food – packaging

applications(Tripathi, Mehrotra, and Dutta 2010). When enzymes used for the treatment of skin injuries were loaded in to a cryogel made up of polyvinyl alcohol and pectin, the gel was found to be retaining the enzymes more effectively for a longer period of time(Martínez et al. 2014). Also, it was observed that the enzymes were bound to pectin rather than to polyvinyl alcohol of the cryogel.

Since more applications are developed for this unique molecule every day, it is essential to look for alternate, abundant and easily available sources so that humanity can get maximum benefits from it. Extraction and characterization of pectin from the peel of banana fruit bunch is discussed in this communication.

2.Materials and methods

2.1. Materials

All reagents are purchased from Merck, India unless otherwise specified.

2.2. Preparation of plant material

Ripe fruit bunch of Nenthran variety of banana/plantain (*Musa sp*.) was collected from a local farmer in Palakkad district, Kerala, India. Peel was separated, washed with mild acid, chopped in to small pieces, pulverised using a food processor, sun dried to constant weight, powdered and kept in an air tight vessel for further use.

2.3. Preparation of extractant solution

Distilled water is mixed with hydrochloric acid to attain a particular range of pH as is shown in table- 1.

2.3.1. Extraction of crude pectin

10g of the powdered peel was weighed, tied up in a bag made of cotton cloth, immersed in a particular volume of water maintained at a pH and heated at a constant temperature (table 1) in a 500 – ml Erlenmeyer flask (Borosil) for a certain duration of time (table 1). After heating, the flask is cooled to room temperature, the bag containing powdered peel was taken out and squeezed to release any drop of liquid. Then pH is brought above 6.0 using Barium Carbonate, centrifuged at 5000 rpm for 10 minutes and the supernatant is collected. It was then evaporated under vacuum to a volume of 50 ml, double volume of isopropyl alcohol was added, shaken well and kept at room temperature for 24 hours. It was then centrifuged at 5000 rpm for 10 minutes, and the supernatant was discarded. The precipitated crude pectin was dried under a stream of air to constant weight, and transferred to air-tight vials for further analysis(Yapo 2009).

2.4. Characterisation

2.4.1. Estimation as calcium pectate

The method originally described by Ranganna S.(Ranganna 1977) was used with further modifications.

1 g of the powdered pectin was dispersed in 10 ml of 0.01 N HCl, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected. The residue was mixed with 10 ml of 0.05 N HCl, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected. The residue was mixed with 10 ml of 0.3 N HCl, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected. The residue was mixed with 10 ml of water, kept in boiling water bath for 20 minutes, cooled to room temperature, centrifuged at 5000 rpm for 10 minutes, and the supernatant collected.

All supernatants were pooled together and made up to 100 ml with water. 30 ml of this solution was pipetted out in to a 500 ml Erlenmeyer flask, 1 drop of phenolphthalein were added as indicator, neutralised with 1 N NaOH. An excess of 3.0 ml of 1N NaOH were added for saponification, shaken well and kept at room temperature for 48 hours. Then 3.0 ml of 1 N acetic acid was added to it, shaken well. After 15 minutes, 4.0 ml of 1N $CaCl_2$ was added with constant shaking and was allowed to stand for 4 hours. Then it was boiled for 2 minutes, filtered using a pre-weighed filter paper (HiMedia Laboratories) under vacuum, washed with hot water, tested with silver nitrate for the presence

of chloride, dried in a vacuum desiccator for constant weight.

$$\% \text{ of calcium pectate} = \frac{W \times V1 \times 100}{V2 \times P}$$

where,
W = weight of calcium pectate,
V1 = total volume of solution prepared,
V2 = volume of solution used for precipitation
P = amount of pectin used

2.4.2. Equivalent mass,(Suman R Yadav, ZH Khan, SS Kunjwani 2015)

0.2 g of the powdered pectin was moistened with 5 ml of ethanol, then dissolved in water (HPLC), and made up to 100 ml. 10 ml of this solution was pipetted out in to a conical flask, 2 drops of phenolphthalein added as indicator, titrated against 0.01 N NaOH. Value noted as V1 and is the measure of the unesterified galacturonic acids.

$$\text{Equivalent mass} = \frac{W \text{ x } 1000}{V1 \text{ x } N1}$$

Where, W = weight of pectin (g) used
V1 = volume of alkali used
N1 = normality of alkali used

2.4.3. Estimation of Degree of Esterification (D.E.),

The method described in Food Chemicals Codex(Birch 2003) 3rd edition, is used with some modifications. 5.0 ml of 0.1 N NaOH is added to the above solution (used for calculation of equivalent mass), shaken well, and kept at room temperature for 3 hours for saponification. Then 5.0 ml of 0.1 N HCl is added to it to neutralise the NaOH, 2 drops of phenolphthalein added as indicator, and titrated against 0.01 N NaOH, value noted as V2. This is the measure of the esterified galacturonic acid.

$$DE = \frac{V2}{V1+V2} 100$$

2.4.4. Estimation Percentage of Anhydrouronic acid (%AUA)*(Joel et al. 2018)*

%AUA is calculated using the above values used for the estimation of equivalent mass and degree of esterification.

$$\%AUA = \frac{176 \text{ x } 0.1 \text{ x } Z \text{ x } 100}{W \text{ x } 1000} + \frac{176 \text{ x } 0.1 \text{ x } Y \text{ x } 100}{W \text{ x } 1000}$$

Where, 176 = molecular mass of AUA,
V1, V2 = volumes of alkali mentioned above
W = weight of pectin used

2.4.5.Sugar Profile Analysis (Corradini, Cavazza, and Bignardi 2012),

50 µg of the pectin sample was hydrolysed using 2N TFA at 100^0C for 5 hours, followed by removal of the acid under a stream of nitrogen gas. The sample was co-evaporated with 50% isopropyl alcohol for the complete removal of the acid. Finally, the sample was dissolved in Milli-Q water and 10 µg was injected on HPAEC-PAD. Dionex ICS-3000 was used for monosaccharide profiling using CarboPacPA-1 column (4mm x 250mm) with 100 mM NaOH and NaOAc gradient.

2.4.5. IR analysis (Kyomugasho et al. 2015),

The powdered peel was mixed with KBr (1,100) and pressed in to pellets. Then it is analysed with a Perkin Elmer (USA) machine and FTIR spectra were collected at the transmission mode in the frequency range of 400-4000 cm^{-1}, resolution = 2 cm^{-1}, No. of scans = 8.

2.4.6 NMR analysis

1D ^{1}H and ^{13}C spectra were obtained at 400MHz, using liquid state NMR spectrometer (Bruker) with D_2O as solvent for the analysis. 2D NMR spectra – HSQC and TOCSY- were obtained at 500MHz, using liquid state NMR spectrometer (Bruker AvansIII 500) with D_2O as solvent for the analysis.

2.4.7. Viscosity measurements,

Weighed amounts of pectin were mixed with water at pH = 3.2 and 16.25 g of sucrose. A series of experiments were set up (in triplicates) according to the table given below (Table 1).

All the above sets were boiled to 105^0C till volume is reduced to 25 ml, and poured in to a test tube, allowed to cool down to room temperature. They were then analyzed using a Rheometer (Anton Paar, MCR52, SN81174546; FW3.65; Slot (2,-1); Adj (1993,0)d, Application RHEOPLUS/32 V3.61 21006273-33024, Accessories TU1=P-PTD200/AIR-SN81174614, Measuring system PP75-SN16019; [d=1 mm], at constant temperature of 25^0C.

2.4.8. Rheological analysis

Ripened pineapple is chopped in to small pieces after removal of outer skin and is made in to a juice in a blender. It is then evaporated, with continuous stirring in a pan placed over a stove to remove water to a certain extent. Then 25g of this juice is weighed in to a beaker, 25g of sucrose is added and further boiled with continuous stirring. Then a powdered mixture of 1g sucrose, and 0.25g pectin is added and boiled again, a small amount of citric acid is added and boiled with continuous stirring to jam of final pH of 3.6 and brix 60%. Different sets of this jam are prepared in triplicates as per the following scheme (table 2),

The Control did not contain any pectin while citrus pectin purchase from Sigma -Aldrich was used in the Standard. The Test contained pectin extracted from banana peel. Strain sweep (shear-strain-amplitude sweep, with controlled-shear deformation CSD) experiments were conducted using Rheometer (Aanton Paar) model MCR 52, plate-plate method, sample thickness of 1mm, at 25^0C, frequency 1Hz, and shear strain range of 0.0001-100%(Dorohovich, Dorohovich, and Kambulova 2016).

Table 1. Preparation of gel using sugar and pectin

Sl. No.	Name of set up	Vol. of acidified water (pH = 3.2)	Amount of sucrose (g)	Amount of pectin (sigma)	Amount of banana pectin (g)	Final volume of gel before pouring (ml)
1	Control	30 ml	16.25	Nil	Nil	25
2	Standard	30 ml	16.25	0.5g	Nil	25
3	Test 1	30 ml	16.25	Nil	0.1	25
4	Test 2	30 ml	16.25	Nil	0.2	25
5	Test 3	30 ml	16.25	Nil	0.3	25
6	Test 4	30 ml	16.25	Nil	0.4	25
7	Test 5	30 ml	16.25	Nil	0.5	25

Table 2. Preparation of Pineapple Jam

Sl. No.	Name	Amt. of sugar(g)	Amt. of pectin(g)	% brix	pH
1	Control	26	nil	60	3.6
2	Standard	26	0.25	60	3.6
5	Test	26	0.25	60	3.6

2.4.9. Texture Profile Analysis(Banaś, Korus, and Korus 2018)

Texture Profile Analysis of the above jams were carried out using UTM-Lloyd instrument, model LR-5k, at a speed of 50mm/min, using a circular probe with a diameter of 80mm.

3. Results and Discussion

3.1. Effect of various extraction conditions upon pectin yield

Table - 3 shows the yield under specified conditions of extraction (Values are averages of six independent analysis ± SEM)

a. Provided all other conditions of extraction being identical, yield has been increased as the duration of heating increased. This is evident from the following pairs of sets, 1 and 16, 6 and 7, 28 and 20, and 24 and 10 (Table 4). In all the above-mentioned cases, the percentage of increase in yield of pectin upon increased duration of heating is noticeable.

Table 3. Yield of pectin (% of dry weight) from peel of Banana.

Set	SLR	pH	Time(min)	Temp(^{0}C)	Yield (%)
1	30	1.5	52.5	54	1.77±0.34
2	40	2	75	68	3.7±0.91
3	30	2.5	52.5	82	3.73±0.27
4	40	2	75	68	2.82±0.13
5	60	2	75	68	4.33±0.4
6	30	1.5	52.5	82	16.4±1.64
7	30	1.5	97.5	82	27.5±0.7
8	20	2	75	68	1.62±0.22
9	50	2.5	97.5	54	1.23±0.16
10	50	2.5	97.5	82	4.97±0.86
11	40	2	75	40	0.97±0.18
12	40	2	75	68	3.43±0.84
13	50	1.5	97.5	54	15.1±0.67
14	50	2.5	52.5	54	1.7±0.09
15	50	1.5	52.5	54	2.68±0.47
16	30	1.5	97.5	54	3.78±0.52
17	40	2	75	68	7.7±1.37
18	50	1.5	52.5	82	28±2.25
19	40	1	75	68	5.47±0.78
20	30	2.5	97.5	54	2.07±0.11
21	50	1.5	97.5	82	24.6±2.46
22	40	3	75	68	3.12±0.28
23	40	2	120	68	8.05±1.53
24	50	2.5	52.5	82	3.57±0.64
25	30	2.5	97.5	82	2.93±0.06
26	40	2	30	68	2.97±0.47
27	40	2	75	68	5.23±0.79
28	30	2.5	52.5	54	2.5±0.39
29	40	2	75	68	7.48±1.16
30	40	2	75	96	17.5±1.87

Table 4. Effect of duration of heating upon yield of pectin

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
1	30	1.5	52.5	54	0.8 (a)	200%
16	30	1.5	97.5	54	1.6 (b)	
6	30	1.5	52.5	82	10.6 (a)	233.02%
7	30	1.5	97.5	82	24.7 (b)	
28	30	2.5	52.5	54	0.7 (a)	242.86%
20	30	2.5	97.5	54	1.7 (b)	
24	50	2.5	52.5	82	1 (a)	240%
10	50	2.5	97.5	82	2.4 (b)	

b. As the temperature of extraction increases, yield also increases. This is evident from the relevant values of the following pairs of sets, 1 and 6, 16 and 7, 11 and 2, 29 and 30, 15 and 18, 13 and 21, and HA9 and 10 (Table 5). At a pH of 1.5 and for a less time period of heating, temperature was a very important limiting factor. This is evident from the sets 1 and 6, 15 and 18 all of which heated for 52.5 minutes. The first pair demonstrated an increase of 926 % while the second one, an increase of 1044.8 % because of the increase of temperature from 54^{0}C to 82^{0}C. However, when the mixtures were heated for a longer period of duration (97.5 min), the increase in percentage of yield decreased to 163 % (13 and 21) or 728 % (16 and 7).

Table 5. Increase in yield presumably due to increased temperature

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
1	30	1.5	52.5	54	1.77 (a)	926%
6	30	1.5	52.5	82	16.4 (b)	
16	30	1.5	97.5	54	3.78 (a)	727.5%
7	30	1.5	97.5	82	27.5 (b)	
15	50	1.5	52.5	54	2.68 (a)	1044.8%
18	50	1.5	52.5	82	28 (b)	
13	50	1.5	97.5	54	15.1 (a)	162.9%
21	50	1.5	97.5	82	24.6 (b)	
11	40	2	75	40	0.3 (a)	233.33%
2	40	2	75	68	0.7 (b)	

29	40	2	75	68	1.8 (a)	466.67%
30	40	2	75	96	8.4 (b)	

c. Yield drastically increases when pH decreases from 2.5 to 1.5. This is clear from the analysis of the following pairs of sets as is shown in tables 6 and 7, 6 and 3, 16 and 20, 7 and 25, 15 and 14, 18 and 24, 13 and 9, 21 and 10. This may be because at the higher pH, the extractant may have lower penetrability in to the cell wall materials and also because the chemical bonds between pectin and other cell wall components become weaker at the lower pH. At pH = 1, the yield was very little (Set 19), probably because the other factors (duration of heating and temperature) might not be in their optimal levels. Also, the higher concentration of the acid might have disintegrated the pectin released. Increase in yield with decrease in pH was noticeable in the sets in table 6. As is evident from the table, a feature common to all sets with increased yield is that they are maintained at a temperature of 82^0C. Even though Set-13 demonstrated an incredible increase of 1227.6% in yield over Set 9, the absolute yield of Set 13 was lower (only 15.1%) probably because of its lower temperature of 54^0C.

Table 6. Noticeably increased yield due to lowered pH

Set	**SLR**	**pH**	**Time (min)**	**Temp (^{0}C)**	**Yield (%)**	**Increase in yield = (b/a) ×100 = c**
3	30	2.5	52.5	82	3.73 (a)	439.7%
6	30	1.5	52.5	82	16.4 (b)	
25	30	2.5	97.5	82	2.93 (a)	938.6%
7	30	1.5	97.5	82	27.5 (b)	
24	50	2.5	52.5	82	3.57 (a)	784.3%
18	50	1.5	52.5	82	28 (b)	
9	50	2.5	97.5	54	1.23 (a)	1227.6 %
13	50	1.5	97.5	54	15.1 (b)	
10	50	2.5	97.5	82	4.97 (a)	495%
21	50	1.5	97.5	82	24.6 (b)	

Table 7. Influence of pH upon yield of pectin from banana peel

Set	**SLR**	**pH**	**Time (min)**	**Temp (^{0}C)**	**Yield (%)**	**Increase in yield = (b/a) ×100 = c**
16	30	1.5	97.5	54	3.78 (b)	182.6%
20	30	2.5	97.5	54	2.07 (a)	
15	50	1.5	52.5	54	2.68 (b)	157.7%
14	50	2.5	52.5	54	1.7 (a)	

d. The effect of SLR on the pectin yield was found to vary with pH. At a pH of 1.5, increase in SLR from 30 to 50 (ml of extractant solution per gram of powered peel) was found to be increasing the yield slightly (table 8). The increase in yield when the SLR is increased to 50 from 30 is not as high as in the case of the other conditions such as pH, duration of heating, temperature etc. because those conditions are more limiting than SLR (within the range studied). The increase in yield of Set -18 may be because of the higher temperature (82^0C) at which the experiment was carried out. Also, the reason for the higher yield (15.1%) of Set-13 (at SLR = 50) may be the increased time period of heating of the extraction medium.

At a pH of 2 or above, it was found that the yield decreases slightly as the SLR increases. This is evident from the analysis in table 9. This must be because of the dilution of protons in the extractant medium. At a higher pH the concentration of protons in the extractant decreases. This, when coupled with increased volume of the extractant solution, results in still lesser concentration of protons affecting the removal of pectin from other cell wall components. But this effect of dilution of protons at higher pH and SLR is solved to a certain extent when both the time of heating and temperature are increased as is evident from the following comparison in table 10. This is also the reason for the result of comparison between Set-3 and Set-24 in table 9. These observations clearly indicate that the effect of dilution of the solution can be overcome by heating the extractant medium for a prolonged time at a higher temperature.

The solid, liquid ratio should be as high as possible because as the volume of the liquid increases, more of it should be evaporated or more alcohol should be used to precipitate the pectin present in it. Also, it consumes more acid to prepare the liquid of desired pH. Therefore, it is essential to know the minimum possible volume of the liquid extractant which can extract maximum amount of pectin from the raw material.

Table 8. Influence of SLR on pectin yield at pH = 1.5

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
1	30	1.5	52.5	54	1.77 (a)	151.4 %
15	50	1.5	52.5	54	2.68 (b)	
6	30	1.5	52.5	82	16.4 (a)	170.7 %
18	50	1.5	52.5	82	28 (b)	
16	30	1.5	97.5	54	3.78 (a)	399.5 %
13	50	1.5	97.5	54	15.1 (b)	

Table 9. decreased yield due to increased SLR

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
29	40	2	75	68	7.48 (a)	57.89%
5	60	2	75	68	4.33 (b)	
28	30	2.5	52.5	54	2.5 (a)	68%
14	50	2.5	52.5	54	1.7 (b)	

3	30	2.5	52.5	82	3.73(a)	95.7%
24	50	2.5	52.5	82	3.57 (b)	
20	30	2.5	97.5	54	2.07 (a)	59.4%
9	50	2.5	97.5	54	1.23 (b)	

Table 10. Effect of SLR upon yield of pectin from banana peel

Set	SLR	pH	Time (min)	Temp (^{0}C)	Yield (%)	Increase in yield = (b/a)×100
25	30	2.5	97.5	82	2.93 (a)	b/a = 169.6%
10	50	2.5	97.5	82	4.97 (b)	

3.2. Estimation as calcium pectate

The percentage of calcium pectate obtained is 56.871 %. During the formation of calcium pectate, adjacent polygalacturonic chains are cross-linked by Ca^{2+} ions(Caffall and Mohnen 2009). In this work, the percentage of calcium pectate obtained is far less than the value given for purified galacturonic acid (about 110%)(Ranganna 1977), probably because many of the galacturonic acid residues in pectin may be methyl esterified and not available for cross linking. Also, the crude pectin extract may contain not only galacturonic acid but other components such as galactose, arabinose, rhamnose etc. all of which are not taking part in the formation of calcium pectate.

3.3. Equivalent Mass,

The equivalent mass of the pectin extracted from the peel of banana was estimated to be 6666.6.

3.4. Degree of esterification,

The DE was found to be 62.5 % by the titrimetric method. Therefore, pectin obtained from underground stem of banana is high methyl pectin.

3.5. % of Anhydrouronic acid,

The % of AUA was estimated to be70.4

3.6. Sugar Profile Analysis,

Pectin extracted from peel of banana was found to contain the following types of sugars (Table 11).

The chromatogram is shown in figure 1.

Table 11. Sugar profile of banana pectin obtained by HPAEC - PAD

Monosaccharide	Quantity in µg
Fucose	0.095
Rhamnose	1.115
Arabinose	0.204
Glucosamine	0.048
Galactose	0.824
Glucose	0.615

Mannose	0.464
Xylose	0.069
Galacturonic acid	0.086
Glucuronic acid	0.315

3.7. IR analysis

The peak at 3398 cm^{-1} indicates that it contains –OH groups, commonly present in carbohydrates(Coates 2004). The peak at 2929 indicates C-H stretch, while 1794 indicates a C=O stretch. Peak at 1641 indicates N-H bend of an amide group and 1419 shows a C-H bend. The peak at 1079 represents the stretching of the bond between C and O in a methoxyl group (CH_3-O-)(Coates 2004). It was already reported that the region with strong absorption between 1200 and 950 cm^{-1}, called finger print region is characteristic for each type of polysaccharide and even though difficult to interpret, is independent of the source of pectin and may be instrumental in the identification of galacturonic acid(Kyomugasho et al. 2015)(M. A. Monsoor, U. Kalapathy 2001)·(Gnanasambandam and Proctor 2000). Presence of these functional groups indicates that the crude extract contains pectin-like substances.

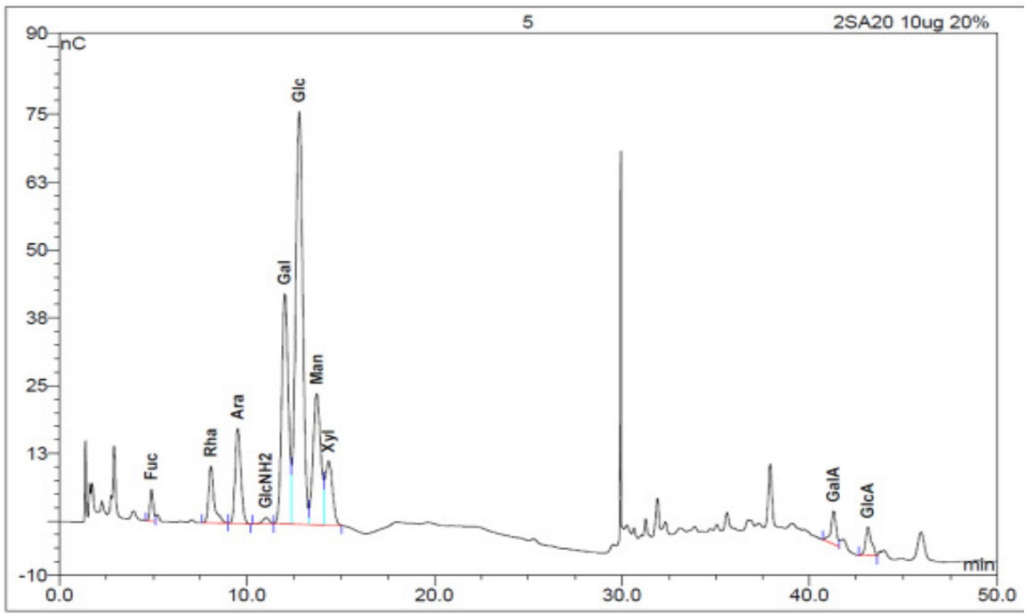


Figure 1. HPAEC – PAD chromatogram of pectin from peel of banana

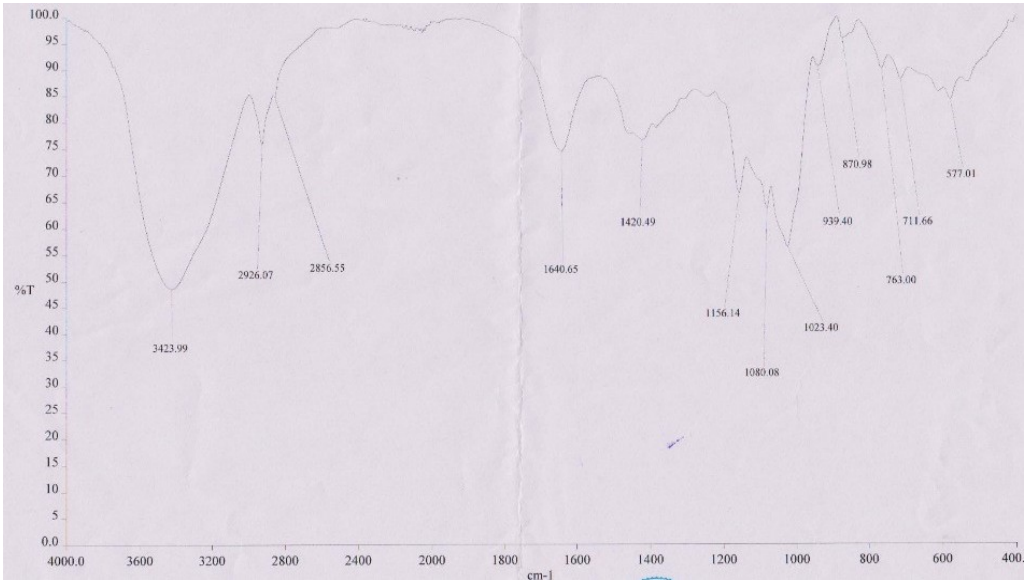


Figure 2. FT IR spectrum of pectin extracted from peel of banana

3.8. NMR Analysis

The ^{1}H NMR spectrum is shown n figure 3.

The ^{13}C spectrum is shown in figure 4.

The HSQC spectrum is given in figure 5

TOCSY spectrum is given in figure 6

Various groups assigned to the chemical shifts are given in table 12(Golovchenko et al. 2007),(BUSH 2016).

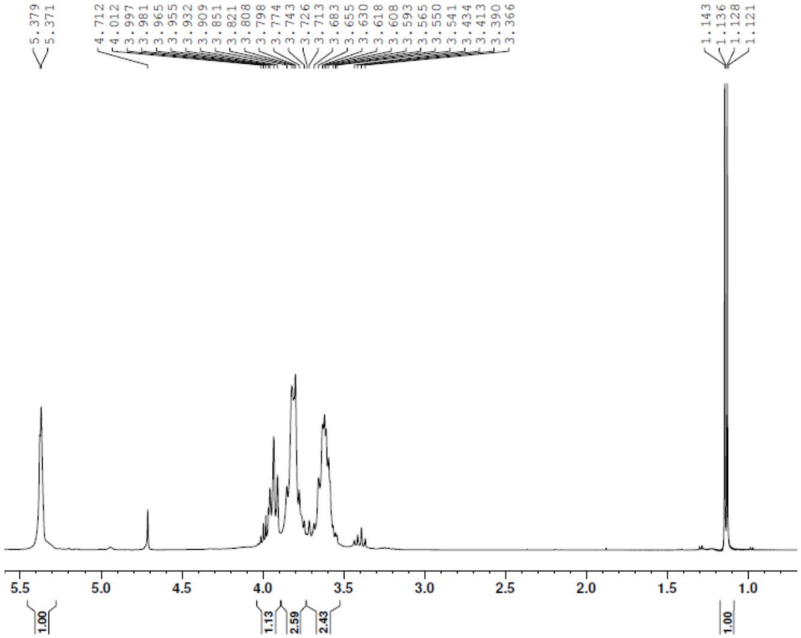


Figure 3. ^{1}H NMR Spectrum of pectin from peel of banana

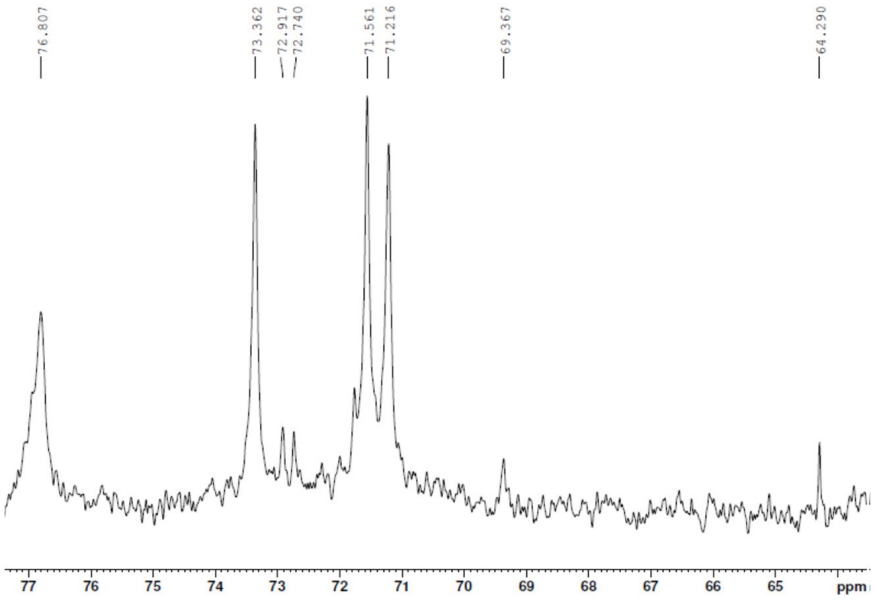


Figure 4. ^{13}C NMR spectrum of pectin from peel of banana

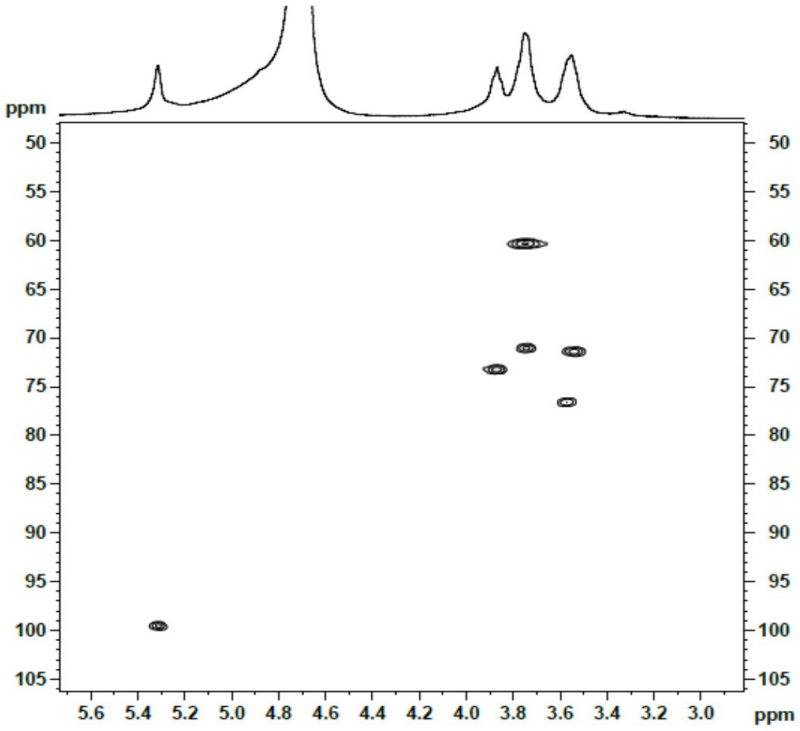


Figure 5. HSQC spectrum of pectin isolated from peel of banana

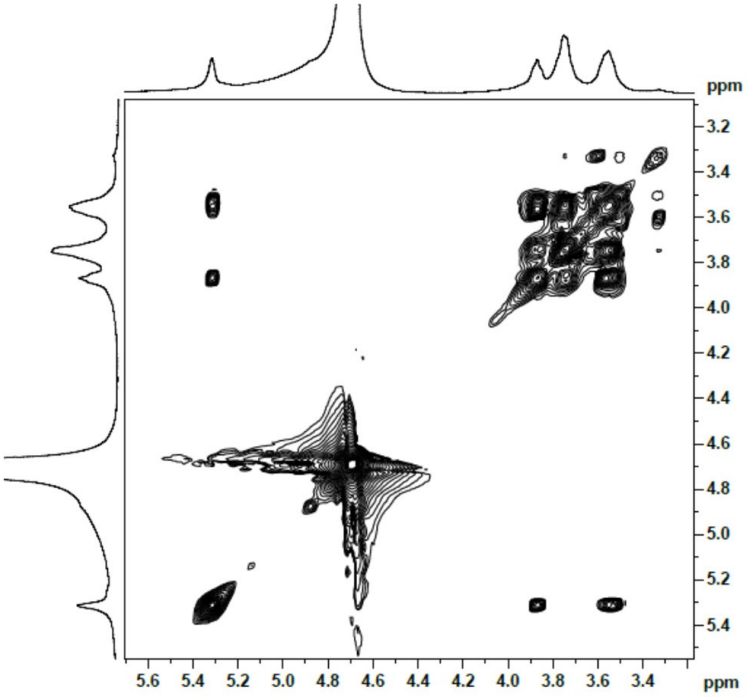


Figure 6. TOCSY spectrum of banana pectin

Table 12. Assignment of chemical shifts of 1H and 13 C NMR spectra

1H Chemical shift	Group identified
3.413	H-4 of →2) - α-L- Rhap-(1→(Rha)
3.43	H4 of → 2) -α - Rhap-(1 →
3.65	H3 of β-Galp-(1→
3.68	H-5 of β-Dgalp - (1→4)-β-Dgalp
3.71	H-5" of α-L-Araf-(1→(Ara)
3.72	H-5 of → 4)- β-DGalpOH
3.74	H-3 of →3)-β-D-Galp-(1→(G)
3.77	H-2 of →4)α-D-GalpA-(1→2) - α-L-Rhap-(1→ (GA)
3.82	H-5 of α-L-Araf-(1→(Ara)
3.85	H-3 of →2) - α-L- Rhap-(1→(Rha)
3.9	H-6" of → 4)- β-DGalpOH
3.93	H4 of β-Galp-(1→
3.96	H-3 of α-L-Araf-(1→(Ara)
4.01	H3 of → 4)-α-GalpA(Ome)-(1→
3.54	H-2 of β-Dgalp - (1→6)-β-Dgalp
3.56	H-2 of β-Dgalp - (1→4)-β-Dgalp
3.59	H-2 of → 4)- β-DGalpOH

3.65	H-3 of β-Dgalp - (1→6)-β-Dgalp
3.72	H6 of β-Galp-(1→
3.77	H-3 of → 4)- β-DGalpOH
3.79	H-6 of β-Dgalp - (1→6)-β-Dgalp
3.8	H-5" of →5)-α-L-Araf-(1→(Ara)
3.9	H-6" of β-Dgalp - (1→6)-β-Dgalp
3.95	H-4 of β-Dgalp - (1→6)-β-Dgalp
3.98	H-3 of →4)α-D-GalpA-(1→GA
13C Chemical shift	Group identified
69.3	C-2 of →4)α-D-GalpA-(1→2) - α-L-Rhap-(1→ (GA)
71.2	C-5 of →2) - α-L- Rhap-(1→(Rha)
71.5	C-3 of →4)α-D-GalpA-(1→2) - α-L-Rhap-(1→ (GA)
72.7	C-2 of →3)-β-D-Galp-(1→(G)
73.3	C2 of →4)-β-D-Galp-(1→4 (G)
76.8	C-5 of →3)-β-D-Galp-(1→(G)
72.9	C-5 of →4)α-D-GalpA-(1→GA
76.8	C-5 of β-Dgalp - (1→4)-β-Dgalp
99.6	non-esterified α-D-GalA

3.9. Viscosity measurements

Results of viscosity measurements are given in table 13.

Test 5, which contains the same concentration of banana as standard, has a viscosity much more than that of the standard which contains citrus pectin. Thus, banana pectin is efficient in increasing viscosity of sugar solutions under the given range of experimental conditions.

3.10. Rheological analysis

Result of Rheological analysis of pineapple jams made using banana pectin (test) is compared with that made using citrus pectin (standard) and that containing no pectin (control)(WINTER 2000).

a. Shear modulus, ratio of stress to strain indicates how strong is the material or how much is the ability of the material to resist a stress. In table 14, shear modulus at a shear strain of about 1.01% is given. Pineapple jam prepared with banana pectin has more shear modulus compared with citrus pectin.

b. Critical stress, also called yield stress, is the minimum stress that must be applied to initiate flow. Pineapple jam prepared with banana pectin has more shear modulus compared with citrus pectin. Jam prepared from banana pectin has more critical stress as is evident from figure 7.

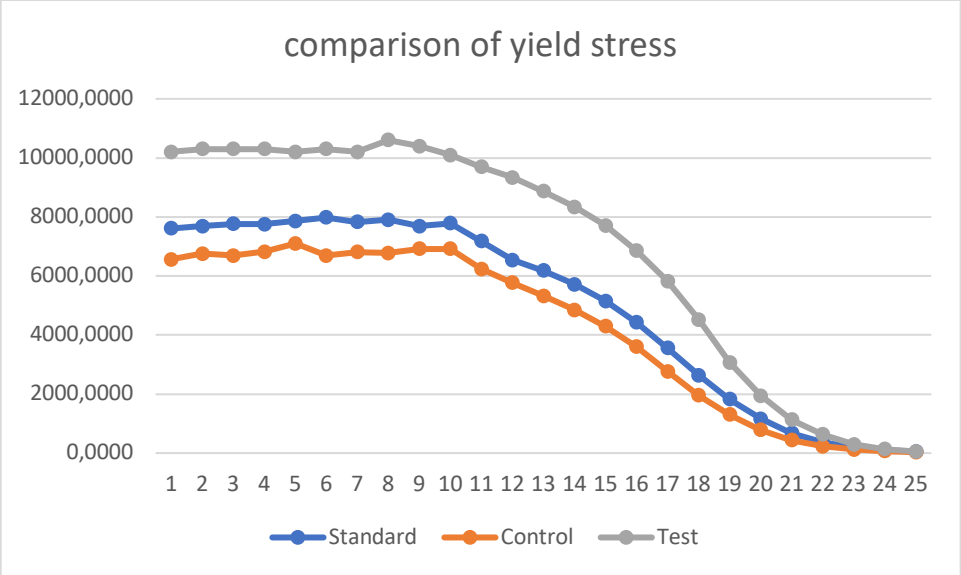


Figure 7. Comparison of yield stress of pineapple jams prepared with banana pectin (test), citrus pectin(standard) and no pectin(control)

3.11. Texture Profile Analysis

Parameters such as hardness, cohesiveness, springiness, gumminess and chewiness are measured. No significant different difference was observed between standard and test in these parameters, as is seen in table 15.

Table 13. Viscosity of sugar solutions containing pectin. All tests contain banana pectin.

Sl. No.	Name of set up	Viscosity at shear rate 1.0 (approx.) Pa.S
1	Control	1.67
2	Standard	11.5
3	Test 1	1.22
4	Test 2	1.99
5	Test 3	2.24
6	Test 4	4.03
7	Test 5	>148

Table 14. Rheological analysis of Pineapple jam prepared with pectin

Sl. No.	Parameter	Control	Standard	Test
1	Shear modulus	30.3000	37.9208	61.2621
2	Critical stress (Pa)	1.2100	1.2400	1.4000

Table 15. TPA of pineapple jams prepared with pectin

	Hardness1 (N)	Hardness2 (N)	Cohesiveness	Springiness (mm)	Gummine ss (kgf)	Chewiness (kgf.mm)
Control	0.617980957	0.531471723	0.768089198	6.244282726	0.04838578	0.302134488

Standard	1.171696864	1.016980649	0.649277941	7.547172493	0.077549126	0.585276632
Test	1.182243837	1.106384146	0.731643238	7.382928507	0.088173365	0.650977649

4. Conclusions

Pectin can be produced from banana peel. Up to 28% of pectin could be extracted under experimental conditions. Different conditions of extraction were found to be affecting the yield. The extracted pectin was found to be high methyl pectin with a %AUA of more than 70% and had a chemical composition similar to pectin from other reported sources. It was also found to be having better rheological properties. India, being the largest producer of banana in the world, has the potential to be the largest exporter as well, if the waste materials such as peel are properly used for the production of pectin. Thus, while increasing productivity and utility of agricultural activities, will also contribute more agro-based industries, employment opportunities and promotes sustainable agriculture practices in the rural and semi-urban India.

5. References

Atmodjo, Melani A., Zhangying Hao, and Debra Mohnen. 2013. "Evolving Views of Pectin Biosynthesis." *Annual Review of Plant Biology* 64(1), 747–79.

Banaś, Anna, Anna Korus, and Jarosław Korus. 2018. "Texture, Color, and Sensory Features of Low-Sugar Gooseberry Jams Enriched with Plant Ingredients with Prohealth Properties." *Journal of Food Quality* 2018, 16–18.

Birch, G. 2003. 59 Food Chemistry *Food Chemicals Codex*.

Brouns, F. et al. 2012. "Cholesterol-Lowering Properties of Different Pectin Types in Mildly Hyper-Cholesterolemic Men and Women." *European Journal of Clinical Nutrition* 66(5), 591–99. http,//dx.doi.org/10.1038/ejcn.2011.208.

BUSH, C. A. 2016. "ChemInform Abstract, High Resolution NMR in the Determination of Structure in Complex Carbohydrates." *ChemInform* 20(51).

Caffall, Kerry Hosmer, and Debra Mohnen. 2009. "The Structure, Function, and Biosynthesis of Plant Cell Wall Pectic Polysaccharides." *Carbohydrate Research* 344(14), 1879–1900. http,//dx.doi.org/10.1016/j.carres.2009.05.021.

Coates, John. 2004. "Encyclopedia of Analytical Chemistry -IInterpretation of Infrared Spectra, A Practical Approach." , 1–23. http,//www3.uma.pt/jrodrigues/disciplinas/QINO-II/Teorica/IR.pdf.

Corradini, Claudio, Antonella Cavazza, and Chiara Bignardi. 2012. "High-Performance Anion-Exchange Chromatography Coupled with Pulsed Electrochemical Detection as a Powerful Tool to Evaluate Carbohydrates of Food Interest, Principles and Applications." *International Journal of Carbohydrate Chemistry* 2012, 1–13.

Dorohovich, Antonella, Viktoriya Dorohovich, and Julya Kambulova. 2016. "The Study of the Rheological Properties of Pectin Gels With Mono - and Disaccharides." *EUREKA, Life Sciences* 4(4), 14–19.

Gnanasambandam, Ravin, and A Proctor. 2000. "Determination of Pectin Degree of Esterification by Diffuse Reflectance Fourier Transform Infrared Spectroscopy." *Food Chemistry* 68(3), 327–32. https,//www.sciencedirect.com/science/article/pii/S0308814699001910 (May 10, 2019).

Golovchenko, V. V. et al. 2007. "Structural Study of Bergenan, a Pectin from Bergenia Crassifolia." *Russian Journal of Bioorganic Chemistry* 33(1), 47–56.

Gunning, A. Patrick, Roy J. M. Bongaerts, and Victor J. Morris. 2008. "Recognition of Galactan Components of Pectin by Galectin-3." *The FASEB Journal* 23(2), 415–24.

Ho, Yen Yi, Chia Min Lin, and Ming Chang Wu. 2017. "Evaluation of the Prebiotic Effects of Citrus Pectin Hydrolysate." *Journal of Food*

and Drug Analysis 25(3), 550–58. http,//dx.doi.org/10.1016/j.jfda.2016.11.014 .

Joel, J M et al. 2018. "Extraction and Characterization of Hydrocolloid Pectin from Goron Tula (Azanza Garckeana) Fruit." *World Scientific News* 101(June), 157–71.

Kapoor, Sabeeta, and Shylaja M. Dharmesh. 2017. "Pectic Oligosaccharide from Tomato Exhibiting Anticancer Potential on a Gastric Cancer Cell Line, Structure-Function Relationship." *Carbohydrate Polymers* 160, 52–61. https,//www.sciencedirect.com/science/article/pii/S0144861716314138 (May 6, 2019).

Kumar, Manoj, Rakesh Kumar Mishra, and Ajit K. Banthia. 2011. "Development of Pectin Based Hydrogel Membranes for Biomedical Applications." *International Journal of Plastics Technology* 14(2), 213–23.

Kyomugasho, Clare et al. 2015. "FT-IR Spectroscopy, a Reliable Method for Routine Analysis of the Degree of Methylesterification of Pectin in Different Fruit- and Vegetable-Based Matrices." *Food Chemistry*.

M. A. Monsoor, U. Kalapathy, and A. Proctor*. 2001. "Improved Method for Determination of Pectin Degree of Esterification by Diffuse Reflectance Fourier Transform Infrared Spectroscopy." *J. Agric. Food Chem* 49(6), 2756–2760.

Martínez, Yanina N. et al. 2014. "Studies on PVA Pectin Cryogels Containing Crosslinked Enzyme Aggregates of Keratinase." *Colloids and Surfaces B, Biointerfaces* 117, 284–89. https,//www.sciencedirect.com/science/article/pii/S0927776514001167 (May 8, 2019).

Maxwell, Ellen G., Nigel J. Belshaw, Keith W. Waldron, and Victor J. Morris. 2012. "Pectin – An Emerging New Bioactive Food Polysaccharide." *Trends in Food Science & Technology* 24(2), 64–73. https,//www.sciencedirect.com/science/article/abs/pii/S0924224411002688 (May 6, 2019).

May, Colin D. 1990. "Industrial Pectins, Sources, Production and Applications." *Carbohydrate Polymers* 12(1), 79–99.

Moreira, Helena R. et al. 2014. "Injectable Pectin Hydrogels Produced by Internal Gelation, PH Dependence of Gelling and Rheological Properties." *Carbohydrate Polymers* 103(1), 339–47.

Mudgil, Deepak, and Sheweta Barak. 2013. "Composition, Properties and Health Benefits of Indigestible Carbohydrate Polymers as Dietary Fiber, A Review." *International Journal of Biological Macromolecules* 61, 1–6. http,//dx.doi.org/10.1016/j.ijbiomac.2013.06.044.

Neves, Sara C. et al. 2015. "Biofunctionalized Pectin Hydrogels as 3D Cellular Microenvironments." *Journal of Materials Chemistry B* 3(10), 2096–2108.

Parkar, Shanthi G. et al. 2010. "Gut Health Benefits of Kiwifruit Pectins, Comparison with Commercial Functional Polysaccharides." *Journal of Functional Foods* 2(3), 210–18. https,//www.sciencedirect.com/science/article/pii/S1756464610000393 (May 6, 2019).

Pattanayak. 2017. Ministry of Agriculture and Farmers' Welfare, Government of India *Horticulture Statistics at a Glance*.

Ranganna. 1977. "Manual Analysis of Fruit and Vegetable Products." In , 77–78.

Ribeiro, Lígia N.M. et al. 2014. "Pectin-Coated Chitosan-LDH Bionanocomposite Beads as Potential Systems for Colon-Targeted Drug Delivery." *International Journal of Pharmaceutics* 463(1), 1–9.

Srivastava, Pranati, and Rishabha Malviya. 2011. "Sources of Pectin, Extraction and Its Applications in Pharmaceutical Industry - an Overview." *Indian Journal of Natural Products and Resources* 2(1), 10–18.

Suman R Yadav, ZH Khan, SS Kunjwani, SM Mular. 2015. "Extraction and Characterization of Pectin from Different Fruits." *International Journal of Applied Research* 1(9), 91–94. http,//www.allresearchjournal.com/archives

/2015/vol1issue9/PartB/1-8-169.pdf.

Tripathi, S., G.K. Mehrotra, and P.K. Dutta. 2010. "Preparation and Physicochemical Evaluation of Chitosan/Poly(Vinyl Alcohol)/Pectin Ternary Film for Food-Packaging Applications." *Carbohydrate Polymers* 79(3), 711–16. https,//www.sciencedirect.com/science/article/pii/S0144861709005335 (May 8, 2019).

Willats, William G.T., J. Paul Knox, and Jørn Dalgaard Mikkelsen. 2006. "Pectin, New Insights into an Old Polymer Are Starting to Gel." *Trends in Food Science and Technology* 17(3), 97–104.

WINTER, H. HENNING. 2000. "THE CRITICAL GEL The Universal Material State between Liquid and Solid." In *99 NATO ASI Meeting*, Les Houches, France, 1–25.

Wong, Tin Wui, Gaia Colombo, and Fabio Sonvico. 2011. "Pectin Matrix as Oral Drug Delivery Vehicle for Colon Cancer Treatment." *AAPS PharmSciTech* 12(1), 201–14. http,//link.springer.com/10.1208/s12249-010-9564-z.

Xiao, Chaowen, and Charles T. Anderson. 2013. "Roles of Pectin in Biomass Yield and Processing for Biofuels." *Frontiers in Plant Science* 4(March), 1–7.

Yapo, Beda M. 2009. "Biochemical Characteristics and Gelling Capacity of Pectin from Yellow Passion Fruit Rind as Affected by Acid Extractant Nature." *Journal of Agricultural and Food Chemistry* 57(4), 1572–78.

Acknowledgement,

1. The 1D - NMR spectra were obtained from NMR Research Centre, IISc, Bangalore, India, while 2D - NMR spectra were obtained from SAIF, IIT Madras, Chennai, India.
2. The FTIR spectrum was obtained from SAIF, SICART, VV Nagar, India.
3. Rheological studies and Texture Profile analysis were conducted at SAIF, CFTRI, Mysore, India

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

ADAPTATION OF A PHOTOVOLTAIC POWERED OZONE GENERATION SYSTEM FOR FOOD STORAGE

L.Nehari[1], M.Brahami[1], IS.Bousmaha[1], H.Labair[2], FZ Boudjella[1], A. Tilmatine[2]*

[1]ICEPS Laboratory, Djillali Liabes University of Sidi Bel-Abbes, Algeria
[2]APELEC Laboratory, Djillali Liabes University of Sidi Bel-Abbes, Algeria
**atilmatine@gmail.com*

https://doi.org/10.34302/2019.11.4.5

Article history:
Received:
29 July 2019
Accepted:
29 September 2019

Keywords:
Power supply;
Ozone generator;
Food storage;
Photovoltaic energy.

ABSTRACT
This paper is aimed to describe a low-cost system, including a power supply, an ozone generator and a photovoltaic panel for the disinfection of a food storage space. The "supply- ozone generator" system is powered by a photovoltaic energy system developed in the laboratory. The experiments were conducted using an ozone treated food storage cabinet and another control cabinet. The obtained results showed that an ozone concentration in the range 2 to 7 ppm make it possible to ensure effective disinfection. The study that was carried out with fresh food products confirmed the effectiveness of such a technique for extending the shelf life of food, which can be used in isolated rural areas that are not provided with electrical energy.

1. Introduction

Ozone (O_3) is a potent and effective germicide oxidant. It has a much higher disinfection potential than chlorine and other disinfectants. Thus, it is widely used in the disinfection of air and water, chemicals and some pharmaceutical applications (JS Chang ET al.1995; A Fridman et al.2005; N Bouregbaa et al. 2014; Roya et al. 2016).

Dielectric barrier discharge (DBD) is considered as the most suitable kind of gas discharges for large-scale industrial applications. This silent discharge is nowadays widely used for ozone generation and several other industry applications such as surface treatment and biomedical application (Brahami, M. N et al. 2015; S Pekárek. 2012; S Boonduang. 2012).

The ozone is produced in industry mainly by electrical discharge and ultraviolet radiation (UV). UV ozone generators are in general not very expensive but ineffective (Drews et al. 2011). They require an exposure of the gas inside an UV reactor of great length while producing ozone with only weak concentration. Thus, the dielectric barrier discharge (DBD) is currently preferred for the production of high concentration ozone and higher gas outputs (Sainct et al. 2011; Fang et al. 2008).

The DBDs are well known for more than a century. Siemens carried out the first experiments on such discharges in 1857 (Kogelschatz et al. 2003). Although this method is very useful, the main disadvantage of DBDs is lack of uniformity. This is because at atmospheric pressure DBD is normally a filamentary discharge (D Trunec et al.2010). Since ozone cannot be stored, it must be generated on site. The reason for the different configurations of dielectric is due to the multiple applications of the DBD (Figure 1).

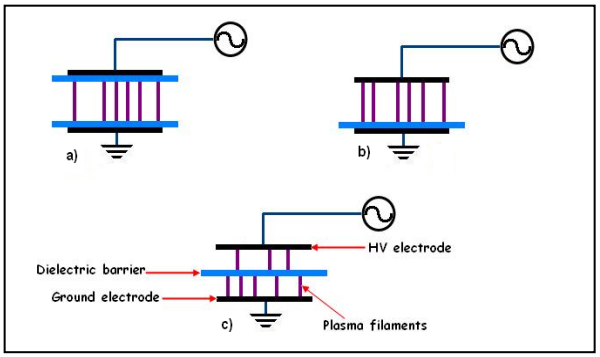


Figure 1. Dielectric barrier discharge with a gas gap

a) The dielectric is located on each electrode
b) The dielectric is located on one of the electrodes
c) The dielectric is located between the two electrodes in the gas

For example, in the case of waste gases sterilization and ozone generation, at least one electrode is covered by a dielectric. While for DBD new-generation lamps, the gas in the lamps is completely isolated from the metallic electrodes, which are covered with a dielectric layer. In this way, gas contamination is prevented and the lifetime of the lamps is enhanced (Williamson et al.2006)

The application field of ozone generation by DBD discharge is wide; the one focused in this work is about air disinfection in food storage rooms.

Generally, households are confronted with the problem of storage of fruits and vegetables. Food storage is carried out in cold rooms at low temperature (below 10 °C) whose electrical operating power is relatively high (2000 W for a volume of 20 m^3), requiring thus a great amount of electrical energy. Therefore, because of the high cost for implementing the necessary equipment for cold rooms, the high electricity consumption and the precarious financial situation of third world countries, it is difficult to build cold rooms with its requested electrical energy in isolated sites. (M Jbilou et al .2018, Tayyari et al, 2017)

The objective of this paper is to develop a low cost system including an electronic power supply feeding an ozone generator, the whole being powered by a photovoltaic energy panel. The system "supply-Ozone Generator-PV Panel" was used for disinfection of the air inside a food storage cabinet.

2. Materials and methods

2.1. Experimental setup

The developed experimental device consists of a photovoltaic generator (1), a power supply (2) and an ozone generator (3) (Figure 2). The experimental setup used in this work is illustrated in Figure 3.

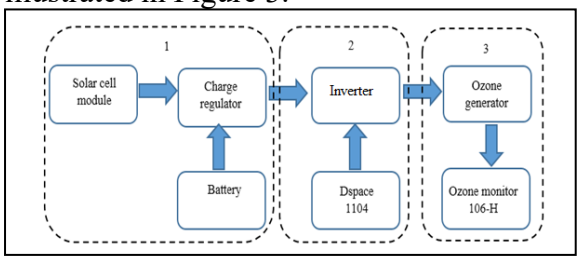


Figure 2. Descriptive diagram of the developed system

1- Photovoltaic Generator, 2- power supply, 3- Ozone Generator

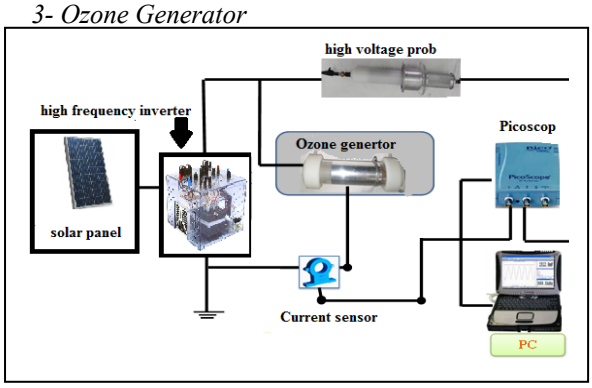
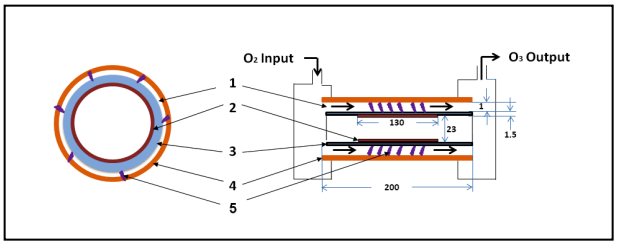


Figure 3. Descriptive schematic of the experimental setup

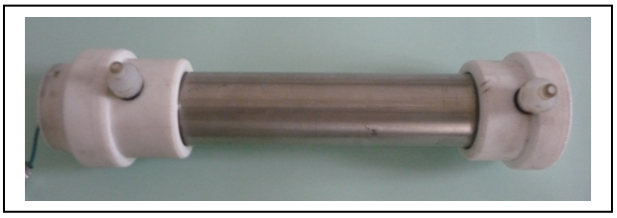
The photovoltaic generator consists of a 135-watt solar panel, a storage battery and a charge controller. This generator provides a voltage of 24 VDC at the inlet of the DC-AC inverter, which transforms it to 2.3 kV AC voltage at a frequency of 14 kHz using a ferrite transformer.

For the ozone generator, we opted for a dielectric barrier discharge gap of 1 mm, which is the value generally used in industry and laboratory research (Figure 4). The inner cylindrical high voltage electrode is an adhesive aluminum sheath with 130 mm length inserted

in a glass tube with a closed contact of their surfaces. The glass tube of thickness 1.5 mm, acting as a dielectric barrier, is 200 mm long and 23 mm inner diameter. The grounded cylindrical electrode is a stainless steel tube of 200 mm length and 28 mm inner diameter.



(a)



(b)

Figure 4. The ozone generator. (a) Descriptive schematic, (b) Photography of the ozone generator

1-Discharge gap (gas flow), 2-High voltage electrode, 3-Glass tube, 4-Grounded electrode, 5-Plasma. (All dimensions are in mm)

2.2. Description of the power supply

Ozone generators are usually supplied by a high-voltage, high-frequency power supply, since high frequencies decrease the necessary power to be used and increase the ozone production rate (Flores-Fuentes et al.2009; Alonso, 2002; Alonso, 2003). Thus, the power density applied to the discharge surface is increased as well as the ozone generation rate, for a given surface area, while the necessary voltage is decreased. The increase in the frequencies up to several kilohertz is now feasible using power electronic switching devices, such as MOSFETs (Ponce-Silva, 2016; Amjad, 2012; Amjad, 2013). The main components of the developed inverter are shown in Figure 5.

- Control block: A DSPACE 1104 board generates the control signals sent to the power switches isolated using opto-couplers.

- Power Interface Block: This part represents an isolation and amplification interface that protects the control circuit (5V) from the power circuit (220V/1A).

- Power unit (inverter): A Semikron block (inverter) based on the IGBTs package equipped with a free-wheeling diode has been used, it converts the DC voltage into AC voltage by means of the DSPACE 1104 board.

As IGBT reliability decreases with increasing temperature, the heating produced in these conductive junctions should be dissipated by heat sinks.

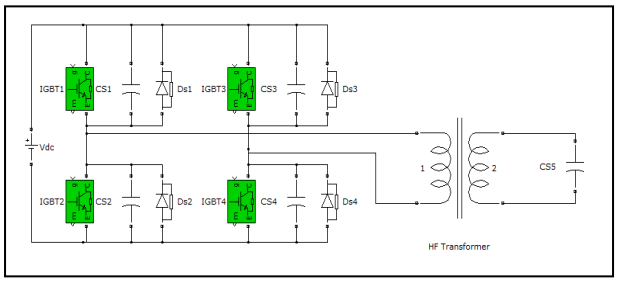


Figure 5. the developed inverter

3. Results and discussion

The switches are controlled by a square wave signal of amplitude 5V delivered by DSpace card at a frequency of 14 kHz, as shown in Figure 6 While Figure 7 illustrates the voltage waveform supplying the ozone generator, which has a sinusoidal shape and can reach values greater than 2 kV.

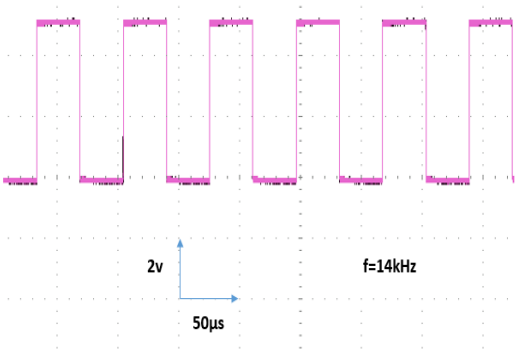


Figure 6. Command signal delivered by DSpace card

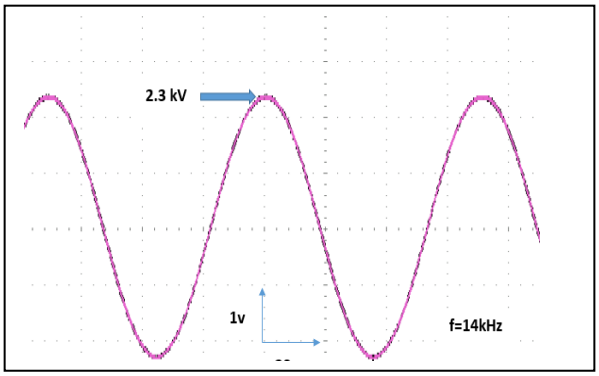


Figure 7. High voltage waveform supplying the ozone generator

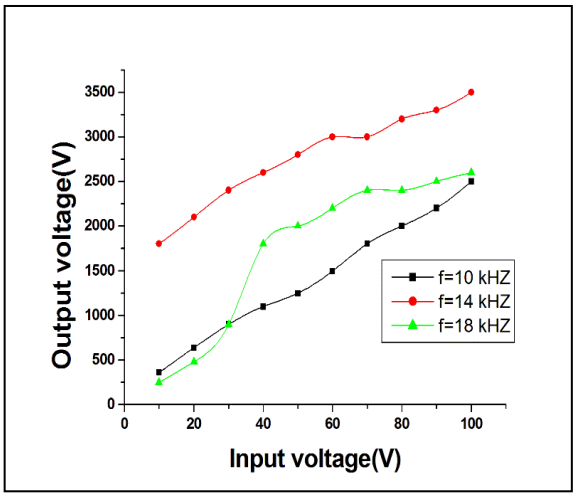


Figure 8. Variation of the output voltage supplying the ozone generator as a function of the inverter input voltage, for different values of the frequency

As shown by the obtained results plotted in Figure 8, representing the variation of the output voltage according to the input voltage, we note that the output voltage increases almost linearly with the input voltage, regardless of the frequency value. It is thus recommended to set the frequency at f = 14 kHz, which represents the resonance frequency giving the maximum voltage value of the ozone generator.

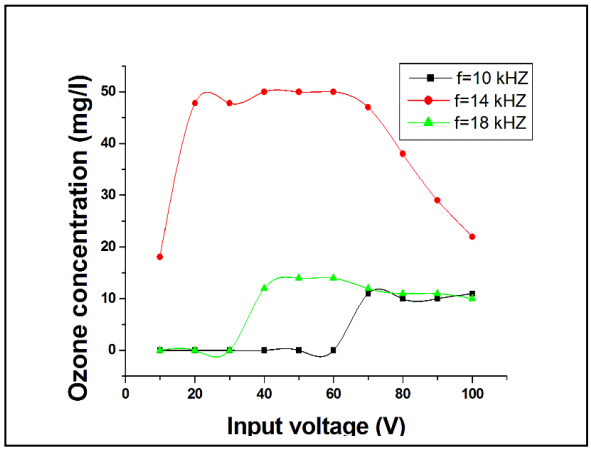


Figure 9. Variation of the ozone concentration as a function of the inverter input voltage Ue for different values of the frequency

These results have been definitively confirmed by the ones plotted in Figure 9 representing the variation of the ozone concentration as a function of the input voltage, for several values of the frequency. Indeed, it's shown that the ozone generator delivers the highest ozone concentration CO^3 = 50 mg/l at a frequency f = 14 kHz and for an input voltage Ue = 24 V. Therefore, we opted for these values in the following experimental study presented in this paper.

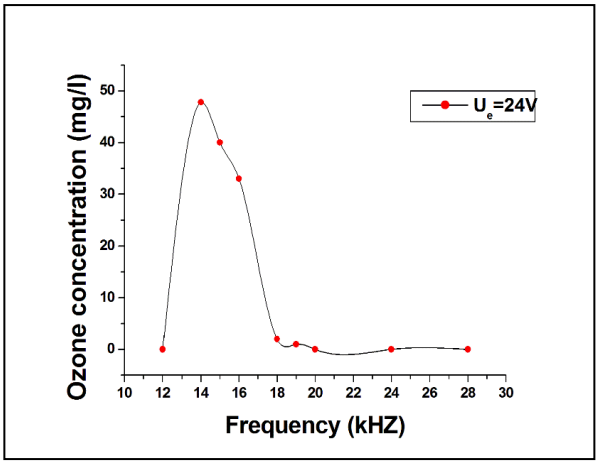


Figure 10. Variation of the ozone concentration as a function of the frequency (Ue= 24V)

Moreover, we plotted the variation of the ozone concentration as a function of the frequency for a constant voltage Ue = 24 V showed in Figure 10. We see that f = 14 kHz is

indeed the optimal resonance frequency giving maximum ozone concentration.

In addition, the developed system comprising the inverter and the ozone generator, was powered by a PV panel for its application for disinfection of food storage spaces to extend their shelflife. This system was tested and studied during a cloudy day, which was subdivided into 3 time slots according to the direction of the sun's rays.

In Figure 11 is represented the variation of the power generated by the PV in the three time intervals during a cloudy day with an average temperature of 18 °C. For each time interval, the inclination angle ß was modified so that the panel surface becomes perpendicular to the sun's rays.

According the results plotted in Figure 11, we see that the average power generated by the PV system remains higher than 60 W, which is a sufficient value to supply the ozone generator that will be used for the air disinfection in food storage room. Power fluctuations are due to intermittent cloud shifts that cover more or less larger portion of the PV panel surface, thereby affecting its performance.

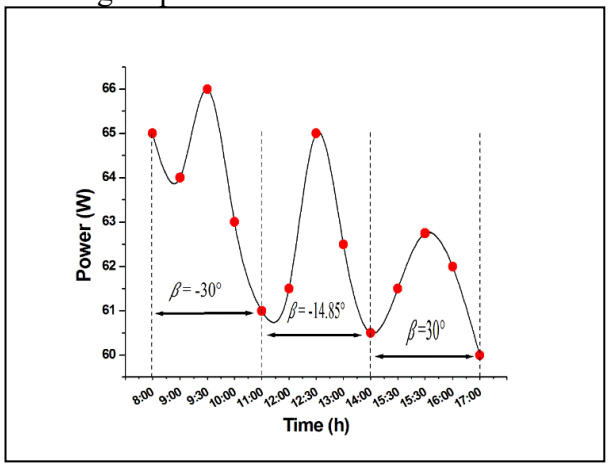


Figure 11. Daily variation of the power generated by the PV system in cloudy weather with adjustment of the inclination angle β

The "inverter- ozone generator" system is used for the treatment of air in a food preservation enclosure. The experimental device used described in Figure 12 is a glass enclosure inside which the food products are placed. A supply system, comprising the ozone generator and the PV energy device shown in Fig. 3, is used to inject ozone into the enclosure through a PVC pipe fixed on the upper wall to diffuse ozone throughout the enclosure.

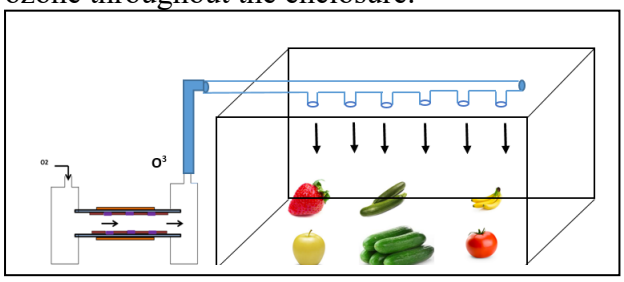


Figure 12. Schematic descriptive of the experimental setup

Ozone is mainly used for the disinfection of air and water, in order to eliminate bacteria and viruses. Nevertheless, the concentration should not exceed a maximum limit that will produce the opposite effect. The recommended amount of ozone in food industry should be comprised between 2 and 7 ppm for effective food preservation, the average ozone concentration in our case has been kept in this range. Note that a second similar untreated chamber was used as a control enclosure.

Fresh food purchased at the local market was placed in both enclosures and kept for a period of 25 days. Photos were taken at regular intervals for visual analysis of food quality. All experiments were performed under stable climatic conditions of temperature (20 ± 8 °C) and humidity (50 ± 15%).

3.1. Application for food preservation

The first step is to determine the operating times of the system to ensure a suitable ozone concentration lying between 2 and 7 ppm. The generator is run for a determined time period Δt_{ON} up to a concentration of 7 ppm and then it is turned off for a period Δt_{Off} untill a concentration of 2 ppm. As soon as the concentration reaches 2 ppm, the generator is restarted again. This regulation is performed automatically using an Arduino based system.

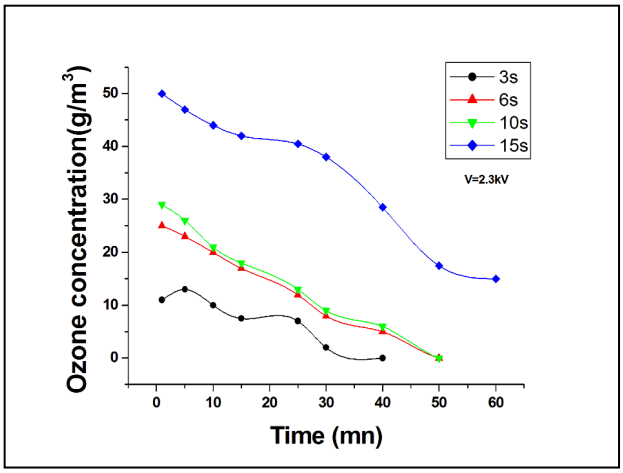


Figure 13. Evolution of the ozone concentration decline as a function of time during the turn off period Δt_{Off}, for different values of the operating time period Δt_{ON}

Figure 13 represents the evolution of the ozone concentration decline as a function of time when the generator is turned off during the period Δt_{Off}, each curve corresponding to an operating time Δt_{ON} of the ozone generator.

As shown in Figure 13, the time period required to reach an ozone concentration of 7 ppm is 15 seconds. Moreover, the time during which the concentration decreases from 7 to 2 ppm, depending on the experimental conditions of temperature and humidity, is approximately equal to 40 minutes.

Based on these results, the time control system of the ozone generator has been set to maintain an ozone concentration between 2 and 7 ppm. Consequently, an operating time of Δt_{ON} of 15 seconds at turn off intervals of 40 minutes was adopted.

Food preservation results were compared with another control untreated air enclosure, in which similar foods were placed. The results were expressed in terms of number of storage days by taking daily pictures. The obtained results are shown by the images in Figure 14.

These results clearly show that food products stored in the ozone-treated enclosure are much more resistant to contamination than the products placed in the untreated enclosure. Ozone eliminates bacteria and slows their growth.

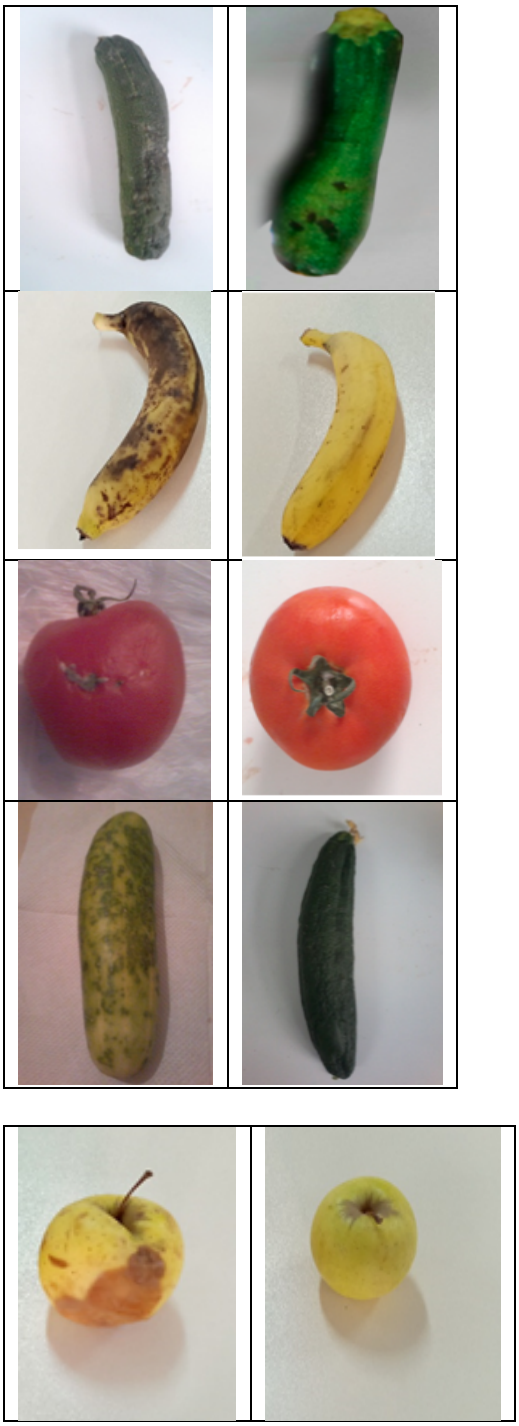


Figure 14. Photographs taken after 25 days period of storage
Left: untreated; Right: ozone treated

The production of ozone by DBD reactor is an effective mean for air disinfection and represents a well-adapted solution in the agri-food sector.

4. Conclusions

Ozone is an effective solution for air disinfection. A system comprising a PV panel supplying a power supply and an ozone generator has been developed and used for the disinfection of food products stored in an ozone-treated enclosure. Moreover, an estimate of the energy produced by the PV panel has been estimated under cloudy conditions. The results obtained have shown that such a system using a high frequency power supply can be envisaged in the storage spaces located in isolated regions. Indeed, encouraging lengthening of the storage duration results have been obtained.

5. References

A. Fridman, A. Chirokov, A. Gutsol.(2005) «Non-thermal atmospheric pressure discharges» Journal of Physics D: Applied Physics,38,pp.1–24.

Alonso, J. M., Rico-Secades, M., Corominas, E., Cardesín, J., & Garcia, J (2002, October) « Low-power high-voltage high-frequency power supply for ozone generation » In Conference Record of the 2002 IEEE Industry Applications Conference. 37th IAS Annual Meeting (Cat. No. 02CH37344) Vol. 1, 257-264.

Alonso, J. M., Valdés, M., Calleja, A. J., Ribas, J., & Losada, J (2003) « High frequency testing and modeling of silent discharge ozone generators » *OZONE SCIENCE & ENGINEERING*, 25(5), 363-376.

Alonso, M., Salam, Z., Facta, M., & Ishaque, K (2012) «A simple and effective method to estimate the model parameters of dielectric barrier discharge ozone chamber» *IEEE Transactions on Instrumentation and Measurement*, 61(6), 1676-1683.

Amjad, M., Salam, Z., Facta, M., & Mekhilef, S (2013) « Analysis and implementation of transformerless LCL resonant power supply for ozone generation» *IEEE transactions on power electronics*, 28(2), 650-660.

Boonduang, S., Limsuwan, S., Kongsri, W., & Limsuwan, P (2012) «Effect of oxygen pressure and flow rate on electrical characteristic and ozone concentration of a cylinder-cylinder DBD ozone generator» *Procedia Engineering*, 32, 936-942.

Bouregba, N., Benmimoun, Y., Meddah, B. Tilmatine, A., & Ouldmoumna, A. (2016) «Ozonation of wastewater in Algeria by dielectric barrier discharge» *Desalination and Water Treatment*, 57(4), 1824-1835.

Brahami, M. N., Hadjeri, S., Nemmiche, S., Brahami, M., & Tilmatine, A. (2015) «Experimental investigation of a PV solar generator for supplying water treatment process» *International Journal of Environmental Studies*, (1), 207-221.

Drews, J., Kusano, Y., Leipold, F., Bardenshtein, A., & Krebs, N. (2011) «Ozone production in a dielectric barrier discharge with ultrasonic irradiation» *Ozone: Science & Engineering*, 33(6), 483-488.

Fang, Z., Qiu, Y., Sun, Y., Wang, H., & Edmund, K(2008) « Experimental study on discharge characteristics and ozone generation of dielectric barrier discharge in a cylinder–cylinder reactor and a wire–cylinder reactor» *Journal of Electrostatics*, 66(7-8), 421-426.

Flores-Fuentes, A., Pena-Eguiluz, R., López-Callejas, R., Mercado-Cabrera, A., Valencia-Alvarado, R., Barocio-Delgado, S., & de La Piedad-Beneitez, A (2008) « Electrical model of an atmospheric pressure dielectric barrier discharge cell» *IEEE Transactions on Plasma Science*, 37(1), 128-134.

J.S. Chang, A.J. Kelly, J.M. Crowley.(1995) «Handbook of Electrostatic Processes» *Ozone Generation and Applications*, vol, 26, 581– 605.

Jbilou, M, Brahami, M.N,Nemmich, S, Brahami, M., & Tilmatine, A (2018) « OZONE FOOD STORAGE SUPPLIED BY PHOTOVOLTAIC ENERGY» *Carpathian Journal of Food Science & Technology*,1-10(4).

Kogelschatz, U (2003) «Dielectric-barrier discharges: their history, discharge physics, and industrial applications» *Plasma*

chemistry and plasma processing, 23(1), 1-46.

Pekárek, S. (2012) «Experimental study of surface dielectric barrier discharge in air and its ozone production» *Journal of Physics D: Applied Physics*, 45(7), 075201.

Roya, Amiri Qandashtani., Esmaeil, Ataye Salehi.(2016) . The effects clo2 & o3 with map on microbial load reduction of mung bean sprouts. *Carpathian Journal Of Food Science And Technology,* 8 (4) , 121-127.

Sainct, F. P., Lacoste, D. A., Laux, C., Mericam-Bourdet, N., Kirkpatrick, M., & Odic, E (2011, July) « Efficiency of ozone generation by dbd and nrp discharges» *In 18th International Colloquium on Plasma Processes (CIP 2011),*(p.3p).

Tayyari, Farinaz, Khazaei, Javad, AJAEI, Peyman, Jouki, Mohammad.(2017) « Effects Of Modified Atmosphere Packaging Systems, Low Temperature And Storage Time On The Quality Of Fresh Minimally Processed Pomegranate Arils» *Carpathian Journal of Food Science & Technology*, 9(1) ,16-26.

Trunec, D, Zajíčková, L, Buršíková, V, Studnička,F,Sťahel,P,Prysiazhnyi,V,& Franta, D (2010) «Deposition of hard thin films from HMDSO in atmospheric pressure dielectric barrier discharge» *Journal of Physics D: Applied Physics*, 43(22), 225403**.**

Williamson, J. M., Trump, D. D., Bletzinger, P., & Ganguly, B. N (2006) « Comparison of high-voltage ac and pulsed operation of a surface dielectric barrier discharge» *Journal of Physics D: Applied Physics*, 39(20), 4400.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

MORPHOLOGY, GROWTH VARIABILITY AND CHEMICAL COMPOSITION OF INDIAN AND NIGERIAN ACCESSION OF OCIMUM SPECIES GROWN IN INDIA

Nensi Patel[1], Nikita Patel[1], Swetal Patel[1], Rajashekhar Ingalhalli[1], Taofeeq Garuba[1,3], Akeem O. Ahmed[1,4], Samson A. Oyeyinka[1,2] , Ramar Krishnamurthy[1*]

[1]C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University Gujarat, India
[2]Department of Home Economics and Food Science, University of Ilorin, Nigeria
[3]Department of Plant Biology, University of Ilorin, Nigeria
[3]Department of Veterinary Microbiology, University of Ilorin, Nigeria
**krishnashanti@gmail.com; krishnamurthy@utu.ac.in*

https://doi.org/10.34302/2019.11.4.6

Article history:
Received:
17 June 2019
Accepted:
25 October 2019

Keywords:
Ocimum sanctum;
Ocimum gratissimum;
Proximate;
Mineral;
Phytochemical.

ABSTRACT

Demand for medicinal plants is increasing by the day, due to their health benefits. In this study, the morphological attributes, proximate, mineral and phytochemical compositions of Indian accessions of *Ocimum sanctum* and *Ocimum gratissimum* as well as that of *Ocimum gratissimum* (Nigerian accession) grown in India under greenhouse conditions were investigated. *Ocimum* leaves showed significant variations in their morphometric attributes and these attributes increased with increasing days after planting. The leaves were good sources of fibre and relatively low in protein. *O. sanctum* showed superior iron content than the other species. Total phenolic and cardiac glycoside contents of the three leaves were very similar but they showed significant variations in their saponins, tannins, flavonoids and alkaloid contents. PCA revealed that Indian accessions of *O. sanctum* and *O. gratissimum* are distinctly separated and different from Nigerian accession of *O. gratissimum* in morphometric data and phytochemical constituents. Nigerian accession had superior phytochemical contents than the Indian accessions and may be further explored for breeding purposes to complement the Indian accessions for enhanced applications in the pharmaceutical industry in India.

1. Introduction

Medicinal plants including the genus *Ocimum*, are well-known to have health-promoting benefits and nutraceutical functions. These functions have been associated with the presence of plant components such as proteins, vitamins, fibres, several kinds of secondary metabolites (Bhattacharya et al., 2014), essential oils and phenolic compounds (Joshi et al., 2011). The genus *Ocimum* belongs to the family Lamiaceae and consist of about 30-35 species indigenous to tropical regions of Asia, Africa, central and south America (Okunlola et al., 2017; Paton, 1992). These plant species have been reported to possess antidiabetic (Gholap and Kar, 2004), antioxidant (Akinmoladun et al., 2007; Bhattacharya et al., 2014; Joshi et al., 2011; Siti et al., 2018) anti-asthmatic (Singh and Agrawal, 1991), anti-inflammatory (Mequanint et al., 2011; Singh and Agrawal, 1991; Singh et al., 1996), antimicrobial (Dambolena et al., 2010; Joshi et al., 2011; Nakamura et al., 1999), anti-stress (Gupta et al., 2007) and anticancer (Prashar et al., 1998) activities.

The nutritional value of genus the *Ocimum* may vary with species, growing location and

processing method. For example, differences in the protein content (7 and 25%) (Dry weight basis) of these species have been reported in different parts of the world including Nigeria (Okunlola *et al.*, 2017) and India (Barua *et al.*, 2015; Siti *et al.*, 2018). In India, *Ocimum sanctum* Linn also known as Tulsi or Holy Basil is an Ayurvedic herb of Southeast Asia with a long history of traditional uses (Singh and Chaudhuri, 2018). It is a common specie that is widely grown in many parts of India. Besides the afore-mentioned therapeutic uses of the species, *Ocimum sanctum* can also be consumed as herbal tea, to treat cough, cold and malaria (Prakash and Gupta, 2005). However, In Nigeria and many African countries, *Ocimum americanum*, *Ocimum basilicum* and *Ocimum gratissimum* are the most important and popular members of these species (Okunlola *et al.*, 2017). Due to the growing demand of medicinal plants in many parts of the world, researchers are now focusing on the possibility of increasing their production either through micro-propagation (Okunlola et al., 2017; Saha et al., 2010; Saha et al., 2012) or macro-propagation (Ehiagbonare, 2007). *Ocimum* species are generally propagated using both seeds and stem cutting, but farmers have problems with cultivating plants from seeds due to their low viability (Okunlola et al., 2017). Many factors such as the method of propagation, specie type, as well as growing conditions may influence the growth pattern and phytochemical constituents of *Ocimum* plants. Okunlola et al. (2017), recently studied the growth and nutritional qualities of *Ocimum basilicum, Ocimum gratissimum,* and *Ocimum americanum*, propagated by stem cutting and seed. According to their report, *Ocimum* species propagated by stem cutting performed better in terms of morphometric attributes than those propagated through the seed. For instance, *Ocimum gratissimum* propagated through the stem had higher number (8 times) of branches and were taller (4 times) than the same specie propagated by seed (Okunlola et al., 2017). However, seed propagated samples contained more phytochemicals than those propagated by stem cutting, suggesting that seed propagation may be better in growing *Ocimum* species for pharmaceutical applications. Hence, in this study, the morphological and growth variability as well as the proximate, mineral and phytochemical composition of *Ocimum sanctum* and *Ocimum gratissimum* were investigated.

2. Materials and methods

2.1. Plant materials

Seeds of three *Ocimum* species were used in this study. Indian accessions of *Ocimum gratissimum* and *Ocimum sanctum* were collected from Maliba Pharmacy College, Uka Tarsadia University (UTU), Gujarat, India, while seeds of one Nigerian accession of *Ocimum gratissimum* was obtained from the botanical garden of University of Ilorin, Nigeria. Body text TNR 12 normal, ident first line 0.66 cm , line spacing Single)

2.2. Seed propagation and processing

Ocimum seeds were sown in the greenhouse of UTU and growth was monitored for 3, 60 and 90 days after planting. Fresh plant leaves of the Indian and Nigerian accessions were collected and washed thoroughly using distilled water. Leaves were separated for different analysis including proximate mineral and photochemical screening as described below. Samples for phytochemical screening were air-dried under shaded condition at room temperature. The dried leaves were crushed into powder and stored in sealed bottles until needed.

2.3. Qualitative and quantitative screening of phytochemicals

Alkaloids, tannins, cardiac glycosides, flavonoids and saponins were determined as previously described (Sofowora, 1993), while the presence of steroids was determined using the method of Khandelwal (2007).

2.4. Proximate composition

Moisture, fat and ash contents of the samples were determined using AOAC (2000) methods. Dry matter contents of the samples were calculated by deducting percentage moisture content from 100. Protein content was determined by the Kjeldahl method (6.25×N) and carbohydrate was calculated by difference. Fibre contents were determined by digestion in sulfuric acid and sodium hydroxide (Kirk and Sawyer, 1991).

2.5. Mineral composition

Mineral content of the samples was determined as described by Amonsou et al. (2014) using Inductively Coupled Plasma (ICP) spectroscopy. Samples were acid-digested by the addition of 1 mL of 55% (v/v) HNO3.

2.6. Statistical analysis

Duplicate samples were prepared and analyses done in triplicate. Data was analysed using one way analysis of variance (ANOVA) and means were compared using the Fisher Least Significant Difference (LSD) test ($p \leq 0.05$) using the Statistical Package for the Social Sciences (SPSS) Version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was used to determine the similarity and differences in the three *Ocimum* accessions based on morphometric data and phytochemical constituents.

3. Results and discussions

3.1. Morphometric characteristics

With the exception of the colour of the leaves and stem, which were generally green and white respectively, the three *Ocimum* species investigated showed significant ($p<0.05$) variations in their morphometric characteristics (Table 1). Regardless of the specie, height, number of leaves, leaf length, leaf width leaf area and number of branches increased with increasing days after planting. Nigerian accession of *O. gratissimum* generally had higher height, number of leaves, leaf length, leaf width and leaf area compared with the Indian accessions (*O. sanctum* and *O. gratissimum*). The height, leaf length, leaf area and number of branches of Indian accession of *O. sanctum* were significantly ($p<0.05$) different from those of *O. gratissimum* throughout the period of planting (30-90 days). Previous research similarly found that *O. gratissimum* performed better in morphometric attributes compared to *O. basilicum* and *O. americanum* (Okunlola et al., 2017). The height (approx. 26-38), number of leaves (approx. 21-24), and number of branches (0-4) observed for *O. gratissimum* (both Nigerian and Indian accessions) examined after 60 days (approx. 9 weeks) of planting in this study, were much higher (2-6 times) than values reported for *O. gratissimum* examined after 8 weeks of planting (Okunlola et al., 2017). Variation in the morphometric data could be due to the differences in days after planting as well as the green house growing conditions such as soil type and seed sowing depth. For example, *O. gratissimum* seed sown at 1 cm depth in humus rich topsoil was reported to have higher germination rate (80%) compared to seed sown on river sand (26.3%) from of the same sowing depth (Ehiagbonare, 2007).

Table 1. Morphometric characteristics of three *Ocimum* species grown by seed propagation

Species	DAP	Height (cm)	NL	LL (cm)	LW (cm)	LA (cm^2)	NB	LC	SC
**Sanctum*	30	$12.19^{g}\pm0.05$	$6.00^{f}\pm1.00$	$3.20^{e}\pm0.26$	$1.67^{e}\pm0.06$	$5.13^{f}\pm0.06$	$0.00^{d}\pm0.00$	Green	White
**Gratissimum*	30	$9.27^{h}\pm0.16$	$8.33^{f}\pm0.58$	$2.46^{f}\pm0.35$	$1.61^{e}\pm0.01$	$4.00^{a}\pm010$	$0.00^{d}\pm0.00$	Green	White
***Gratissimum*	30	$12.31^{g}\pm0.12$	$11.33^{e}\pm0.58$	$3.03^{ef}\pm0.32$	$2.13^{d}\pm0.06$	$6.27^{fg}\pm0.31$	$0.00^{d}\pm0.00$	Green	White
**Sanctum*	60	$34.53^{e}\pm0.36$	$23.33^{cd}\pm1.52$	$5.33^{d}\pm0.40$	$3.90^{e}\pm0.10$	$20.67^{e}\pm0.05$	$6.67^{b}\pm0.57$	Green	White
**Gratissimum*	60	$26.02^{f}\pm0.12$	$21.33^{d}\pm1.52$	$7.53^{bc}\pm0.40$	$3.80^{e}\pm0.17$	$28.50^{d}\pm0.50$	$0.00^{d}\pm0.00$	Green	White
***Gratissimum*	60	$37.95^{d}\pm0.12$	$24.33^{c}\pm0.58$	$8.93^{a}\pm0.31$	$5.20^{b}\pm0.20$	$46.67^{b}\pm1.52$	$4.33^{c}\pm1.52$	Green	White
**Sanctum*	90	$69.36^{b}\pm0.66$	$58.67^{a}\pm3.06$	$7.20^{a}\pm0.20$	$3.90^{e}\pm0.10$	$27.37^{d}\pm0.40$	$10.67^{a}\pm1.52$	Green	White
**Gratissimum*	90	$48.05^{c}\pm0.14$	$42.67^{b}\pm0.58$	$7.90^{b}\pm0.30$	$3.90^{e}\pm0.10$	$30.93^{c}\pm0.12$	$5.33^{bc}\pm0.58$	Green	White
***Gratissimum*	90	$74.87^{a}\pm0.37$	$58.33^{a}\pm2.08$	$9.10^{a}\pm0.50$	$5.47^{a}\pm0.31$	$50.93^{a}\pm2.08$	$10.33^{a}\pm0.57$	Green	White

Mean ± S.D. Means with same superscript within the same column are not significantly ($p<0.05$) different.
DAP: Days after planting; LN: Number of leaves; L: Leaf length; LW: Leaf width
LA: Leaf Area; LC: Leaf Colour; SC: Stem colour; NB: Number of branches *Indian accession** Nigerian Accession

3.2. Proximate composition

The proximate composition data of the three Ocimum species are presented in Table 2. Expectedly, the moisture content of the three *Ocimum* leaves were generally high (average 77%). *O. gratissimum* (both Nigerian and Indian accessions) had significantly ($p<0.05$) lower moisture values (approx. 74-78%) than O. sanctum (approx. 80%). Beside the moisture content, which was the major component of the leaves, ash (average 11%), followed by fibre (4.72-8.47%) were present in fairly good quantities. Protein (0.13-0.21%), carbohydrate (1.63-2.53%) and fat (average 3.31%) were found in relatively small quantities (Table 2). Nigerian accession and Indian accession of *O. gratissimum* had almost similar composition except in their protein and fibre contents, which could be attributed to inherent differences in the plant species. This seems plausible, since both plants were grown under the same conditions. Although the protein content of the leaves were generally low, Nigerian accession of *O. gratissimum* had slightly higher protein content than other *Ocimum* species. Similarly low levels (0.20-1.21%) of protein have been reported for different *Ocimum* leaves (Idris et al., 2011; Oboh et al., 2009). Some authors, however, reported higher protein values (2.88-9.10) for different *Ocimum* leaves (Emeka and Chimaobi, 2012; Mlitan et al., 2014; Okunlola et al., 2017; Shuaib et al., 2015). Generally, fresh leafy vegetables have been reported to have low levels of protein, which are mostly in the form of enzymes, rather than acting as a storage pool, as in grains and nuts (Oboh et al., 2009).

3.3. Mineral composition

There were significant ($p<0.05$) differences in the mineral composition of the three *Ocimum* leaves (Table 3). Nitrogen (4.28-5.58%), followed by calcium (2.92-3.58%), phosphorus (0.38-4.83%) and potassium (2.05-3.51%) were the major mineral elements in the leaves. Sodium (0.02-0.92%) and magnesium (1.17-1.49%) were found in relatively small quantities. High levels of potassium in human diet is important for the protection against life-threatening diseases such as hypertension, cardiac dysfunctions and osteoporosis (Demigne et al., 2004; Lewu et al., 2010). Among the microelements, iron (263.01-863.00 ppm) was the major mineral in the leaves. Iron content of *O. sanctum* was substantially higher (about 3 times) than values recorded for the *O. gratissimum* species. The consumption of such foods rich in micronutrients such as iron helps to build a strong immune system and facilitate nutrient absorption, utilisation and digestion (Njoku and Ohia, 2007). Furthermore, iron is known to play a vital role in haemopoiesis, control of infection and cell mediated immunity (Barua et al., 2015; Bhaskaram, 2001). Thus, in addition to the well-known medicinal properties of these leaves, they can also be explored in haematinic applications.

The leaves were fairly good sources of manganese (16.00-40.67 ppm), zinc (19.60-39.33 ppm) and copper (25.96-36.27 ppm). The zinc, copper and iron values obtained in this study are in agreement with the literature (Idris et al., 2011; Kashif and Ullah, 2013; Vidhani et al., 2016). Nigerian accession of *O. gratissimum* was lower in nitrogen phosphorus, sodium, magnesium and iron, but higher in manganese and copper compared with the Indian accessions. Nitrogen is important for amino acid and protein production and plays a pivotal role in many critical functions such as photosynthesis in plant. Thus, the lower amount of nitrogen in the *O. gratissimum* (Nigerian accession) may explain why it protein content was higher than the Indian accessions (Table 2).

3.4. Phytochemical composition

The three *Ocimum* leaves showed significant ($p<0.05$) variations in their phytochemical composition, except in their total phenolic and cardiac glycoside contents, which were very similar (Fig. 1). In general, *O. gratissimum* (Nigerian and Indian accessions) displayed significantly ($p<0.05$) higher alkaloids, tannin, flavonoids and saponin contents compared with *O. sanctum* (Indian accession). However, Nigerian accession of *O. gratissimum* was higher in tannins, flavonoids and saponin

contents than the other two species. Pachkore and Dhale (2012), working with three *Ocimum* species reported lower tannin values for *Ocimum gratissimum* (0.12 mg/100 g) and *Ocimum sanctum* (0.42 mg/100 g), when compared to values in this study. However, other authors reported substantially higher tannins (3.29-4.60 mg/100 g) and saponins (4.50-6.86 mg/100 g) for *Ocimum gratissimum* cultivated by seed and stem cutting (Okunlola et al., 2017). Variation in the phytochemical constituents may be associated with inherent genetic differences among the species studied in the respective studies as well as the environmental condition of the various growth locations (Okunlola et al., 2017). The phenolic and flavonoid contents recorded in this study were almost 3-6 times higher than values reported for *Ocimum* leaves in earlier studies (Okunlola et al., 2017; Pachkore and Dhale, 2012).

The relatively higher phenols and flavonoids of the leaves suggest that these species will have high antioxidant activities. Phenols and flavonoids are important groups of secondary metabolites, synthesized by plants and have been previously associated with antioxidative activities (Akinmoladun et al., 2007; Bhattacharya et al., 2014; Joshi et al., 2011; Siti et al., 2018).

3.5. Principal component analysis

Morphometric data and phytochemical constituents of the three accessions were analysed using principal component analysis (PCA). PCA revealed that Indian accessions of *O. sanctum* and *O. gratissimum* are distinctly separated and different from Nigerian accession of *O. gratissimum* in morphometric data and phytochemical constituents, despite growing the three plant under the same greenhouse conditions (Figure not shown). The *O. sanctum* and *O. gratissimum* are also different from each other based on these parameters. The first two principal components (PC) accounted for approximately 92% of the total variability of the data (Fig. 2). PC1 accounted for approximately 59% of the total variation and was predominantly a function of total phenolic content, tannin, cardiac glycoside, flavonoids, saponins, plant height, leaf length, leaf width and leaf area. The PC2, which only accounted for 32% of the total variation was made up of alkaloids, number of leaves and number of branches.

Table 2. Proximate composition of leaves of three *Ocimum* species at 90 days after planting (%)

Parameters	**Sanctum*	**Gratissimum*	***Gratissimum*
Dry matter	19.77^{b}±0.21	27.85^{a}±0.55	26.07^{a}±1.33
Moisture	80.23^{a}±0.21	78.15^{b}±0.55	73.93^{b}±1.33
Protein	0.17^{ab}±0.04	0.13^{b}±0.02	0.21^{a}±0.04
Ash	10.07^{b}±0.05	11.27^{a}±0.31	11.40^{a}±0.53
Fibre	4.72^{b}±0.10	4.88^{b}±0.24	8.47^{a}±0.40
Fat	3.18^{b}±0.07	3.3^{ab}±0.13	3.46^{a}±0.11
Carbohydrate	1.63^{b}±0.30	2.25^{a}±0.07	2.53^{a}±0.39

Mean ± S.D. Means with same superscript within the same row are not significantly ($p<0.05$) different.
*Indian accession ** Nigerian Accession

Table 3. Mineral composition of leaves of three *Ocimum* species at 90 days after planting

Minerals	**Sanctum*	**Gratissimum*	***Gratissimum*
Nitrogen (%)	5.58^{a}±0.39	4.84^{b}±0.07	4.28^{c}±0.02
Phosphorus (%)	2.05^{b}±0.03	4.83^{a}±0.02	0.38^{c}±0.02
Potassium (%)	2.04^{c}±0.04	3.51^{a}±0.02	2.61^{b}±0.03
Sodium (%)	0.92^{a}±0.06	0.08^{b}±0.02	0.02^{c}±0.01
Calcium (%)	3.58^{a}±0.03	2.92^{c}±0.07	3.13^{b}±0.06

Magnesium (%)	1.49[a]±0.03	1.40[b]±0.03	1.17[c]±0.03
Iron (ppm)	863.00[a]±1.15	285.00[b]±1.53	263.01[c]±0.58
Manganese (ppm)	16.00[c]±2.00	29.00[b]±1.00	40.67[a]±1.53
Zinc (ppm)	39.33[a]±0.58	19.60[c]±1.22	25.63[b]±0.67
Copper (ppm)	25.96[b]±0.97	26.57[b]±0.58	36.27[d]±1.18

Mean ± S.D. Means with same superscript within the same row are not significantly ($p<0.05$) different.
*Indian accession
** Nigerian Accession
***Values are expressed in %

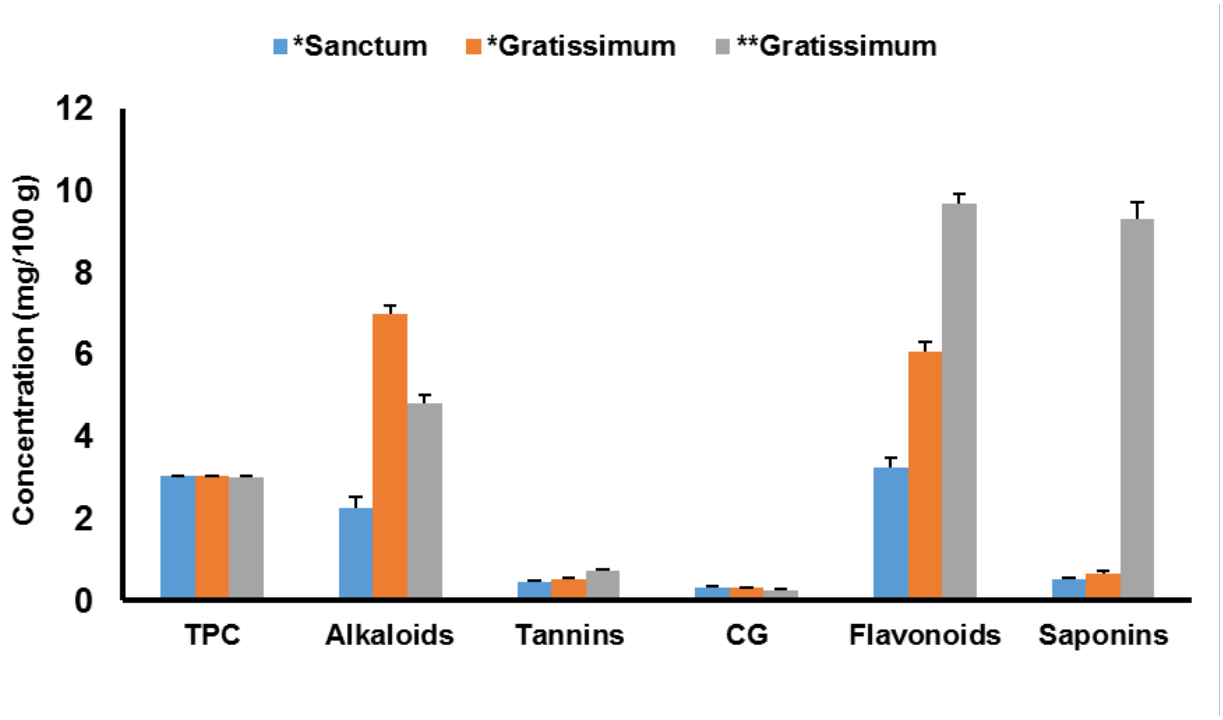


Figure 1. Phytochemical contents of three *Ocimum* species at 90 days after planting
Error bars indicate standard deviation (N= 3)
TPC: Total phenolic content
CG: Cardiac glycosides
*Indian accession
** Nigerian Accession

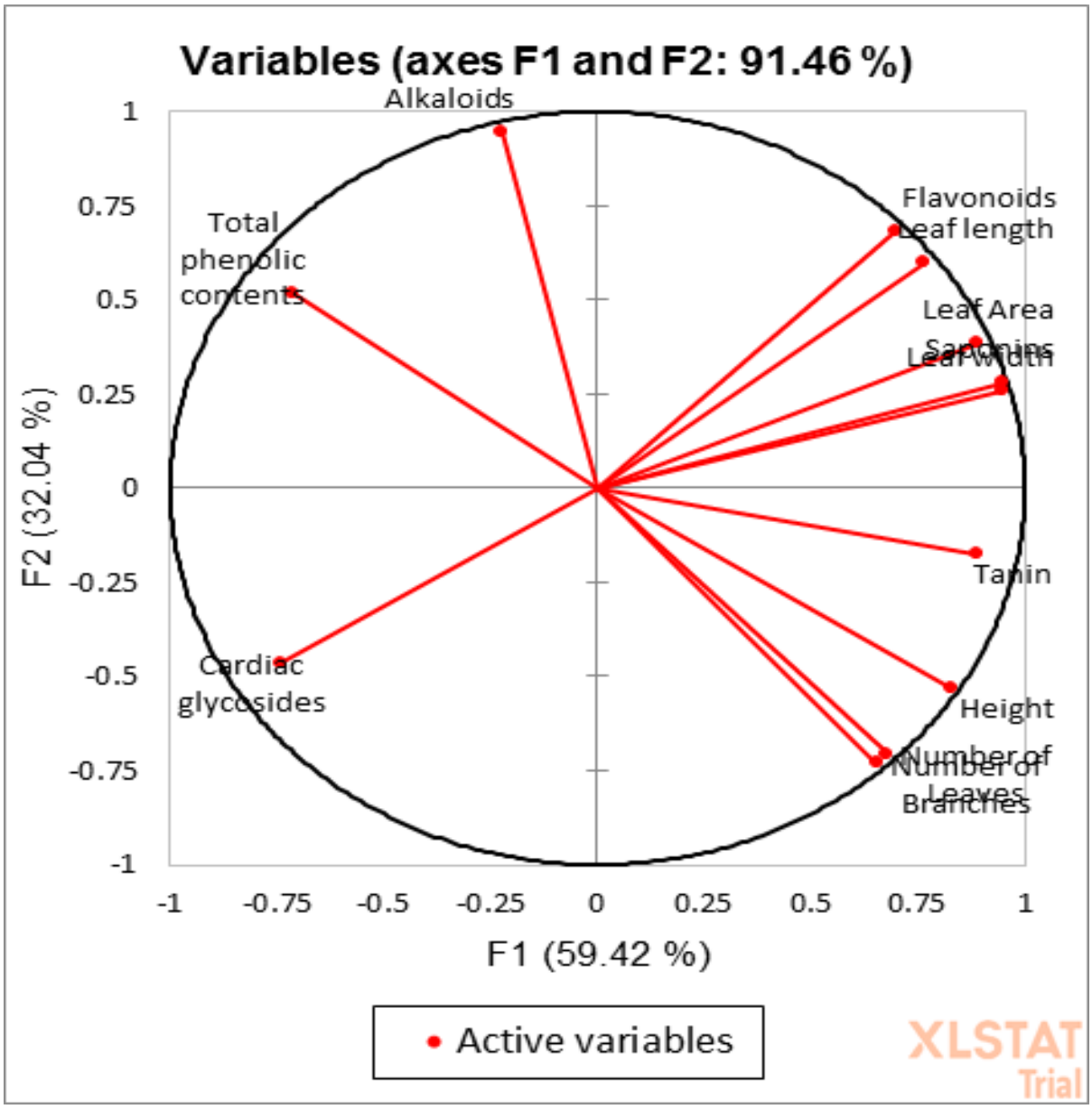


Figure 2. Principal component analysis of morphometric data and phytochemical constituents of *Ocimum* species

4. Conclusions

Nigerian accession of *O. gratissimum* had different morphometric attributes compared with the Indian accessions (*O. sanctum* and *O. gratissimum*), though they were grown under the same greenhouse conditions. All the leaves had relatively low protein content and are fairly good sources of iron, but *O. sanctum* showed superior iron content than the other species. Nigerian accession had superior phytochemical contents than the Indian accessions and may be further explored for breeding purposes through plant tissue culture, to complement the Indian accessions for enhanced applications in the pharmaceutical industry in India.

5. References

Akinmoladun, A. C., Ibukun, E., Afor, E., Obuotor, E., and Farombi, E. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Scientific Research and Essays*, 2, 163-166.

Amonsou, E. O., Siwela, M., and Dlamini, N. (2014). Chemical composition and microstructure of Bauhinia grains. *Journal of Food Science and Technology*, 51, 2263-2269.

AOAC. (2000). Offcial methods of analysis. 17th edition. In: Asscoaition of official Analytical Chemists, Rockville.

Barua, C. C., Bora, M., Saikia, B. N., Hazarika, M., and Barua, I. (2015). Quantitative analysis of proximate and mineral composition of a few important medicinal plants of north east region. *International Journal of Applied Biology and Pharmaceutical Technology*, 6, 188-193.

Bhaskaram, P. (2001). Immunobiology of mild micronutrient deficiencies. *British Journal of Nutrition*, 85, S75-S80.

Bhattacharya, A., Aggarwal, A., Sharma, N., and Cheema, J. (2014). Evaluation of some anti-oxidative constituents of three species of Ocimum. *International Journal of Life Sciences*, 8, 14-17.

Dambolena, J. S., Zunino, M. P., López, A. G., Rubinstein, H. R., Zygadlo, J. A., Mwangi, J. W., . . . Kariuki, S. T. (2010). Essential oils composition of Ocimum basilicum L. and Ocimum gratissimum L. from Kenya and their inhibitory effects on growth and fumonisin production by Fusarium verticillioides. Innovative Food Science and Emerging *Technologies*, 11, 410-414.

Demigne, C., Sabboh, H., Re´me´, S., and Meneton, P. (2004). Protective effects of high dietary potassium in Nutritional and metabolic aspects. *Journal of Nutrition*, 134, 2903–2906.

Ehiagbonare, J. (2007). Macropropagation of Ocimum gratissimum L: A multi purpose medicinal plant in Nigeria. *African Journal of Biotechnology*, 6, 13-14.

Emeka, N. G., and Chimaobi, A. (2012). Chemical composition and variability among some Ocimum gratissinum accessions. *International Journal of Medical and Aromic Plants*, 2, 460-467.

Gholap, S., and Kar, A. (2004). Hypoglycaemic effects of some plant extracts are possibly mediated through inhibition in corticosteroid concentration. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, 59, 876-878.

Gupta, P., Yadav, D. K., Siripurapu, K. B., Palit, G., and Maurya, R. (2007). Constituents of Ocimum sanctum with antistress activity. *Journal of natural products*, 70, 1410-1416.

Idris, S., Iyaka, Y., Ndamitso, M., and Paiko, Y. (2011). Nutritional Composition of the Leaves and Stems of Ocimum gratissimum. *Journal of Emerging Trends in Engineering and Applied Sciences*, 2, 801-805.

Joshi, S., Bothara, S., and Surana, S. (2011). Evaluation of aqueous extract of Ocimum sanctum in experimentally induced parkinsonism. *Journal of Chemical and Pharmaceutical Research*, 3, 478-487.

Kashif, M., and Ullah, S. (2013). Chemical composition and minerals analysis of Hippophae rhamnoides, Azadirachta indica, Punica granatu and Ocimum sanctum leaves. *World Journal of Dairy and Food Science*, 8, 67-73.

Khandelwal, K. (2007). Practical pharmacognosy techniques and experiments (17th ed.). Nirali Prakashan, India.

Kirk, S., and Sawyer, R. (1991). Pearson's composition and analysis of foods, England Longman scientific and technical: Longman Group Ltd.

Lewu, M., Adebola, P., and Afolayan, A. (2010). Effect of cooking on the mineral contents and anti-nutritional factors in seven accessions of Colocasia esculenta (L.) Schott growing in South Africa. *Journal of Food Composition and Analysis*, 23, 389-393.

Mequanint, W., Makonnen, E., and Urga, K. (2011). In vivo anti-inflammatory activities of leaf extracts of Ocimum lamiifolium in

mice model. *Journal of Ethnopharmacology*, 134, 32-36.

Mlitan, A., Sasi, M., and Alkherraz, A. (2014). Proximate and minor mineral content in some selected basil leaves of Ocimum gratissimum L, in Libya. *International Journal of Chemical Engineering and Applications*, 5, 502.

Nakamura, C. V., Ueda-Nakamura, T., Bando, E., Melo, A. F. N., Cortez, D. A. G., and Dias Filho, B. P. (1999). Antibacterial activity of Ocimum gratissimum L. essential oil. *Memórias do Instituto Oswaldo Cruz*, 94, 675-678.

Njoku, P., and Ohia, C. (2007). Spectrophometric estimation studies of mineral nutrient in three cocoyam cultivars. *Pakistan Journal of Nutrition*, 6, 616-619.

Oboh, F. O., Masodje, H. I., and Enabulele, S. A. (2009). Nutritional and antimicrobial properties of Ocimum gratissimum leaves. *Journal of Biological Sciences*, 9, 377-380.

Okunlola, A., Ogungbite, O., and Hassan, G. (2017). Growth and nutritional qualities of three ocimum species as affected by methods of propagation. *East African Agricultural and Forestry Journal*, 82, 36-46.

Pachkore, G., and Dhale, D. (2012). Phytochemicals, vitamins and minerals content of three Ocimum species. Internation *Journal of Science Innovations and Discoveries*, 2, 201-207.

Paton, A. (1992). A synopsis of Ocimum L. (Labiatae) in Africa. Kew *Bulletin*, 47, 403-435.

Prakash, P., and Gupta, N. (2005). Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian Journal of Physiology and Pharmacology*, 49, 125.

Prashar, R., Kumar, A., Hewer, A., Cole, K. J., Davis, W., and Phillips, D. H. (1998). Inhibition by an extract of Ocimum sanctum of DNA-binding activity of 7, 12-dimethylbenz [a] anthracene in rat hepatocytes in vitro. *Cancer letters*, 128, 155-160.

Saha, S., Dey, T., and Ghosh, P. (2010). Micropropagation of Ocimum kilimandscharicum Guerke (labiatae). Acta Biologica Cracoviensia *Series Botanica*, 52, 50-58.

Saha, S., Kader, A., Sengupta, C., and Ghosh, P. (2012). In vitro propagation of Ocimum gratissimum L. (Lamiaceae) and its evaluation of genetic fidelity using RAPD marker. *American Journal of Plant Sciences*, 3, 64-74.

Shuaib, O., Adeniran, O., Musah, M., Yerima, H., Sani, H., and Amusat, K. (2015). Comparative nutritional and anti-nutritional analysis of Ocimum grattissimum and Ocimum basilicum. *Academia Arena*, 7, 77-81.

Singh, D., and Chaudhuri, P. K. (2018). A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.). *Industrial Crops and Products*, 118, 367-382.

Singh, S., and Agrawal, S. S. (1991). Anti-asthmatic and anti-inflammatory activity of Ocimum sanctum. *International Journal of Pharmacognosy*, 29, 306-310.

Singh, S., Majumdar, D., and Rehan, H. (1996). Evaluation of anti-inflammatory potential of fixed oil of Ocimum sanctum (Holybasil) and its possible mechanism of action. *Journal of Ethnopharmacology*, 54, 19-26.

Siti, M. Y., Rabeta, M., and Antora, R. (2018). Effects of different drying methods on the proximate composition and antioxidant activities of Ocimum basilicum leaves. *Food Research*, 2, 421-428.

Sofowora, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa (2nd ed.). Spectrum Books Ltd, Ibadan, Nigeria.

Vidhani, S. I., Vyas, V. G., Parmar, H. J., Bhalani, V. M., Hassan, M. M., Gaber, A., and Golakiya, B. A. (2016). Evaluation of some chemical composition, minerals fatty acid profiles, antioxidant and antimicrobial activities of Tulsi (Ocimum sanctum) from India. *American Journal of Food Science and Technology*, 4, 52-57.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

DENSITY, STEADY AND DYNAMIC STATE SHEAR RHEOLOGICAL PROPERTIES OF GONGURA (HIBISCUS SABDARIFFA) LEAVE PUREE AS A FUNCTION OF TEMPERATURE & TSS

J. Meher[1]*, A. Keshav[1], B.Mazumdar[1]

[1]*Food Research and Intensification Lab, Department of Agricultural Engineering, Kalasalingam University*
**jaga@klu.ac.in*

https://doi.org/10.34302/2019.11.4.7

Article history:
Received:
17 February 2019
Accepted:
10 October 2019

Keywords:
Gongura Leaves;
Density;
Puree;
Rheology;
TSS.

ABSTRACT

Rheological properties must be assessed for the processing and handling of different pastes and purees. Cox Merz rule was applied and variation in dynamic and steady-state properties with temperature (283-358K) was evaluated for Gongura leaves puree. Mathematical models were applied in relation to experimentally obtain TSS and density and it was concluded that the linear correlation was best suited. With an increment in TSS and decrement in temperature, there is an increment in the density of the puree. HB model was found to be best fitted and described the flow behavior of the puree, within the temperature range ($R^2 > 0.98$). In the frequency range (1-50 Hz), the product shows weak gel behavior. Modified Cox Merz rule can be useful where it was revealed that the steady-state viscosity is identical to complex viscosity raised to power α.

1.Introduction

Gongura leaf (GL) is a species of hibiscus, utilized as a part of many dishes in Sri Lanka, India, and neighboring nations. Besides, in the vast majority of the nations, it is called 'Roselle'. The young leaves are eaten raw in salads and crushed or chopped with the tender stem as chutney. It is added to curries as flavor enhancer after fried along with the chopped onion in the first stage of preparation and in some Malaysian dishes as a seasoning. Medically it is used for the treatment of different chronic diseases in Ayurvedic medicines (Puro et al., 2014). It is a natural remedy for cancer, blood pressure, and anemia as it is a very good source of folic acid and iron and improves the digestive system, as it is carminative in nature. Amongst the different chutney available in south India, the most widely consumed chutney in breakfast is the GL based chutney, which is prepared by blending the leaves using blender processor until the preferred consistency reached.

Fresh GL is perishable in nature and has a short time frame of shelf life, and the quality of the leaves deteriorates due to microbial and physiological behavior during the period of storage and transportation. In addition, the dried form of leaves has a poor (inferior) aroma and hence widely not suited to use in a different food product. Hence, there is a need for minimally processing of the fresh leaves into puree to preserve and maintain the quality of food with freshness. These food items are in the form of puree when processed, handled, stored and sold. These are easily consumable and remain fresh until consumed by the customer. As people are lean towards the ready to use a form of products, so it can also save time and money. A pureed form of chutney can be used for topping, seasoning and has recipes for the fast-food industry(Espinosa,2011). A puree is a suspension of soft particles in viscous gel or

serum and is obtained from fruit/vegetable by processes like heat treatment and tissue restructuration (Colin Henrion et al 2007).

Physical properties of foods like viscoelasticity and density are the key parameters utilized as a part of the designing, evaluation, and optimization of processing parameters. In addition, the physical properties are quality indices of food (Alvarado and Romero, 1989., Pourmohammadi et al., 2017). This information is used for the emergent of the latest food items without having the most wanted qualities or for quality enhancement of previously present products. Also, for defining the effectiveness of food systems if designing information are accessible. The density of GL puree is extremely influenced by both TSS (total soluble solid) & temperature. Therefore, it becomes necessary to obtain the physical property values of GL puree as a function of temperature and TSS (Hajieghrary & Homayouni-Rad (2016)). Previously different authors have worked on the consequence of temperature and soluble solid concentration on its density of different fruits (Constenla et al., 1989) and developed some Empirical equations that linked the density of TSS & temperature. A wide range of temperature was chosen as our puree experienced different processing conditions with a change in viscosity. Along with varying temperature and concentration, the shear rate of the puree also has a greater impact on its processing operation like pipe flow, pumping, stirring and mixing. The viscosities of the puree were measured at temperatures between 283 to 358K. Since this is the range of temperature that the fluid products experienced during the evaporation study (Nindo, 2004). In low acid foods (pH<4.5), the main purpose is the destruction of pathogenic bacteria, while below pH 4.5 the destruction of spoilage microorganisms or enzyme deactivation is usually more important. Also, Past research work by the researcher on the impact of pH on the viscosity of sweet potato puree and quince puree demonstrated that the consistency of puree increase with pH value (Ice et al., 1980). However, very few works have been done on the processed form of vegetables with varying temperatures, TSS and pHs.

Dynamic rheological properties of food can be used for ensuring the total cyclic chain of food processing that started from harvesting to storage (Campo-Deaño and Tovar 2009). The small-amplitude oscillatory shear run is a kind of rheological test in which strain and stress value change with time in the LVR (linear viscoelastic region). It is more imperative to know the viscoelastic properties of purees, which is essential for the food processor for altering planning parameters, checking consistency and the mouthfeel characteristics of prepared food. Hence, dynamic rheology is usually applied to estimate the viscoelastic characteristics of paste/puree. Broad temperature ranges are experienced all through the preparing and storage of pureed foods, and hence, the temperature influence on flow properties must be recorded (Rao 1999). Different worked has done on different viscoelastic food materials such as vegetable-based baby foods(Ahmed and Ramaswamy 2006a), sweet potato-based infant food puree (Ahmed and Ramaswamy, 2006b) and strained pureed baby foods (Ahmed and Ramaswamy 2007a) in the range of temperatures (278-353K). In view of differentiation in puree composition and distinguishing situations inside the puree, the rheological behavior is very particular in association with one TSS to another. Because of the complicated nature of purees and their change subsidiaries, it is inaccessible for the food preparation industry throughout the food formulation. Henceforth, knowing the rheological characteristics of GL is necessary for assessing its proposed utilization and use as food stabilizers or thickeners. An analysis of the viscoelastic properties of vegetable puree in the LVE region keeps a check on the process. It also helps in maintaining the consistency and stability of liquid and formulated food.

The present work emphasizes on determining a) the density of GL puree and to model the effect of temperature (283-358K)

and TSSs (4.6^0Brix) b) the dynamic rheological properties of GL puree in the LVE range by both amplitude sweep and frequency sweep tests and its dependency on TSS (4.6^0Brix) and temperature.

2. Materials and methods

The fresh GL was procured from a Kota market, Chhattisgarh, India. Vegetables were cleaned with distilled water. An adequate amount of distilled water was transferred into a container and heated to the desired temperature (363K) for the blanching of vegetable leaves. A desired amount of leaves was sunk in an abundance of water & then go through blanching, in the separate blanching media for 5 min. Peroxidase inactivity test was performed to know the exact duration and viability of the sample. Immediately after blanching, the leaves are quenched in the cold water to stop the degradation of essential components present in it. Then, the blanched vegetable was ground in a wet grinder (Remi Lab Equipment, India) for a specified amount of time until the puree is formed. The pureed material was then passed through the sieves of 14 m size, to obtain a puree of uniform consistency. Samples used were obtained from GL, with a TSS of 5.6^0Brix.

2.1. Physiochemical analysis

Chlorophyll content was analyzed using the method described by Arnon (1949). The pH and TSS (^{0}Brix) of the samples were determined using a handy pH meter and Otago refractometer (Tokyo, Japan) at 303K, respectively. Samples of 1.6, 2.6, 3.6 & 4.6^0 Brix were obtained by diluting the concentrated form of puree by using distilled water.

The density of the puree was evaluated by pycnometers of 25 ml measure from 283 to 358K, at temperatures increment of 15K. All the pycnometer has been before calibrated with pure water, to check potential contrasts in their volume, by a heating method. The instant measurement was then carried of Pycnometers by an analytical balance (0.0001 g precision). Each experiment was repeated thrice at 283, 298, 313, 328, 343 and 358K with TSS of 1.6, 2.6, 3.6, 4.6 and 5.6 ^{0}Brix. Four distinct models, for example, linear, exponential, exponential quadratic & quadratic are attempted to fits the test data utilizing MS office 2007. The fitting accurateness of computed parameters was estimated through the interpretation of R^2 and plot of prediction error with confidence intervals ($p = 0.05$).

2.2. Rheology procedures

The rheological analysis of GL puree was performed by means of a modular compact four-bladed vane (ST22-4V-40) rheometer (MCR 102 (Anton Paar, GmbH, Germany) The vane was dipped into the cylinder and was kept at 10mm above from the bottom of the cylinder (Steffe, 1996).

At the required temperature, the concentrated puree (35ml) was poured into the sample cup (40ml). The sample puree was carefully transferred into the cup so that no air bubble was present. The surplus puree was removing with a spatula. The temperature of the cup & samples were maintained to equilibrate with the experimental temperatures (283, 298, 313, 328, 343 & 358K) before measuring the rheological parameters. Fresh samples were taken with appropriate care for all testing to stay away from the consequences of the aging & high shear rate during sample loading.

2.2.1. Steady-state measurements

The steady-state shear experiments were conducted in the range between .01-100/s (Steffe, 1996). In order to avoid thixotropy(data not shown), the samples were sheared for 5 minutes at 300/s thixotropy. Herschel–Buckley's (HB) model (Eq. 1) was employed to calculate the flow behavior of the purees. HB model embodies power law, Newton &, Bingham models and used for the explanation of the rheological properties of the food products.

$$\sigma = \sigma_0 + K * \gamma^n \quad (1)$$

2.2.2. Dynamic oscillatory measurements

The dynamic viscoelastic components of blanched puree (TSS=3.6^{0}Brix) were measured such as loss tangent (tan d), complex viscosity (h*), G' (storage modulus), & G" (loss modulus), at a temperature range of 283-333K. During each experimentation run, fresh samples were utilized to keep away from the consequence of maturing & high shear rate throughout sample loading, Both, frequency, & strain sweep test was performed as previously mentioned temperatures. Strain sweep tests were conducted at a steady frequency of 1 Hz to decide the limit of the linear viscoelastic regime of the sample (LVR). Frequency sweep tests were done at a steady strain of 0.02% in the range (0.01 to 10 Hz) in the field of LVR at temperatures(283-358K) to defined the mechanical spectra of G' & G" (Pa) values. Frequency sweep tests at a steady strain in the LVE region were completed to decide the viscoelastic nature of GL puree. The G' can be utilized as a measure of the elastic part of the sample & correspondingly, the G" describes the viscous portion of the specimen. The information of rheological evaluations was analyzed utilizing the RheoPlus programming of Anton Paar GmbH for calculation of dynamic rheological properties & examine the rheological results. Each oscillatory measurement was completed in triplicate. The viscoelastic behavior (G' & G") of food & dispersions were modeled as a power function of oscillatory frequency (Eqs. 2-3) (Rao, 1999).

$$G' = G_0'\omega^{n'} \quad (2)$$

$$G'' = G_0''\omega^{n''} \quad (3)$$

Where G_0' (Pa), G_0'' (Pa), ω and n'& n" (dimensionless) are the storage modulus, viscous modulus, oscillation frequency, and exponents which denote the influence degree of ω on both modulus respectively.

2.2.3. Applicability of the Cox-Merz rule

According to Cox-Merz rule, at a specific shear rate (γ), when $\gamma = \omega$, then the complex viscosity at a particular ω is equal to η_a at a specific shear rate (Rao, 2005). The rheological properties of food can be ascertained by steady-state or oscillatory shear experiments; subjected to the validation of Cox-Merz rule (Gunasekaran and Ak, 2000). Both the experiments are adjuvant because of having certain limitations in each kind.

Appraisal of the Cox-Merz rule was assessed by the outcomes got from steady-state shear and viscoelastic investigation.

$$\eta_a(\gamma) = \eta*(\omega)|_{\gamma=\omega} \quad (4)$$

3.Results and discussions

3.1. Effects of temperature and TSS on its density of GL puree

The experimentally obtained results comprising the impact of temperature and TSS on the density of the GL puree were presented in Table 1. It is very much clear from the data that the density of the GL puree increased with increment in TSS and decrement in temperature. Regardless of the temperature scale, the density was observed to be well influenced by the TSS of the puree. For an example case point, at 283K, density increased from 1.102g/cm^3 at 1.6 ^{0}Brix to 1.379 g/cm^3 at 5.6 ^{0}Brix, which involves an increase of 31.1% in case of GL puree. Similarly, there are increases in density nearly 25-30% are observed in other temperature range.

At a fixed TSS of 5.6 ^{0}Brix, the density decreased by 1.376%, with an increment of temperature from 283K to 358K. Similar types of results are obtained in case of clear grape juice (Zuritz et al., 2005), whereby it was reported that, with increment in TSS from 22.9 to 70.6 ^{0}Brix at a fixed 353K, density increment by 24.11%. Density is extremely vital in determining the material handling, packaging requirement and during wet processing of the food sector (Karuna et al., 1996). Various models were used to fit the data with the experimental value of density with temp and concentration of GL puree(Aguado and Ibarz, 1988). These models are quadratic,

linear, quadratic exponential and exponential as revealed in the Eqs. (5–8):

$$\rho = a + bX + cX^2 \quad (5)$$

$$\rho = a + bX \quad (6)$$

$$\ln \rho = a + bX + cX^2 \quad (7)$$

$$\ln \rho = a + bX \quad (8)$$

Where a, b, and c are constants; ρ is density in g/cm^3; and X is the variable to model, temperature (K) or TSS (^{0}Brix) respectively.

For a fixed TSS, density decreases with an increase in temperature (Table 1). Various models (Eqs. 5–8) are used to fit the experimental data. Regression coefficient values range from 0.98 to 0.99 from the regression analysis. Increasing TSS causes an increase in the values of parameter a & b. However, in the case of quadratic and exponential quadratic models, c values were dismissed as it was discovered unimportant (10^{-6} requests). In this way, it may be recommended that a linear model, on account of its appropriate fitting and consistency, may help to find density as a factor of temperature. Concerning the effect of TSS, density change under set temperature increased with the increment of TSS under a relative temperature effect (Table. 2). So, a straight model can be proposed for the GL puree density as a part of TSS. As per previous findings at 25^{0}C, the density variation of cleared and depectinised juice of Malus floribunda with concentration is observed to be linear (Cepeda and Villarán, 1999).

Table 1. Experimental values for density (in g/cm3) at different concentrations and temperature for Gongura leaves puree.

Temperature (K)	**Density (g/cm^3)**				
	1.6 ^{0}Brix	**2.6^{0}Brix**	**3.6^{0}Brix**	**4.6^{0}Brix**	**5.6^{0}Brix**
283	1.102	1.1724	1.23984	1.30813	1.37933
298	1.098	1.164858	1.233416	1.301152	1.372576
313	1.09375	1.16215	1.23129	1.29988	1.37108
328	1.08975	1.156608	1.225166	1.292902	1.363726
343	1.0855	1.1539	1.22304	1.29163	1.36213
358	1.0815	1.148358	1.216916	1.284652	1.355376

Table 2. Fitting parameters for equations to predict the variation of density of Gongura leaves puree with temperature and TSS.

Models	**^{0}Brix**	**a**	**-b*10^{-4}**	**C*10^{-6}**	**R^2**
$\rho = a + bX$	1.6	1.1062	5	-	0.9999
	2.6	1.1756	5	-	0.9828
	3.6	1.2435	4	-	0.9825
	4.6	1.3117	4	-	0.9677
	5.6	1.3832	3	-	0.9735
$\rho = a + bX + cX^2$	1.6	1.1062	5	0.000000000003	0.9999
	2.6	1.1772	6	2	0.9861
	3.6	1.2437	5	0.3	0.9826
	4.6	1.3117	4	0.000000000003	0.9677
	5.6	1.3831	4	-0.07	0.9735
$\ln \rho = a + bX$	1.6	1.1062	4	-	0.9999
	2.6	1.1755	4	-	0.9833

	3.6	1.2435	4	-	0.9826
	4.6	1.3118	3	-	0.9677
	5.6	1.3833	3	-	0.9734
$\ln \rho = a + bX + cX^2$	1.6	0.1009	5	-0.07	0.9999
	2.6	0.1632	4	1	0.9862
	3.6	0.2181	-0.0004	0.0000001	0.9826
	4.6	0.2713	-0.0003	-0.00000007	0.9677
	5.6	0.3244	-0.0003	-0.00000007	0.9735

3.2. Rheological studies on purees

3.2.1. Steady-state shear properties

Figure 1, demonstrates the flow curves of shear rate and stress of GL puree at 283-358K. Obviously, the GL puree demonstrated shear-diminishing conduct (n < 1) with a yield value. The yield stress characterized as slightest shear stress important to start product flow, linked with the breaking of the material's inner structure (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Elastic deformation happens underneath the yield stress making it act like an elastic solid; however material flows over the yield stress making it act like a viscous liquid(Bayod et al., 2007). If there should arise an occurrence of multiphase material like vegetable puree/paste, which is formed by a dispersion of insoluble parts (materials of cell wall) in a water solution (serum, containing sugars, minerals, proteins, and solvent polysaccharides), is having a yield stress (Sun and Gunasekaran, 2009). Table 3, describes the estimations of the HB model parameters for GL puree in the assessed temperature range. A higher estimation of R^2 (more than 0.98) was obtained for all cases. A nearby value for yield stress, flow behavior index (n), and consistency coefficient (k) were acquired tentatively when contrasted with values from the literature for vegetable items (Table 4; 283-358K).

High consistency is a typical characteristic of GL as resembled by yield stress (59.245 Pa) and consistency index (35.94746 Pa sn) value at 283K. With the increase in temperature, there is an increase in flow behavior index, while there is a decrease in yield stress and consistency index. Although the shear thinning behavior reduces with temperature, it is still present at 358K (n = 0.45893).In order to model the temperature effect on the rheology of food one must mull over how apparent viscosity changes with temperature. In order to evaluate non-Newtonian fluid, each of the parameters present in Hershey Buckley needs to be modeled individually. Therefore, each of these parameters was modeled as a function of temperature. Arrhenius Equation (Eq. (9) expresses the variation of consistency index (K) with temperature.

$$\ln K = \left(\frac{E_a}{R} \right) * T^{-1} + B \qquad (9)$$

Here, Ea, R, T, and B represent the activation energy ($kJ.mol^{-1}$), constant of the ideal gases ($J.g^{-1}mol^{-1}K^{-1}$), absolute temperature (K) and the Arrhenius Constant (Pas^n) respectively.

In comparison to other vegetable products (Table 5), the activation energy of GL puree was found to be smaller (Ea = 5.062KJ mol^{-1}). Thus it can be concluded that in comparison to other vegetable products, the internal structure of GL puree is more affected by temperature. The lower value of the consistency index explained in terms of an increase of Brownian motion with temperature, resulting in the less developed structure at a higher temperature (Massa et al. (2010)).

$$\ln K = \left(\frac{5.062}{R} \right) * T^{-1} + 0.01 \qquad (10)$$

Rao (1999) showed that the flow behavior index does not vary with temperature but for GL, Figure 2, depicts an increase in flow behavior index with temperature and a linear model can be applied. (Eq. (11); $R^2 = 0.97$):

$$n = 0.1874 + 0.0008 * T \quad (11)$$

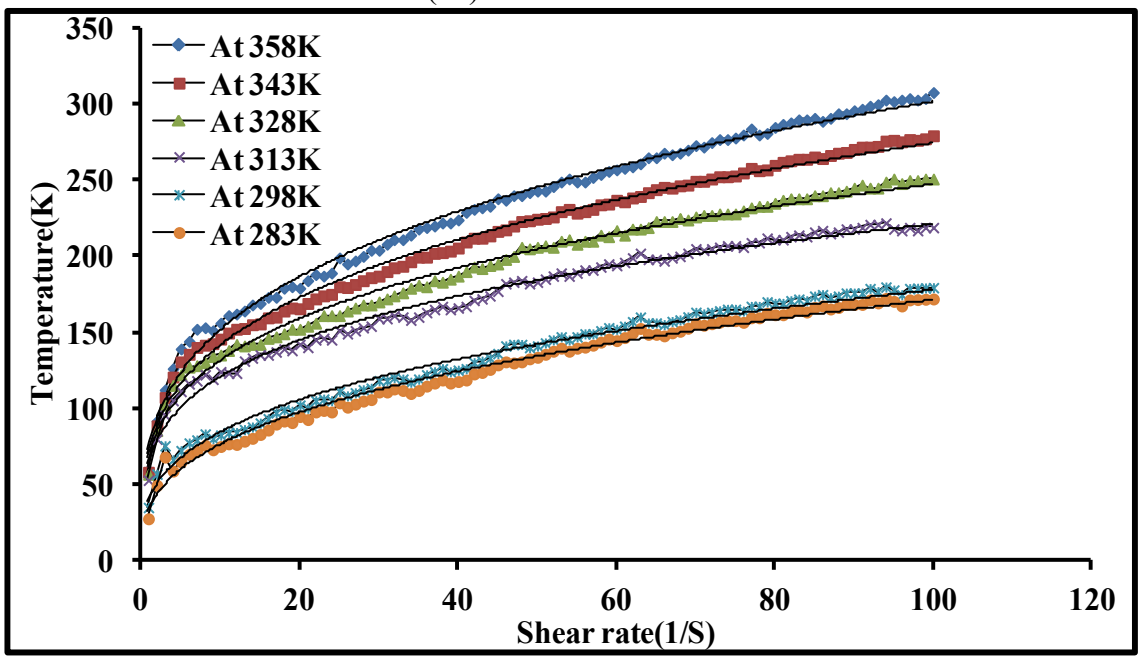


Figure 1. Flow curves of Gongura leave puree (TSS=4.6^0 Brix, T=283-358K).

Table 3. Values for the parameters of the Herschel–Buckley model for the GL puree

T (K)	σ_0 (Pa)	K (Pa s^n)	n	R^2
283	59.245	35.94746	0.39898	0.987
298	56.987	32.50153	0.41778	0.988
313	55.9874	26.32514	0.43012	0.997
328	49.2896	23.30368	0.44171	0.989
343	24.3214	19.23751	0.44785	0.995
358	16.851	17.72813	0.45893	0.979

Table 4. Values for the parameters of the Herschel–Buckley model for vegetable products.

Product	T (K)	σ_0(Pa)	k (Pa.s^n)	n	References
Sweet potato puree	278-353	0.54-1.82	0.44-1.52	0.34-0.54	Ahmed and Ramaswamy(2006)
Coriander leaf puree	303-353	10.9- 36.1	-	0.455-0.735	Rudra et al.(2008)
Mint leaf puree		26.0- 489.0	-	0.137- 0.451	
Fenugreek paste	283-303	1.050-3.675	2.351-7.478	0.706-0.834	Işıklı and Karababa(2005)
Ginger paste	298-338	63.3-159.2	29.30-269.88	0.52-0.66	Ahmed(2004)
Rocket leave puree	298-343	2.89-4.26	167-402	0.10-0.22	Ahmed et al.(2013)
Vegetable-based infant puree	278-338	2.08-14.26	13.56-76.09	0.21-0.75	Alvarez et al. (2013)
Tamarind juice concentrates	283-363	0.91-3.88	0.27-9.28	0.43-0.78	Manohar et al.(1991)

Beetroot juice concentrates	298-328	0.013-0.080	0.008-0.189	0.70-0.99	Kumar and Kumar(2015)
Gongura leaves puree	283-318	72.41-100.40	11.56-16.04	0.157-0.523	Present work

Table 5. Activation energy (Ea) of the Arrhenius model ($A = A_0 \exp(Ea/RT)$) for the consistency Index (K) in Vegetable products.

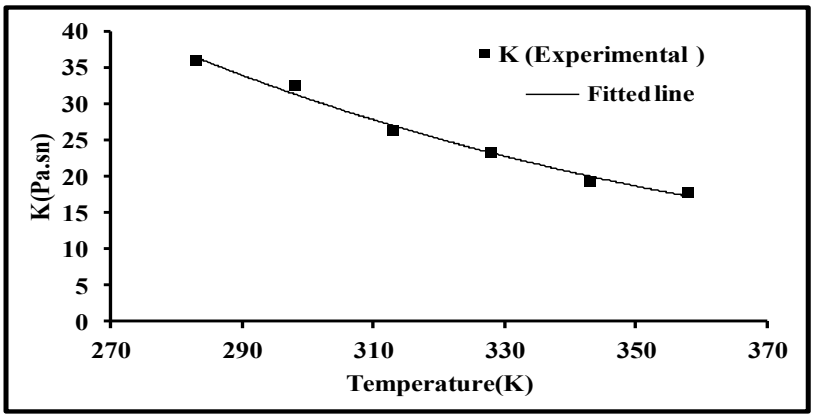
Product	Ea (k) (kJ mol^{-1})	References
Tomato paste	8.600–13.000	Dak et al. (2008)
Curry leaves puree	16.18-30.14	Meher et al. (2017)
Jabuticaba pulp	13.00	Sato and Cunha (2007)
Tomato juice	7.353.3	Augusto et al.
Gongura leaves puree	5.062644	Present work

In the literature, no modeling of yield stress has been carried out as its behavior is not definite. In the case of peach puree(21^{0}Brix), it remains constant (between 278 and 328K; Massa et al., 2010), in the case of Butia puree it shows a continuous declining behavior (at 283 and 333K; Haminiuk et al., 2006) while in case of potato puree it shows an unpredictable behavior (298 and 338K; Canet et al., 2005). Here it is important to note that the yield stress depends on the product as well as the temperature range selected.

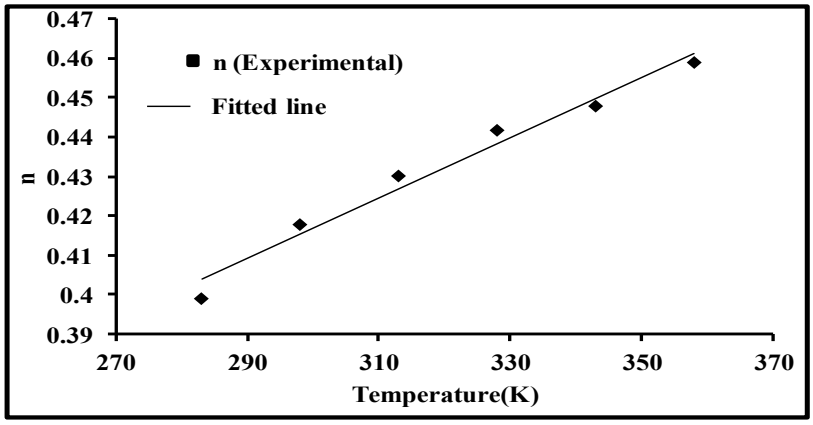
Even though the shear stress declines with increasing temperature, the Arrhenius equation cannot be utilized to model the behavior. For the tomato juice, yield stress was modeled using Arrhenius Equation (the other works listed in Table 4), however the same cannot be a) applied for the case of Gongura leaves as the behavior is different.

Figure 2 (c) shows the behavior of yield stress versus temperature as sigmoidal (Eq. (12); R^2 = 0.98). It can be seen from the graph that the yield stress remains constant (55.98–59.24 Pa) within the temperature range of 280 -313K. After 313K, yield stress declines monotonously up to the temperature of 358K. Beyond 358 K, it again attains a constant value (16.851 Pa). The viscoelastic study confirms this observation (Figure 3). After fitting the experimental data, the sigmoidal equation formulated is expressed in equation (12).

$$\sigma_0 = \frac{42.21}{1+e^{(T-33)/5.76}} + 15.97 \qquad (12)$$



b)



c)

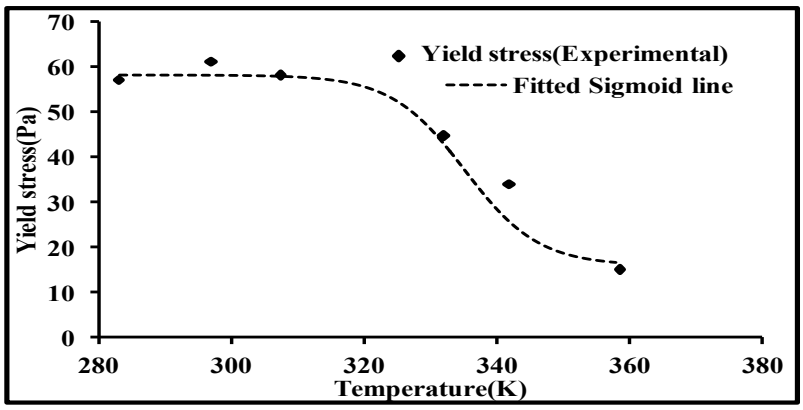


Figure 2 (a-c). Parameters of the Herschel–Bulkley model as a function of temperature.

3.2.2. Viscoelastic properties

Figure 3, shows the mechanical spectra of GL at 283 and 358K. It can be seen from the graph that the G' is higher than the values of G". Thus, it can be inferred that the elastic behavior supersedes viscous behavior. Hence, the behavior of GL puree is confirmed as a weak gel (Rao, 1999). Parallel findings have been done for other food products such as potato puree (Alvarez et al., 2004), baby foods (Ahmed and Ramaswamy, 2006), tomato concentrates (Bayod et al., 2008) and tomato juice (Augusto et al., in press-b). Apparent and complex viscosity decreases with an increase in shear rate/frequency. It can be seen from figure 5 that G' is dominant over G'' at temperatures (283K and 358K). Hence, G' and G" were modeled as a power function of oscillatory frequency as shown by Eq. (2-3). The R^2 values were found to be greater than 0.93 when the experiments were replicated (thrice).

Experimental values in agreement with literature values were obtained (Table 6). The n" values were always greater than n' (Table 6), which shows that the viscous nature of GL comparatively significant in high frequencies. At high temperatures, the consistency becomes constant, as K' and K" value converges to a constant value (Table 4, Figure 4). The estimations of n' and n" are consistent with temperature (280-360K), according to reporting (n; Rao, 1999). The mean values for n' and n" from Figure 4, are 0.127 and 0.06495 respectively. The K' and K" values exhibit a sigmoidal decay behavior with temperature, for the yield stress. As can be found in Figure 4, both K' and K" demonstrated moderately predictable values in the temperature (283-313K). It shows that the GL viscoelastic properties are a low dependent of temperature at this range, reflecting low inner structure changes. In reality, it is similar conduct

observed for the yield stress (Figure 2). Again, it is watched that the basic changes in the K' and K" values are done at the temperatures of 313 and 358K, supporting the yield stress finding. This conduct could be all around demonstrated by a power sigmoidal function (Eqs. (12) and (13); $R^2 > 0.97$). It is intriguing to watch that, even for K' and K", the parameters identified with the sigmoidal shape are a remarkable same (the proportional and power parameters in temperature). It exhibits that the reduction in the extents of G' and G" in connection with temperature follows a similar pattern:

$$k' = \frac{1051.8}{1+e\left(T-315\right)/10.4875} + 341.809 \quad (13)$$

$$k'' = \frac{219.91}{1+e\left(T-315\right)/13.02} + 74.42 \quad (14)$$

The watched conduct is not the same as those saw in different products, in spite of the fact that there are only a couple of studies that have demonstrated the estimations of K', K", n' and n" as an element of temperature. Ahmed et al. (2007) and Ahmed and Ramaswamy (2006) depicted that the temperature impact was not efficient in the assessment of K', K", n' and n" of baby food (293-353K). Augusto et al. (2011) have shown the estimations of n' and n" as a quadratic function (second-order polynomial) in association with a temperature in peach juices with fibers (273– 313K). The estimations of K' and K" were shown using the Arrhenius Equation. Emphasize that the property conduct regarding temperature is a function of the product itself and in addition to the investigated temperature range.

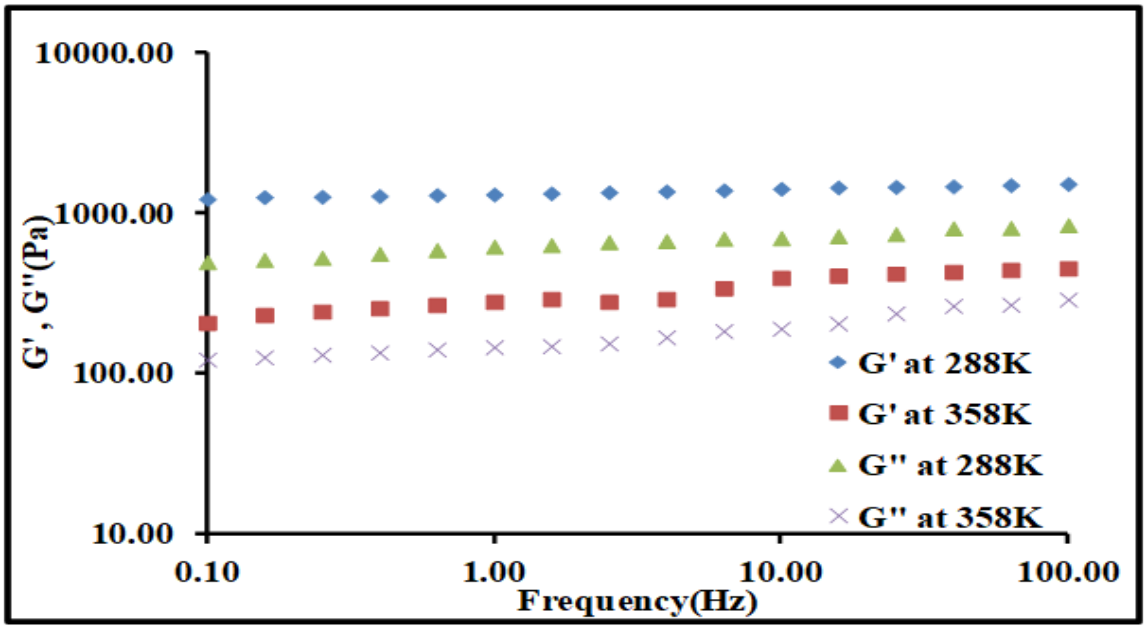
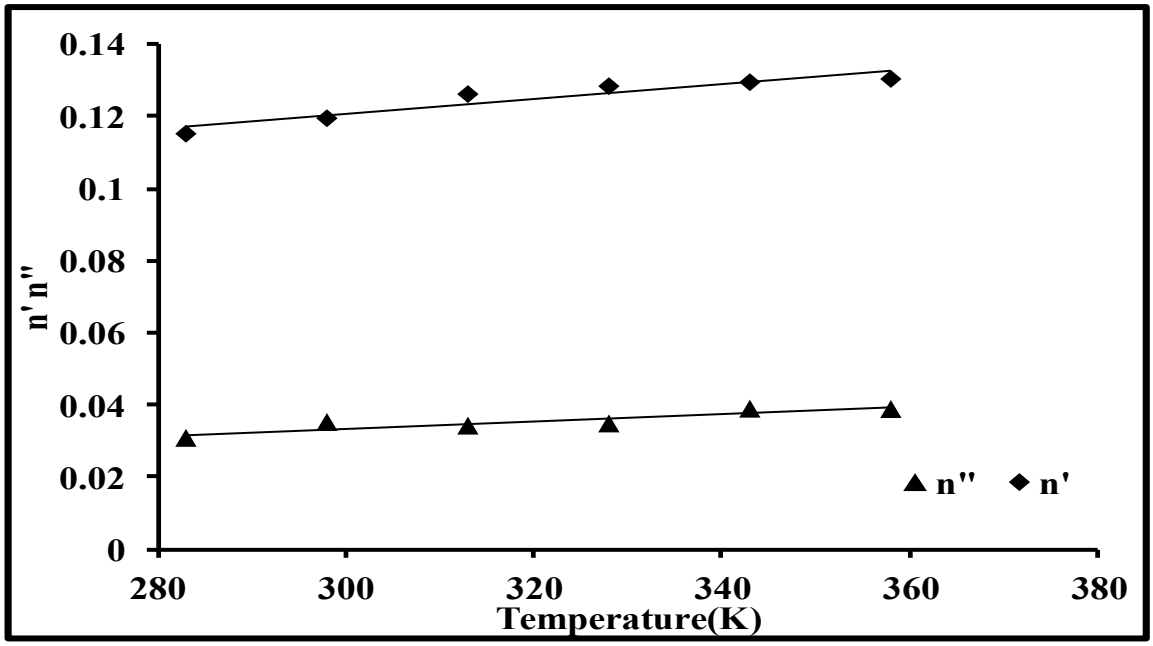


Figure 3. Mechanical spectra of Gongura leave puree at 283 and 358K.

a)



b)

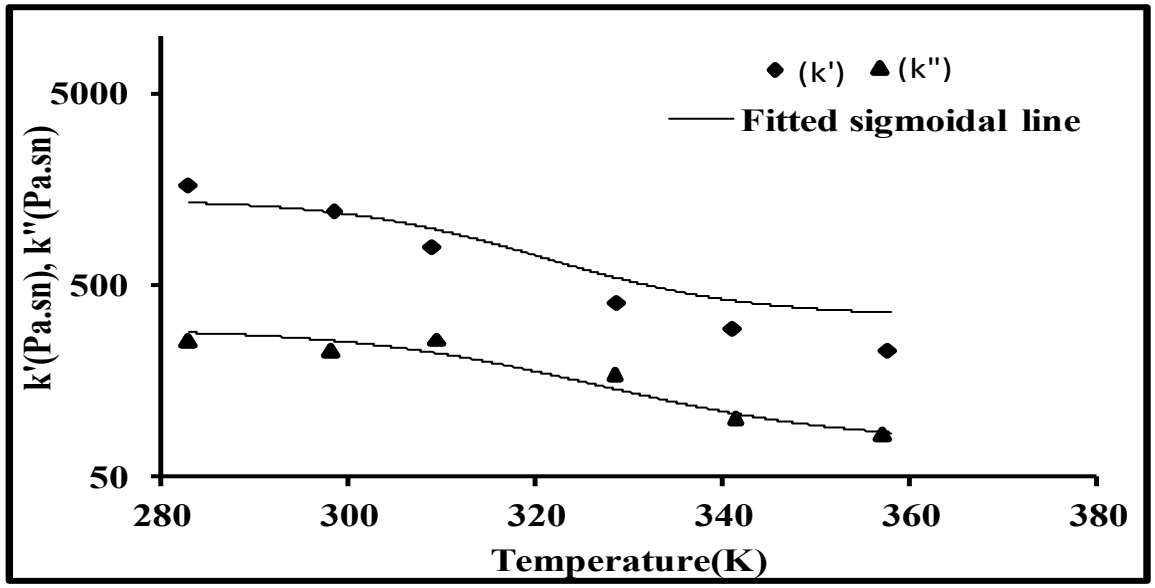


Figure 4(a-b). Temperature dependency of the parameters of the power-law model for storage (G') and loss (G") modules as a function of oscillatory frequency (ω).

Table 6. Values for the power-law model for storage (G') and loss (G") modules of GL puree as a function of oscillatory frequency (ω) (1 Pa)

Product		**T (K)**	**K'(Pa.sn')**	**n'**	**K"(Pa.sn")**	**n"**	**References**
Vegetable based baby food		293–298K	131.0-13500	0.06–0.14	19.7-1750	0.16-0.22	Ahmed & Ramaswamy(2006)
Tomato products	**Paste**	293–298K	9763.3-11607.5	0.121-0.139	1659.9-2539.0	0.2077-0.2546	Bayod et al. (2008)
	Ketchup		560.0-735.5	0.102-0.108	106.6-159.5	0.3032-0.3313	
Peach juice		273–	263.9-	0.14-	59.8-	0.24-	Augusto et al.

	313K	1567.1	0.28	616.6	0.54	(2011)
Gongura leaves puree	283-358K	357.37-1316.2	0.030-0.103	85.461-277.35	0.0662-0.1284	Present work

3.2.3. Applicability of the Cox-Merz rule

As per the Cox– Merz rule, when the shear rate is equal to frequency than the steady shear viscosity is almost equivalent to dynamic shear viscosity (Cox and Merz, 1958). Cox– Merz governs is connected to complex food. This experimental model is utilized for relating substantial deformation (the steady shear flow) and the small and linear deformation (Gunasekaran and Ak, 2000). Whether or not Cox-Merz rule can be applied in GL puree (equation (4)) was checked by comparing the plots of apparent viscosity against shear rate and complex viscosity against angular frequency. Modifications in Cox-Merz rule are required when it is to be applied in complex food systems as compared to simple cases of polymeric dispersions where the rule can be applied directly (Rao, 2005). The inapplicability of the Cox-Merz rule for complex dispersions is due to the structural decay caused by the high amount of applied strain(Ahmed and Ramaswamy, 2006), the occurrence of high-density agglomerates(Da Silva and Rao, 1992). Rheological properties of GL puree are very different from polymeric solutions and resemble the properties shown by structured systems (Ahmed and Ramaswamy, 2006). With modifications in the Cox-Merz rule, the oscillatory and steady-state rheological properties can be correlated with complex food. (Rao, 2005). A slight modification has been proposed by Bistany and Kokini (1983) in the actual rule where the original terms have been raised to some power α. Table 7 depicts that the R2 was found to be more than 0.96 for the GL puree for the modified Kox-Merz rule.

$$\beta \cdot \left[\eta_a(\gamma)\right]^{\alpha} = \eta^*(\omega)\big|_{\gamma=\omega} \qquad (15)$$

With reference to other food products (tamarind juice, potato puree, fruit-based baby foods), GL is found to be consistently similar to α & β values (Table 7). In the modified Cox-Merz rule, α value corresponds to the behavior difference whereas β value resembles the magnitude difference between the complex and the apparent viscosities, It can be inferred from Figure 5, that the α and β values are constantly decreasing with temperature. Whereas, no particular trend was found to fit α and β values as per the observations of Alvarez et al. (2004). Thus, it can be seen that both oscillatory and steady-state experiments can be used for the determination of the rheological properties of GL puree.

Table 7. Values for the parameters of the modified Cox-Merz rule for Vegetable products

Products	**T (K)**	**α**	**β**	**R^2**
Potato puree	298-338	0.90–1.35	2.15–39.83	Alvarez et al. (2004)
Tamarind juice	283-363	0.67–1.06	0.86–30.82	Ahmed et al. (2007)
Apple baby food	278-353	1.15–1.20	4.64–6.99	Ahmed et al. (2007)
Gongura leaves puree	283-358	1.05-1.12	2.59-9.84	Present work

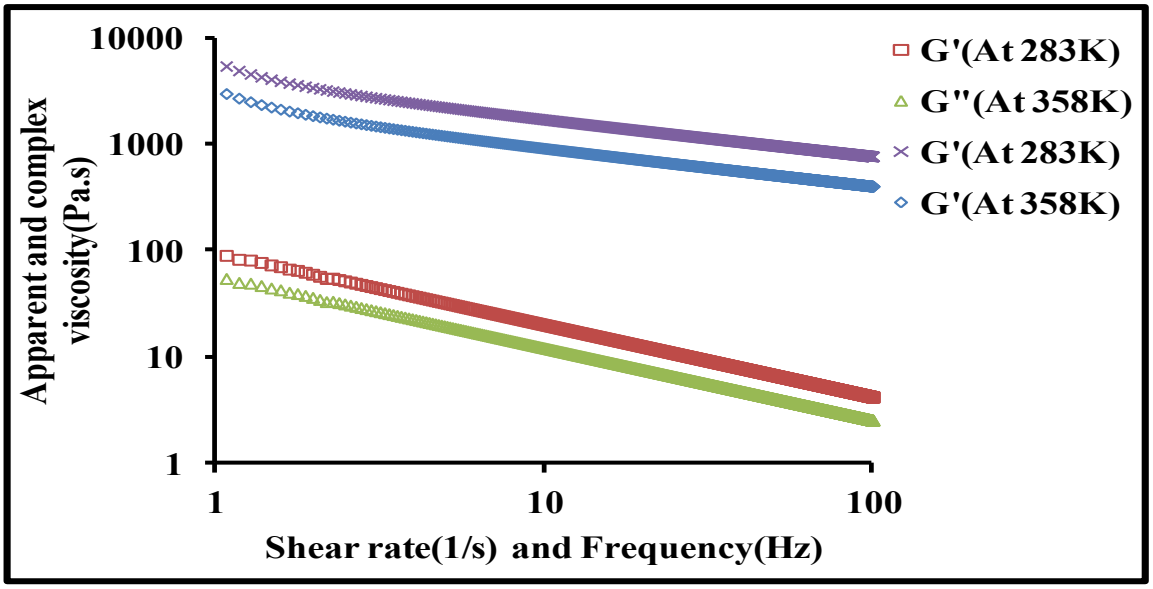


Figure 5. Complex viscosity as a function of oscillatory frequency and apparent viscosity as a function of shear rate of Gongura leaves puree at 283 and 358K.

4. Conclusions

The current finding has assessed the density of a GL puree as a component of temperature and TSS. The increment in TSS and decrement in temperature brings about density increment. With respect to all scope of temperatures, density was observed to be emphatically influenced by puree TSS. GL puree demonstrates shear-thinning conduct and found to display HB flow behavior with yield stress decrease with increment in temperature (diminish from 59.245 to 16.851 Pa, was seen as the temperature was raised from 283 - 358 K). K value of puree lessens from 35.94746 to 17.72813 Pa s^n and n was found to change from 0.39898 to 0.45893 as the temperature was raised from 283 to 358 K. The GL puree viscoelastic conduct was described as a weak gel and in oscillatory frequency, its G' and G" was portrayed by utilizing a power function. A power modified Cox-Merz s was shown that the puree rheological properties could be coordinated by either oscillatory or steady-state shear tests. The information obtained is conceivably helpful for future examinations on the properties of food and process design.

5. References

Aguado, M. A., Ibarz, A. (1988). Variación de la densidad de un zumo de manzana con la temperatura y concentración. *Alimentacion, Equipos y Tecnologia*, *3*, 209-216.

Ahmed, J., Al-Salman, F., Almusallam, A. S. (2013). Effect of blanching on thermal color degradation kinetics and rheological behavior of rocket (Eruca sativa) puree. *Journal of Food Engineering*, *119*(3), 660-667.

Alvarez, M. D., Canet, W. (2013). Time-independent and time-dependent rheological characterization of vegetable-based infant purees. *Journal of Food Engineering*, *114*(4), 449-464.

Ahmed, J., Ramaswamy, H. S. (2006). Viscoelastic and thermal characteristics of vegetable puree-based baby foods. *Journal of Food Process Engineering*, *29*(3), 219-233.

Ahmed, J., Ramaswamy, H. S. (2006). Viscoelastic properties of sweet potato puree infant food. *Journal of Food Engineering*, *74*(3), 376-382.

Ahmed, J., Ramaswamy, H. S. (2007). Dynamic and steady shear rheology of fruit puree based baby foods. *Journal of Food*

Science and Technology-Mysore-, *44*(6), 579.

Ahmed, J., Ramaswamy, H. S., Sashidhar, K. C. (2007). Rheological characteristics of tamarind (Tamarindus indica L.) juice concentrates. *LWT-Food Science and Technology*, *40*(2), 225-231.

Alvarado, J. D. D., Romero, C. H. (1989). Physical properties of fruits I. density and viscosity of juices as functions of soluble solids content and temperature. *Latin American Applied Research*, *19*(15), 15-21.

Alvarez, M. D., Fernández, C., Canet, W. (2004). Rheological behavior of fresh and frozen potato puree in steady and dynamic shear at different temperatures. *European Food Research and Technology*, *218*(6), 544-553.

Arnon, D. (1949). Estimation of Total chlorophyll. *Plant Physiology*, 24(1), 1-15.

Augusto, P. E., Falguera, V., Cristianini, M., Ibarz, A. (2011). Influence of fiber addition on the rheological properties of peach juice. *International Journal of Food Science & Technology*, *46*(5), 1086-1092.

Augusto, P. E., Falguera, V., Cristianini, M., Ibarz, A. (2011). Viscoelastic properties of tomato juice. *Procedia Food Science*, *1*, 589-593.

Balaghi, S., Mohammadifar, M. A., Zargaraan, A., Gavlighi, H. A., Mohammadi, M. (2011). Compositional analysis and rheological characterization of gum tragacanth exudates from six species of Iranian Astragalus. *Food Hydrocolloids*, *25*(7), 1775-1784.

Bayod, E., Månsson, P., Innings, F., Bergenståhl, B., Tornberg, E. (2007). Low shear rheology of concentrated tomato products. Effect of particle size and time. *Food Biophysics*, *2*(4), 146-157.

Bistany, K. L., Kokini, J. L. (1983). Dynamic viscoelastic properties of foods in texture control. *Journal of Rheology*, *27*(6), 605-620.

Campo-Deaño, L., Tovar, C. (2009). The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska Pollock and Pacific Whiting surimi. *Food Hydrocolloids*, *23*(7), 1641-1646.

Canet, W., Álvarez, M. D., Fernández, C., Luna, P. (2005). Comparisons of methods for measuring yield stresses in potato puree: effect of temperature and freezing. *Journal of Food Engineering*, *68*(2), 143-153.

Cepeda, E., Villaran, M. C. (1999). Density and viscosity of Malus floribunda juice as a function of concentration and temperature. *Journal of Food Engineering*, *41*(2), 103-107.

Colin-Henrion, M., Cuvelier, G., Renard, C. M. G. C. (2007). Texture of pureed fruit and vegetable foods. *Stewart Postharvest Review*, *5*, 1-14.

Constenla, D. T., Lozano, J. E., Crapiste, G. H. (1989). Thermophysical properties of clarified apple juice as a function of concentration and temperature. *Journal of Food Science*, *54*(3), 663-668.

Cox, W. P., Merz, E. H. (1958). Correlation of dynamic and steady flow viscosities. *Journal of Polymer Science*, *28*(118), 619-622.

Da Silva, J. A. L., Rao, M. A. (1992). Viscoelastic Properties of Food Hydrocolloid Dispersions en Rao MA. Steffe, JF.(Eds.) Viscoelastic Properties of Foods 285-315.

Espinosa, L., To, N., Symoneaux, R., Renard, C. M., Biau, N., & Cuvelier, G. (2011). Effect of processing on rheological, structural and sensory properties of apple puree. *Procedia Food Science*, *1*, 513-520.

Gunasekaran, S., Ak, M. M. (2000). Dynamic oscillatory shear testing of foods—selected applications. *Trends in Food Science & Technology*, *11*(3), 115-127.

Hajieghrary, F., Homayouni-Rad, A. (2016). Physical, rheological and sensory properties of non-alcoholic malt beverage formulated with date syrup. *Carpathian Journal of Food Science & Technology*, *8*(4).

Haminiuk, C. W. I., Sierakowski, M. R., Maciel, G. M., Vidal, J. R. M. B., Branco, I. G., Masson, M. L. (2006). Rheological

properties of butia pulp. *International Journal of Food Engineering*, *2*(1)

Ibarz, A., Miguelsanz, R. (1989). Variation with temperature and soluble solids concentration of the density of a depectinised and clarified pear juice. *Journal of Food Engineering*, *10*(4), 319-323.

Ice, J.R., Hamann, D.D., Purcell, A.E. (1980). Effects of pH, enzymes, and storage time on the rheology of sweet potato puree. *Journal of Food Science*, 45(6), 1614-1618.

Işıklı, N. D., Karababa, E. (2005). Rheological characterization of fenugreek paste (çemen). *Journal of Food Engineering*, *69*(2), 185-190.

Karuna, D., Noel, G., Dilip, K. (1996). Food and nutrition bulletin, 17: 2. *United Nation University*.

Koocheki, A., Razavi, S. M. A., Milani, E., Moghadam, T. M., Abedini, M., Alamatiyan, S., Izadkhah, S. (2007). Physical properties of watermelon seed as a function of moisture content and variety. *International Agrophysics*, *21*(4), 349.

Kumar, S., Kumar, P. (2015). Rheological modeling of non-depectinized beetroot juice concentrates. *Journal of Food Measurement and Characterization*, *9*(4), 487-494.

Manohar, B., Ramakrishna, P., Udayasankar, K. (1991). Some physical properties of tamarind (Tamarindus indica L.) juice concentrates. *Journal of Food Engineering*, *13*(4), 241-258.

Massa, A., González, C., Maestro, A., Labanda, J., Ibarz, A. (2010). Rheological characterization of peach purees. *Journal of texture studies*, *41*(4), 532-548.

Nindo, C. I., Tang, J., Powers, J. R., Bolland, K. (2004). Energy consumption during Refractance Window® evaporation of selected berry juices. *International Journal of Energy Research*, 28(12), 1089-1100.

Pourmohammadi, K., Najafi, M. B., Majzoobi, M., Koocheki, A., Farahnaki, A. (2017). Evaluation of dough rheology and quality of sugar-free biscuits: isomalt, maltodextrin, and stevia. *Carpathian Journal of Food Science & Technology*, *9*(4).

Puro, K., Sunjukta, R., Samir, S., Ghatak, S., Shakuntala, I., Sen, A. (2014). Medicinal uses of Roselle plant (Hibiscus sabdariffa L.): a mini review. *Indian Journal of Hill Farming*, *27*(1), 81-90.

Rao, M. A. (2014). Flow and functional models for rheological properties of fluid foods. In *Rheology of fluid, semisolid, and solid foods* (pp. 27-61). Springer, Boston, MA.

Rao, M. A., Rizvi, S. S., Datta, A. K., & Ahmed, J. (2014). *Engineering properties of foods*. CRC press.

Rudra, S. G., Sarkar, B. C., Shivhare, U. S., Basu, S. (2008). Rheological properties of coriander and mint leaf puree. *Journal of food process engineering*, *31*(1), 91-104.

Steffe, J. F. (1996). *Rheological methods in food process engineering*. Freeman press.

Sun, A., Gunasekaran, S. (2009). Yield stress in foods: measurements and applications. *International Journal of Food Properties*, *12*(1), 70-101.

Tabilo-Munizaga, G., Barbosa-Cánovas, G. V. (2005). Rheology for the food industry. *Journal of food engineering*, *67*(1-2), 147-156.

Zuritz, C. A., Puntes, E. M., Mathey, H. H., Pérez, E. H., Gascón, A., Rubio, L. A., Cabeza, M. S. (2005). Density, viscosity, and coefficient of thermal expansion of clear grape juice at different soluble solid concentrations and temperatures. *Journal of Food Engineering*, *71*(2), 143-149.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

COATING MATERIALS AS A POTENTIAL PRE-TREATMENTS FOR REDUCING OIL UPTAKE OF FRIED POTATO CHIPS

Saad Ahmed Saad Hallabo[1], Mohamed Mohamed Ahmed El-Nikeety[1], Samah Said Mahmoud Allam[2] and Asmaa Gamal Abd El-hamied[2*]

[1]Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Oils and Fats Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt
**asmaagamal2009@yahoo.com*

https://doi.org/10.34302/2019.11.4.8

Article history:
Received:
17 August 2019
Accepted:
10 October 2019

Keywords:
Pre-Treatment Coating Materials;
Fried Potato Chips;
Oil Uptake;
CMC;
XG.

Abstract

Different concentrations of carboxymethyl cellulose (CMC), xanthan gum (XG) and soya protein isolate (SPI) were used as coating materials for potato chips before subjected to frying process. The effect of these coating materials on oil uptake%, moisture loss%, sensory attributes of fried potato chips were investigated and results revealed that, they were efficient and their efficiency were increased with the increment in their concentrations and using 1% CMC and 1% XG as coating materials reduced the oil uptake of fried potato chips from 35.88 to 23.25 and 25.00%, respectively compared with the un-coated potato chips and those coated with 5% SPI; 35.88 and 30.92%, respectively and gave a reduction rate of oil uptake35.20 and 30.32%, respectively. Also coating with 1% CMC improved the sensory attributes of fried potato chips and gave a high overall acceptability compared with the other coating materials and control sample. The best performance of coating pre-treatment materials were selected and applied for frying experiment for 6 hr daily for 4 consecutive days at 180±5°C and their effect on physical and chemical properties of oils used for frying process were monitored and results revealed that, using 5% SPI, 1% XG and 1% CMC as coating materials had a different effect during frying process; while using 5% SPI had a sever effect on physical and chemical properties of oil used for frying, 1% XG had a moderate effect and 1% CMC had a slight effect.

1.Introduction

Fried Potato chips are considered one of the most important products among all the snack food industry. During frying, the water present in the raw material evaporates, and is partially replaced by oil, constituting up to 40% of the finished product, and consequently affecting its properties (Lin Yu *et al*., 2016). As a result, consumption of deep-fat fried foods has been associated with coronary heart diseases, obesity and type 2 diabetes. Selection of an appropriate food coating before frying may act as a barrier to moisture loss, which is important commercially, and also reduce fat uptake during frying. (Ananey-Obiri *et al*., 2018).

During frying, the water loss process passes three different steps. The first step corresponds to potato heating, involving mainly the loss of water at the cutting surfaces. During the second step, an intense formation of water bubbles and an exponential decrease of water content with time are occurring. The third step occurs after the formation of the crust, hindering the movement of the vapor bubbles, created by the internal gas pressure (Costa

et al., 1996). Physical changes induces by frying process such as crust formation have been closely correlated to oil penetration, and this imparts the characteristic appetizing nature of the food. Also fried foods develop desirable organoleptic properties such as color, crispiness, texture, and fried flavor resulting in popularity among consumers (Kassama and Ngadi, 2016).

Absorption of oil is influenced by a variety of factors; oil quality, product and frying temperature, oil degradation, frying time, frying duration, initial moisture content of food ingredients, product shape and content, porosity of coating, and the method of frying (Bouchon *et al.*, 2003; Mellema, 2003; Math *et al*., 2004; Bouaziz *et al*., 2016).

Coating materials; protein based and polysaccharides based can be successfully used in food products as base components, alone or in a mixture. These materials have function properties such as thickening, gelling, stabilizing, film forming, dispersing and texture modifying (Kurek *et al*., 2017).

Coating agents is one of the effective methods used to reduce oil absorption in fried products and can be used as an alternative solution to comply with both health concerns and consumer preferences (Angor, 2016).The effectiveness of a coating material is determined by its mechanical and barrier properties, which depend on its composition and microstructure, and by the characteristics of the food product to which it binds. Hydrophilic biopolymers can be used as water binders in coating to reduce water loss from the coat. If we would be able to reduce water loss, oil uptake would also be reduced. Most commercial biopolymer coatings that are claimed to act like this to reduce fat uptake, are polysaccharide coatings (Sobowale and Omotoso, 2018).On the other hand, coating material can be used as emulsifiers in composite films, the surface tension between the oil and the food could also be reduced, consequently contributed to decrease oil uptake (Skurtys *et al*., 2010).

The objective of this study was to investigate the effects of pre-treatment agents such as; soya protein isolate (SPI), carboxy methyl cellulose (CMC) and xanthan gum (XG) on the oil uptake%, moisture content%, sensory attributes of fried potato chips and the quality characteristics of oil used for deep frying different pre-treatments coated potato chips compared with the un-coated potato chips (control sample) for 24 hr at 180±5°C.

2.Materials and methods

2.1.Materials

Potatoes (Rosetta or Red variety) were purchased from the local market, Giza, Egypt. Refined, bleached and deodorized (RBD) sunflower oil free of added antioxidants was obtained from Arma Company, 10th of Ramadan, Sharkia Governorate, Egypt. Carboxymethyl cellulose (CMC), xanthan gum (XG) and soya protein isolate (SPI) were purchased from Loba Chemie, India. Chemicals and solvents of analytical grade were purchased from El-Gomheriya Company for Chemical and Drugs, Egypt.

2.2.Methods

Potatoes were washed, peeled and cut into chips with 1.5±0.1 mm thickness using a manual slicing machine and divided into portions.

Coating solutions were prepared for each coating material with different concentrations; 5, 10% of SPI, 0.5, 1% of CMC and 0.5, 1% of XG in distilled water. Each solution was heated to 90ºC for 5 min with continuous stirring and cooled to room temperature. Each portion of potato chips was immersed for 30 seconds in one of the prepared coating solution with a ratio of 2:1(wt/vol) followed by air drying for 3 min. The last portion of potato chips was treated in the same procedure without using coating materials (control sample).

Frying process**:** Three kilograms of sunflower oil with (6.50% Palmitic acid, 3.50% Stearic acid, 26.20% Oleic acid and 62.26% Linoleic acid) were used in deep fat frying process which was carried out for the 6 pre-treatments potato chips and potato chips without treatment (control sample) using a domestic fryer (Model 7122 A, tefal super 500 delux, France) in batches with a ratio of 1:30 potato weight/oil volume. Each batch lasted for 3 min at 180±5°C. Frying different pre-treatments and control sample were lasted for 6 hr daily for 4

consecutive days without replenishment and 100 ml of oil was withdrawn at the end of frying day and after oil was let to call down to room temperature, they stored in dark glass bottles at -4 °C till analysis.

Moisture content % and oil uptake % of potato chips samples, refractive index (RI) at 25°C, free fatty acids (FFA) % (as oleic acid) and peroxide value (PV) (meq O_2/kg oil) were determined according to the method described by the AOAC (2016).

Fried potato chips (coated and un-coated) were evaluated by twenty panelists for sensory evaluation in terms of appearance, color, flavor, taste, crispiness and overall acceptability. Panelists were requested to assess each coded sample, and record the degree of difference using a 10-points Hedonic scale. On this scale, 1 represented dislike extremely and 10 represented like extremely (Singthong and Thongkaew, 2009). Results were statistically analyzed using CoState statistical software (CoHort Software, Monterey, CA, USA). The statistical calculations included the analysis of variance (ANOVA) one way completely randomized. $P<0.05$ was considered to be significant using Duncan's test. All data were expressed as means values± Standard Deviation (SD), as described by Snedecor and Cochran (1982).

Fatty acids were carried out by preparation of methyl ester followed by the identification of methyl esters using an Agilent 6890 series gas chromatograph apparatus equipped with a DB23 (60 m X 0.32) (ISO, 2011).

Color of oil samples was measured by using a Lovibond Tintometer model F, 5.25 inch cell and expressed as total color 5×red+1×yellow Lovibond units according to (Latha and Nasirullah, 2014).

Polymer contents (PC) % of oil samples were determined following the method mentioned by Pel-Fan and Nawar (1986).Total polar compounds (TPC) % of oil samples were determined by using the column chromatography method described by (Waltking and Wessels, 1981).

Viscosity (cP) was monitored using Brook-filed Viscometer RVDV Spindle SC4-21 connected to water bath Brook-field TC500. Viscosity determination was carried out at 25± 0.1 °C according to the method described by Howard (1991).

3.Results and discussion

3.1.Influence of coating materials on moisture loss and oil uptake% of fried potato chips

Data in Table (1) show the effect of coating materials on moisture content and oil uptake% of potato chips after being fried in sunflower oil at 180±5°C and data indicated that, potato chips immersed in 1% CMC as edible coating material had the highest reduction rate% in oil absorption followed by potato chips treated with 1% XG and those treated with 10% SPI; 35.20, 30.32 and 24.72%, respectively. Results in Table (1) revealed that, increasing CMC, XG and SPI concentrations were accompanied by decreasing the oil content of fried coated potato chips. The thermal gelation properties of coating materials led to the formation of a small amount of wide punctures with low capillary pressures, which resulted in less oil entrance to the pores as illustrated by (Mellema, 2003).

Table 1. Effects of coating pre-treatments on oil uptake% and moisture content % of fried potato chips

Coating agents		CMC		XG		SPI	
	Un-coated	0.5%	1%	0.5%	1%	5%	10%
Oil uptake %	35.88	26.31	23.25	28.85	25.00	30.92	27.01
Oil uptake reduction %	0.00	26.37	35.20	19.59	30.32	13.82	24.72
Moisture content%	0.94	1.60	1.94	1.55	1.87	1.06	1.20

CMC: carboxy methyl cellulose
XG: xanthan gum
SPI: soya protein isolate

Moisture content of fried potato chips was affected by coating agents; 0.5, 1% CMC, 0.5, 1 % XG and 5, 10% SPI used as pre-treatments before frying process (Table 1). Moisture content of the

control (un-coated) fried potato chips was found to be 0.94%. The moisture content of fried samples was increased with the increase in the concentration of coating materials used. The highest moisture retention was found by using 1% CMC (1.94%), this positive effect could be related to the high water binding capacities of CMC, preventing the replacement of moisture with oil during the frying process. Moisture loss and oil absorption have inverse relationship as previously reported by Bouaziz *et al*., (2016).

Hydrocolloids have been proved to reduce the oil uptake during the frying process as reported by several workers, Hua *et al.* (2015) observed the effectiveness of pectin in reducing oil uptake during frying of potato chips; Maity *et al*. (2015) found similar relationship between moisture loss and oil uptake in deep fat fried jackfruit chips coated with hydrocolloids and Bouaziz *et al*. (2016) also found minor oil uptake in fried potato chips treated with almond gum as coating agent during frying process.

3.2.Effect of coating agents on sensory attributes of fried potato chips

Sensory attributes of fried potato chips are presented in Table (2). High heat transfer rates during frying developed and improved desirable sensory properties of fried products (Hubbard and Farkas, 2000). Changes in sensory attributes of fried potato chips after coating could be attributed to the different behavior between coating materials and starch during frying (Hua *et al*., 2015). Fried potato chips coated with 1% CMC had the highest scores in all sensory attributes and overall acceptability (with a significant difference $P<0.05$), which suggested that fried coated potato chips may be more attractive than the un-coated one. The overall acceptability of potato chips coated with either CMC or XG was higher than those coated with SPI and there was an increase in sensory attributes of the coated potato chips with the increment of CMC and XG concentrations.

Fried products' color is one of the most important physical attribute that greatly influence consumer perception and can summarily lead to rejection of the product (Sobowale and Omotoso, 2018). The high color value was observed in fried potato chips coated with 1% CMC and 1% XG while the lowest value was obtained for potato chips coated with 10% SPI, the color development of SPI-coated potato chips may be attributed to the Maillard reaction occurred between reducing sugar and amino acid of the SPI coated potato chips. These results are in accordance with those reported by (Sobowale and Omotoso, 2018) in regarding the increment in sensory acceptability of potato chips coated with egg and carboxyl-methylcellulose. Also Kilincceker *et al*. (2009) reported that the smell, taste and flavor of the frozen fish fillets samples were improved by using coating materials compared to un-coated sample, and the desired color formed. On the other hand, Hua *et al*. (2015); Bouaziz *et al*. (2106) found that, the un-coated potato chips had the highest scores in all attributes and overall acceptance.

Table 2. Sensory evaluation of coated and un-coated fried potato chips

Coating agents / Sensory attributes	Un-coated	CMC		XG		SPI	
		0.5%	1%	0.5%	1%	5%	10%
Appearance	7.70 ± 0.9^{ab}	8.00 ± 0.53^{ab}	8.80 ± 0.53^{a}	7.30 ± 0.65^{a}	7.50 ± 0.74^{ab}	6.80 ± 0.93^{b}	6.30 ± 1.55^{b}
Color	8.00 ± 0.52^{ab}	8.00 ± 0.73^{ab}	9.00 ± 0.21^{a}	8.50 ± 0.84^{ab}	8.70 ± 0.91^{a}	7.20 ± 0.63^{bc}	6.20 ± 0.83^{c}
Flavor	8.00 ± 0.50^{a}	8.00 ± 0.2^{ab}	8.50 ± 1.00^{a}	7.20 ± 0.30^{ab}	$7.50\pm1.00a^{b}$	7.23 ± 0.3^{b}	7.00 ± 0.40^{b}
Taste	7.80 ± 0.20^{b}	8.20 ± 0.30^{ab}	8.50 ± 0.10^{a}	7.60 ± 0.30^{b}	7.80 ± 0.10^{b}	7.00 ± 0.50^{c}	7.00 ± 0.50^{c}
Crispiness	7.93 ± 0.4^{ab}	8.10 ± 0.20^{ab}	8.50 ± 0.70^{b}	7.50 ± 0.5^{b}	7.80 ± 0.30^{ab}	7.56 ± 0.40^{ab}	7.50 ± 0.30^{ab}
Overall Acceptability	8.50 ± 0.20^{ab}	8.00 ± 0.20^{c}	8.70 ± 0.10^{a}	8.30 ± 0.10^{bc}	8.00 ± 0.10^{c}	7.00 ± 0.20^{d}	6.50 ± 0.30^{e}

Means ±SD. values in the same row with different superscript letters are significantly different ($p< 0.05$)

3.3.Effect of using coating materials on the physical and chemical characteristics of oil used in frying process

Frying experiment was conducted using potato chips coated with coating materials in concentrations that gave the best performance in reducing oil uptake% and have a high over all acceptability by panelist compared with the uncoated potato chips (control sample).

Accordingly, potato chips coated with 1% CMC, potato chips coated with 1% XG and those coated with 5% SPI were selected for frying process for 6 hr daily for 4 consecutive days.

Frying oils' properties were affected by various frying conditions; temperature, time and the nature of fried products. Changes in these properties were monitored and results were tabulated in Tables (3, 4).

The effect of frying conditions and coating materials on the physical properties (refractive index {RI}, viscosity and total color) of oils used for frying at 180±5°C for 24 hr (six hr per day for four consecutive days) are shown in Table (3). Data in Table (3) illustrated that, during frying process, RI, viscosity and total color were increased as frying time increased, and there were a gradually increased in both RI and viscosity, while the total color had different trend as they was sharply increased after frying for 12 hr and then gradually increased through the last 12 hr of frying both un-coated and coated potato chips with different coating materials. The increments of RI, viscosity and total color were more pronounced with oils used for frying the un-coated potato chips and potato chips coated with 5% SPI rather than oils used for frying potato chips coated with either 1% CMC or 1% XG throughout the 24 hr of frying process.

The effect of frying conditions and coating agents on the chemical characteristics (free fatty acids {FFA} (% as oleic acids), peroxide values {PV} (meq O_2/kg oil), polymer content {PC}% and total polar compounds {TPC}%) of oils used in frying process were monitored and results were tabulated in Table (4).

Table 3. Physical characteristics of oils used in frying coated and un-coated potato chips at 180±5°C for 24 hr (4 consecutive days)

Pre-treatment	Frying time (hr)	Refractive index (at 25° C)	Viscosity (cP)	Total color
Fresh oil	**0**	1.4730	59.00	7.0
Un-coated	**6**	1.4739	61.50	11.5
	12	1.4750	65.00	40.0
	18	1.4762	72.00	65.0
	24	1.4776	79.00	100.0
Coated with 1% CMC	**6**	1.4737	61.00	11.0
	12	1.4743	63.50	38.0
	18	1.4452	67.91	62.5
	24	1.4770	75.64	90.0
Coated with 1% XG	**6**	1.4738	61.24	11.5
	12	1.4747	64.00	40.0
	18	1.4758	68.45	64.0
	24	1.4773	76.25	96.5
Coated with 5% SPI	**6**	1.4741	63.00	12.0
	12	1.4751	66.00	49.0
	18	1.4770	69.45	73.0
	24	1.4781	81.50	112.0

Table 4. Chemical characteristics of oils used in frying coated and un-coated potato chips at 180±5°C for 24 hr (4 consecutive days)

Pre-treatment	Frying time (hr)	Free fatty acids (% as oleic acids)	Peroxide value (meq O_2/kg oil)	Polymer contents %	Total polar Compounds %
Fresh oil	**0**	0.06	1.19	0.00	2.55
Un-coated	**6**	0.30	4.10	1.05	10.53
	12	0.57	10.60	2.25	18.37
	18	0.89	16.10	3.85	22.11
	24	1.18	25.13	7.08	27.50
Coated with 1% CMC	**6**	0.23	3.92	0.90	9.85
	12	0.46	9.18	2.19	17.92
	18	0.85	14.37	3.60	20.78
	24	1.03	23.09	6.72	25.52
Coated with 1% XG	**6**	0.31	4.20	0.94	10.24
	12	0.55	9.78	2.27	18.10
	18	0.91	15.23	3.69	21.94
	24	1.12	24.38	6.84	26.98
Coated with 5% SPI	**6**	0.53	5.93	1.70	17.00
	12	0.76	13.42	3.21	26.21
	18	1.17	18.60	4.31	30.09
	24	1.49	16.70	8.21	35.52

Results in the same Table revealed that, FFA% were sharply increased throughout the first 6 hr of frying the un-coated and coated potato chips with different coating materials and after that they were gradually increased during the rest of frying hours till the end of frying time; 24 hr. This sharp increment was very obvious when frying potato chips coated with 5% SPI followed by un-coated potato chips and potato chips coated with 1% XG (1.49, 1.18 and 1.12, respectively) and the least affect was shown when frying potato chips coated with 1% CMC (1.03).

This trend of increment was also noticed when monitoring the changes in PC% for oils used in our frying experiment, regarding the sharp increase in PC% during the first 6 hours followed by gradually increase during the rest of the experiment time (24 hr). Also, these changes in PC% were more pronounced in oils used for frying potato chips coated with 5% SPI followed by un-coated potato chips and potato chips coated with 1% XG (8.21, 7.08 and 6.84, respectively). Polymers are mainly responsible for the increase in viscosity, refractive index, specific gravity and contribute to the foaming tendency of heated oil (Wang *et al*., 2016). From Table (4) we can also noticed that PV, which was used as a measurement of the primary products of oxidation, was monitored for oil samples used in frying process and the tabulated results in Table (4) illustrated that PV of oils used in frying were increased sharply after 6 hr of frying either untreated or pre-treated potato chips with different coating materials and these were more pronounced when frying potato chips coated with 5% SPI, followed by potato chips coated with 1% XG and un-coated potato chips (5.93, 4.20 and 4.10 meq O_2/kg oil, respectively). Also, we could observed that PV of all oil samples had the same trend as the previously mentioned chemical characteristics; FFA% and PC% with one exception that PV of oil used for frying 5% SPI coated potato chips had declined in the last 6 hours of frying process from 18.60 to 16.70 meq O_2/kg oil at the end of frying time 24 hr. Peroxides are unstable and can break down to carbonyl and aldehydic compounds as a result of secondary oxidation (Li *et al.,* 2014). These data are in agreement with those reported by (Khazaei *et al.,* 2016), who indicated that, the coating treatment was effective in reducing lipid oxidation and as previously stated by Kim *et al*. (2011), hydrocolloid coatings significantly reduced the heat transfer coefficients as well as oil uptake, which could be contributed to the lower lipid oxidation in the coated fried food compared to un-

coated (control) samples. In parallel, Aminlari *et al*. (2005) found that potato chips coated with sodium caseinate had 14% less oil than control samples. In coated chips the water retention and the protein content was significantly increased, and the peroxide value was decreased between 30% and 50%.

Total polar compounds% (TPC) of oils which indicates the total amount of degradation compounds in frying oils were also determined and results were tabulated in Table (4). Results showed that, coating potato chips with 5% SPI had severally affected the TPC% of oils used in frying process when compared with oils used for frying un-coated potato chips, potato chips coated with 1% XG and those coated with 1% CMC; 35.52, 27.50, 26.98 and 25.52, respectively.

From Tables (3,4) we could observed that frying potato chips coated with 1% CMC slightly affect the physical and chemical characteristics of oil used for frying for 24 hr at 180±5°C compared with the other coating materials and control sample without coating.

4.Conclusions

Coating as a pre-treatment procedure was important for fried food manufacture in reducing the oil uptake of fried potato chips. The oil uptake had a reciprocal relationship with the moisture content retained in the fried potato chips. Using 1% CMC as an edible coating material was more effective in reducing oil uptake% and improved the sensory attributes of fried potato chips compared with the other coating materials with different concentrations used in our experiment and slightly affected the physical and chemical properties of oil used for frying at 180±5°C for 24 hr.

Although, SPI was effective in reducing oil uptake% and increasing its concentration was accompanied by increasing its efficiency in oil uptake reduction rate, it was severally affected the physical and chemical characteristics of oils used in frying process and can't be practically used in commercial scale.

So, during frying process coating pre-treatments could be used in decreasing the oil uptake% while maintaining the high quality of the fried potato chips which would be benefit for the consumer, adding value to the snake market as a healthy food product and CMC could be used as a potential coating material in reducing oil uptake of fried potato chips during frying process.

5.References

Aminlari, M., Ramezani, R., Khalili, M. H. (2005). Production of protein-coated low-fat potato chips. *Food Science and Technology International*, 11(3), 177-181.

Ananey-Obiri, D., Matthews, L., Azahrani, M. H., Ibrahim, S. A., Galanakis, C. M., Tahergorabi, R. (2018). Application of protein-based edible coatings for fat uptake reduction in deep-fat fried foods with an emphasis on muscle food proteins. *Trends in Food Science and Technology,* (80), 167-174.

Angor, M. M. (2016). Reducing fat content of fried potato pellet chips using carboxymethyl cellulose and soya protein isolate solutions as coating films. *Journal of Agricultural Science*, 8(3), 168-168.

AOAC (2016). Official Methods of Analysis of AOAC International 20th Ed. Association of Official Analytical Chemists, Gaithersburg, Maryland. U.S.A.

Bouaziz, F., Koubaa, M., Neifar, M., Zouari-Ellouzi, S., Besbes, S., Chaari, F., Kamoun, A., Chaabouni, M., Chaabouni, S. E, Ghorbel, R. E. (2016). Feasibility of using almond gum as coating agent to improve the quality of fried potato chips: Evaluation of sensorial properties. *LWT-Food Science and Technology,* (65), 800-807.

Bouchon, P., Aguilera, J. M., Pyle, D. L. (2003). Structure oil absorption relationships during deep-fat frying, *Journal of Food Science*, 68(9), 2711-2716.

Costa, R. M., Oliveira, F. A. R., Gekas, V. (1996). Water loss during frying of thin potato slices. In The first main meeting of process optimisation and minimal processing of foods. *Porto: Escola Superior de Biotecnologia*, (3), 70-75.

Howard, D.W. (1991). A look at viscometry. *Food Technol*., 82-84.

Hua, X., Wang, K., Yang, R., Kang, J., Yang, H.

(2015). Edible coatings from sunflower head pectin to reduce lipid uptake in fried potato chips. *LWT-Food Science and Technology*, (62), 1220-1225.

Hubbard, L. J., Farkas, B. E. (2000). Influence of oil temperature on heat transfer during immersion frying. *Food Process J. Preserv,* (24), 143-162.

ISO (12966-2/2011). International standard. Animal and vegetables fats and oil preparation of methyl esters of fatty acids.

Kassama, L. S., Ngadi, M. (2016). Relationship between oil uptake and moisture loss during deep fat frying of deboned chicken breast meat. *Advances in Chemical Engineering and Science*, 6(04), 324-334.

Khazaei, N., Esmailli. M., Emam-Djomeh, Z. (2016). Effect of active edible coatings made by basil seed gum and thymol on oil uptake and oxidation in shrimp during deep-fat frying, *Carbohydrate polymer*, (137), 249-254

Kilincceker, O., Dogan, I., Kucukoner, E. (2009). Effect of edible coatings on the quality of frozen fish fillets. *LWT- Food Science and Technology*, (42), 868- 873.

Kim, D. N., Lima, J. Bae, I. Y., Lee, H.G., Lee, S., (2011). Effect of hydrocolloid coatings on the heat transfer and oil uptake during frying of potato strips. *Journal of Food Engineering*, (102), 317-320.

Kurek, M., Scetar, M., Gali, K. (2017). Edible coatings minimize fat uptake in deep fat fried products: Areview. *Food Hydrocolloids,* (71), 225-235.

Latha, R. B., Nasirullah, D. R. (2014). Physico-chemical changes in rice bran oil during heating at frying temperature. *J. Food Science and Technology,* 51(2), 335-340.

Li, y., Ma, w., Qi, B., Rokayya, S., Li, D., Wang, J., Feng, H., Sui, H., Jiang, L. (2014). Blending of soybean oil with selected vegetable oils: impact on oxidative stability and radical scavenging activity. *Asian Pacific Journal of Cancer Prevention*, (15), 2583-2589.

Lin Yu, Jinwei, L. I., Ding, S., Hang, F., d Fan, L. (2016). Effect of guar gum with glycerol coating on the properties and oil absorption of fried potato chips. *Food Hydrocolloids,* (54), 211-219.

Maity, T., Bawa, A. S., Raju, P. S. (2015). Use of hydrocolloids to improve the quality of vacuum fried jackfruit chips. *International Food Research Journal*, 22(4), 1571-1577.

Math, R. G., Velu, V., Nagender, A., Rao, D. G. (2004). Effect of frying conditions on moisture, fat, and density of papad. *Journal of Food Engineering*, 64(4), 429-434.

Mellema, M. (2003). Mechanism and reduction of fat uptake in deep-fat fried foods. *Trends in Food Science and Technology*, (14), 364-373.

Pel-Fan, W., Nawar, W. W. (1986). A technique for monitoring the quality of used frying oils. *J. Am. Oil Chem. Soc.*, (63), 1363- 1367.

Singthong, J., Thongkaew, C. (2009). Using hydrocolloids to decrease oil absorption in banana chips. *LWT-Food Science and Technology*, 42(7), 1199-1203.

Skurtys, O., Acevedo, C., Pedreshi, F., Enrione, J., Osorio, F., Aguilera, J. M. (2010). Food hydrocolloid edible films and coatings. *New York: Nova Science Publishers Inc*.

Snedecor, G. W., Cochran, W. G. (1982). Statistical methods. 6th ed., *lowa State Univ*., Press, lowa, U.S.A.

Sobowale, S. S., Omotoso, B.O. (2018). Effect of pre-drying and hydrocolloids batter coating during heat and moisture transfer of coated fried potato chips. *Journal of Bioprocessing &Biotechnique*s, (8), 1-9.

Waltking, A. E., Wessels, H. (1981). Chromatographic separation of polar and non polar components of frying fats. *J. Assoc. of Chem*., (64) 1329-1330.

Wang, S. N., Sui, X. N., Wang, Z. J., Qi, B. K., Jiang, L. Z., Li, Y. (2016). Improvement in thermal stability of soybean oil by blending with camellia oil during deep fat frying. *European Journal of Lipid Science and Technology*, (118), 524-531.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

INFLUENCE OF CSN3, LGB, PRL, GH, TG5 GENES ALLELES ON DAIRY PRODUCTIVITY AND ENERGY VALUE OF COW'S MILK

Evgeny O. Krupin[1*], Shamil K. Shakirov[2]

[1]Tatar Scientific Research Institute of Agriculture*
[2]FRC Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russia
evgeny.krupin@gmail.com

https://doi.org/10.34302/2019.11.4.9

Article history:
Received:
1 August 2019
Accepted:
1 November 2019

Keywords:
Milk production;
Fat;
Protein;
Genotype;
Cow

ABSTRACT

The aim of our research was to study the dependence of Kholmogorsk breed dairy cows' productivity on polymorphism of kappa-casein (CSN3), beta-lactoglobulin (LGB), prolactin (PRL), somatotropin (GH), thyroglobulin (TG5) genes. It was found that B allele (BB genotype) of PRL gene caused maximum increase in milk production (19.7%). Maximum increase in milk productivity (15.0%, P <0.001), was found at L allele (LL genotype) of GH gene. Significant increases in the presence of A and B alleles with AA (14.3%) and BB (12.7%) genotypes of CSN3 and LGB genes were detected. The maximum increase in fat mass fraction (0.22%) in milk was found at ABL homozygous genotype of PRL gene, while significant increase of fat content was detected at C allele (CC genotype) of TG5 gene (0.15%, P <0.05). The maximum increase of protein mass fraction at B allele (BB genotype) of PRL gene (0.11%) was detected. The maximum increase in milk protein was found at B and L alleles (BB and LL genotypes) of LGB and GH genes (0.06% with P <0.01 and P <0.001, respectively). Analysis of milk energy value showed that A and B alleles and AA (2.80 MJ / kg) and BB (2.80 MJ / kg) genotypes of CSN3 gene and BB genotype of LGB (2, 82 MJ / kg) and PRL genes (2.93 MJ / kg), as well as the T allele and TT genotype for the TG5 gene (3.17 MJ / kg) are associated with this indicator.

1.Introduction

All over the world, cow farming mainly targets milk production. The average milk production level of cows differs between regions, partly due to different cow genetics (Perišić et al, 2011). Cow genetics basically determines the potential of their milk production, in fact - the amount of milk received from animals. It is known that the level of milk productivity is a polygenic trait controlled by various genetic loci (Dybus et al, 2004; Singh et al, 2014), some of which are already known (Singh et al, 2014; Khatami et al, 2005), and some are only classified as candidate genes (Viale et al, 2017). Based on literature data, the following economically valuable traits gene loci were selected for our work: kappa-casein (CSN3) (Chasovshchikova et al, 2017), beta-lactoglobulin (LGB) (Di Gregorio et al, 2017), prolactin (PRL) (Chasovshchikova et al, 2017), somatotropin (GH) (Akyuz et al, 2015; Metin Kiyici et al, 2019), thyroglobulin (TG5) (Zhang et al, 2015).

On the other hand, realization of a dairy cow as its genetically determined potential for milk production depends on the technology of keeping and growing, as well as the feeding programs used. In general, the increase in the economic efficiency of livestock production is closely related to the intensity of livestock reproduction and the terms of

the productive use of animals, which largely depends on the level of milk productivity (Turenkova & Vasilieva, 2014). So, the fundamental factor ensuring stable and profitable dairy cattle breeding is the introduction of technological innovations that reduce losses by improving the health and productivity of the herd. Therefore, there is an acute issue of reassessing the available genetic resources of dairy cattle breeding, both of individual breeding animals, and of breeds, and types in general. The severity of the problem is due to the fact that all the work carried out by livestock breeders on Holsteinization is associated with an increase in the amount of milk received from animals, and it is not carried out in isolation, but against the background of changing in technology as a whole and of introduction of various innovations in feed production and feeding (Furaeva, 2013).

It is well known and repeatedly proven that the composition of milk depends on the technology of feeding in general, which can be used to control the content of the mass fraction of fat in milk and the fatty acid composition of milk fat itself. In addition, feed strategies have been developed to regulate the content of the mass fraction of protein in milk (Chilliard et al, 2007).

The insufficient production of high-quality feed and their inefficient use leads to a decrease in milk productivity and high feed costs per unit of production and, ultimately, has a negative impact on the economy of the entire production (Giniyatullin, 2016).

As world experience shows, the achievement of success in animal husbandry in general and in increasing the milk production of animals and reducing the cost of production of animal products in particular, only 30 ... 35% determined by achievements in breeding and genetics, but on 50 ... 60% they depends on feeding. The organization of a balanced feeding of dairy cows in different physiological periods determines their high milk productivity, and, consequently, an increase in the production of livestock products. In addition, the balanced feeding of animals is one of the main factors ensuring the effectiveness of breeding, and acts as a fundamental element of a set of measures to increase the milk productivity of animals, improve existing breeds and types (Maslyuk & Tokareva, 2018).

So, the aim of our work was to study changes in the productive qualities of dairy cows depending on the polymorphism of responsible for the phenotypic manifestation of economically valuable traits genes. To solve this problem we established genotypes of dairy cows at the loci of genes studied and we analyzed the dynamics of cow's milk productivity and mass fractions of fat and protein in milk obtained from them and calculation of the energy value of milk from different genotypes. Also we have determined dependences of milk productivity, the content of the mass fraction of fat and protein in milk and the energy value of milk at these genotypes.

2.Materials and methods

Population studied, sample size and animal feeding

All studies were performed using dairy cows of the Tatarstan type of Kholmogorsk breed. Experimental animals were kept on a leash. For the formation of one miniature herd of 81 experimental animals, animals were allocated to the group, which is an independent production unit. The composition of this group was a reduced copy of the herd on which the study was conducted. When forming the miniature herd, the age of the animal and the level of their productivity were taken into account. The relative heterogeneity of the miniature herd allowed us to assess the influence of the studied factors under conditions, approximated to production.

The main diet of all dairy cows consisted of roughage (1.5 kg of alfalfa hay), succulent feed (8.0 kg of alfalfa haylage; 9.0 kg of grass mix haylage; 12.0 kg of corn silage) and concentrated feed (6, 0 kg of mixed feed for dairy cows; 2.0 kg of corn grain; 1.0 kg of beer dry draff; 1.0 kg of oilseed rape; 0.5 kg of steamed oats). In addition, a complex feed supplement was introduced into the animal feeding ration (0.7 kg per day), which was consists of grain fermentation products, peat, waste from food production and micronutrients.

The average milk productivity of experimental animals at the beginning of experiment was 28.6

kg. The average age of dairy at the beginning of experiment was 5 years. The average number of days of milking cows at the start of the experiment was 178 days.

The milk production of each animal was individually assessed during control milking using "DeLaval" milking equipment.

The treatment of experimental animals was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (European Treaty Series - No. 123).

Milk Quality and Composition

Individual milk samples were analyzed for fat and protein contents using "Lactan 1-4" milk quality analyzer ("Sibagropribor", Russia). For milk samples preparation volume of 25 cm^3 was heated in a water bath to a temperature of (45 ± 5) ° C and thoroughly mixed by transfusion from vessel to vessel (at least 3 times). Then the sample was cooled to a temperature of (22 ± 4) ° C. The prepared sample was placed in device. After 2.5-3.5 minutes, results were read from the device indicator. After each measurement, the device was washed with water and detergent according to the instructions that came with the device.

Calculation of the energy value of milk obtained from animals was made on the basis of the "Guidelines for hygienic nutrition control in organized groups" (approved by the USSR Ministry of Health on December 29, 1986 No. 4237-86).The energy value of milk was determined by multiplying the content of proteins, fats and carbohydrates (g/kg) by the corresponding coefficients of their energy value, which were equal to: for proteins - 4 kcal/g, for fats - 9 kcal / g, for carbohydrates - 4 kcal / g. Carbohydrate (lactose) content in milk was determined using infrared analyzer in accordance with GOST 32255-2013 "Milk and dairy products. An express instrumental method for determining the physicochemical identification indicators using an infrared analyzer (with Change No. 1)" at JSC "Elit GPP" in Vysokogorsky Municipal District of Tatarstan Republic.

Calculation of energy value was carried out according to the following formula:

E = ((4x (P) + 9 x (F) + 4 x (C)) x 4184) / 1000000,

where:

E - energy value of a milk dish, MJ/kg;

P, F, C - the amount of proteins, fats and carbohydrates in milk, respectively, g;

4; 9 and 4 are the energy value coefficients of proteins, fats and carbohydrates, respectively, kcal/g;

4184 - the number of J per 1 kcal;

1000000 - conversion rate J to MJ.

DNA isolation

DNA was extracted from animal blood taken from vena coccygea using a reagent kit "DNA-Sorb-B" (NextBio, Russia) for DNA extraction from the clinical material in accordance with the manufactures recommendation.

Genotyping

Animal genotypes were determined by the of economically valuable traits genes loci: kappa-casein (CSN3), beta-lactoglobulin (LGB), prolactin (PRL), somatotropin (GH), thyroglobulin (TG5). The establishment of animal genotypes was carried out by polymerase chain reaction (PCR), followed by hydrolysis of PCR products. The reaction composition for PCR, consisting of template DNA, dNTPs, Taq polymerase with supplied buffer, was prepared with a total volume of 20 μl.

For the analysis of the CSN3 gene locus, a set of primers with the following nucleotide sequence was used: F: 5'-ATCATTTATGGCCATTCCACCAAAG-3 '(25 n.); R: 5'-GCCCATTTCGCCTTCTCTFTAACAGA-3 '(26 n.). To analyze the LGB gene locus, a set of primers with the following nucleotide sequence was used: F: 5'-GTCCTTGTGCTGGACACCGACTACA-3 '(25 n.); R: 5'-CAGGACACCGGCTCCCGGTATATGA-3 '(25 n.). To analyze the PRL gene locus, a set of primers

with the following nucleotide sequence was used: F: 5'-CGAGTCCTTATGAGCTTGATTCTT-3 ', (24 n); R: 5'-GCCTTCCAGAAGTCGTTTGTTTTC-3 '(24 n.). For the analysis of the GH gene locus, a set of primers with the following nucleotide sequence was used: F: 5'-GCTGCTCCTGAGGGCCCTTC-3 '(20 n.); R: 5'-CATGACCCTCAGGTACGTCTCCG-3 '(23 n.). To analyze the TG5 gene locus, a set of primers with the following nucleotide sequence was used: F: 5'-GGGGATGACTACGAGTATGACTG-3 '(23 n.); R: 5'-GTGAAAATCTTGTGGAGGCTGTA-3 '(23 n).

Amplification was performed under optimal temperature and time conditions for each individual set of oligonucleotide primers on a MyCycler T100 programmable thermal cycler (Bio-Rad Laboratories, USA). The amplification products obtained were digested with restriction enzymes Hinf I, HaeIII, Rsa I, Alu I at 37 ° C for 16 h for the CSN3, LGB, PRL, GH, and BstX2I genes and at 60 ° C for the TG5 gene, respectively.

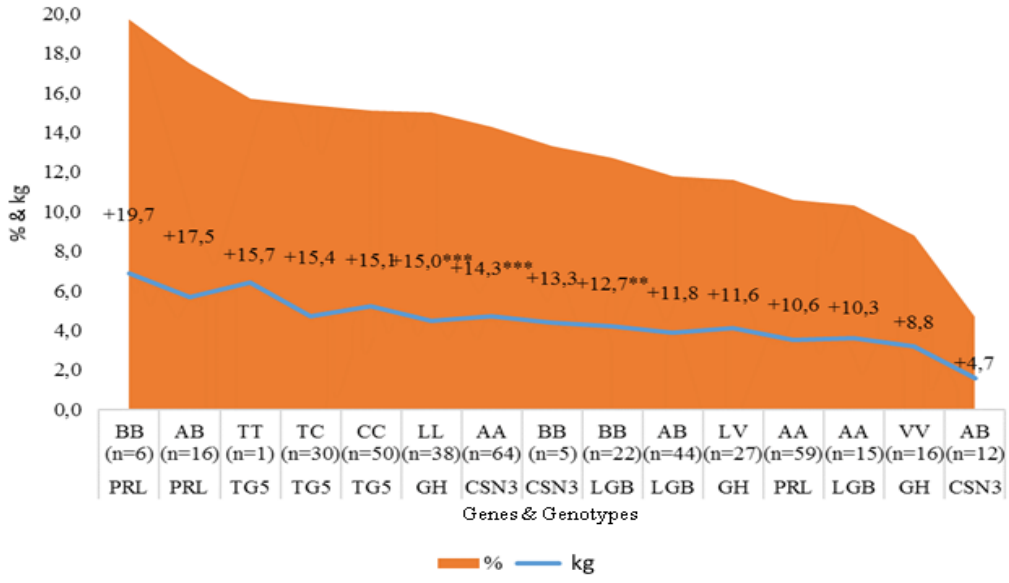
Genotyping was evaluated by running a small aliquot of PCR-RFLP product on 2.6% agarose gel in the presence of ethidium bromide in 1xTBE buffer at 20 V/cm for 30 min. Visualization and results' fixation was carried out using UV transilluminator and Gel Doc documentation system.

Statistical Analysis

Results are expressed as means ± standard deviation. The results obtained were processed using mathematical and statistical methods using the Microsoft Excel program.

3.Results and discussions

The data obtained shows that changes in milk productivity of dairy cows which were on balanced feeding diet recommended by us depended on the animal's genotype. The maximum increase in cows' productivity in terms of mass fractions of both fat and protein in milk (Figure 1) was 19.7% (or 6.9 kg) and was characteristic to animals with the BB genotype of the PRL gene.



*Note: ** - P <0.01; *** - P <0.001*

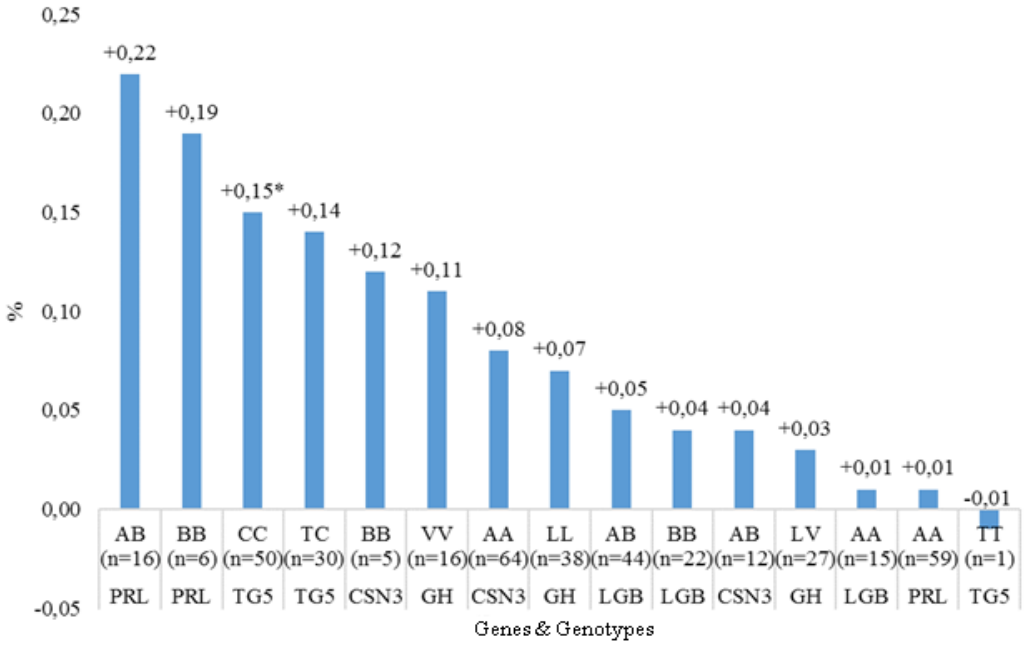
Figure 1. Changes in milk production of dairy cows in terms of the basis fat and protein mass fraction in milk.

As to TG5 gene, this change was most pronounced in the dairy cow with TT genotype - 15.7% (or 6.4 kg), and slightly smaller but statistically significant - in animals with LL genotype of GH gene - 15.0% (or 4,5 kg, P <0.001). It is necessary to note that in animals with the AA genotype of CSN3 gene milk production (14.3% or 4.7 kg) was also 0.7% lower, and these changes in productivity dynamics were statistically significant (P <0.001). As to LGB gene, the highest increase in milk productivity was found in animals with the genotype BB, which was 12.7% (or 4.2 kg, P <0.01).

Along with the dynamics of milk productivity, differences due to the influence of genotypes on mass fraction of fat and protein in cow's milk have been established. For example, the largest mass fraction of fat in milk of dairy cows was found in cows with the AA genotype of CSN3 gene (3.73%). Similar influence of the LGB gene was detected in animals with a homozygous BB genotype (3.79%). Also, the maximum content of the mass fraction of fat in milk depends on PRL gene in animals with the same genotype was found (4.03%), while for GH gene maximum of the mass fraction of fat in milk was found in animals with the heterozygous LV genotype (3.92%). The highest content of fat mass fraction in milk was characteristic to animals with a homozygous genotype for the TG5 gene (4.59%).

Analysis of the content of the mass fraction of protein in the milk of experimental animals dependent on alleles of genes studied showed that in animals with a homozygous AA genotype for the CSN3 gene, the protein content in milk was 3.31%. In dairy cows with homo and heterozygous genotypes AA and AB, respectively, of LGB gene, the content of protein mass fraction in milk was 3.27%, as well as in animals with LL and VV genotypes of GH gene. It should be noted that in animals with TT and BB genotypes for TG5 and PRL genes, the content of the protein mass fraction in milk were found to be the highest - 3.35% and 3.30% respectively, which repeats the trend in the content of the fat mass fraction in milk.

Data about dynamics of the content of fat mass fraction in milk of dairy cows (Figure 2) shows that, the maximum increase of fat content in milk of animals with the heterozygous genotype AB of PRL gene was observed (0.22%).

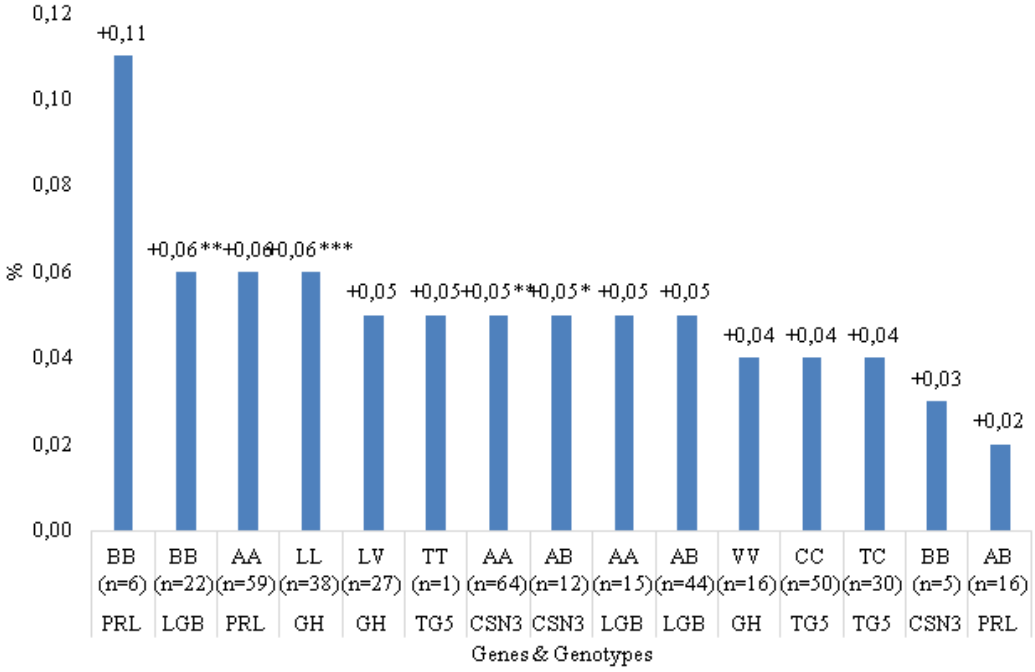


Note: * - P <0.05

Figure 2. Change in the fat mass fraction in milk of dairy cows.

As to TG5 gene, a significant increase in the content of fat mass fraction in milk was detected in homozygous animals with the CC genotype, which was 0.15% (P <0.05). In cows with a homozygous BB genotype for the CSN3 gene, the fat content in milk increased by 0.12%, in homozygous animals with a VV genotype of GH gene it increased by 0.11%, and in heterozygous animals with AB genotype of LGB gene it increased by 0.05%. However, other than described above significant changes have not been found.

Analyzing the changes in content of the protein mass fraction in milk of dairy cows (Figure 3) it was found that the most significant increase in this indicator was in animals with the homozygous BB genotype of PRL gene, which increased by 0.11%, however, this change was not statistically significant.



Note: * - P <0.05; ** - P <0.01; *** - P <0.001

Figure 3. Change in the protein mass fraction in milk of dairy cows.

A significant increase in milk yield was observed in cows with homozygous BB and LL genotypes of LGB and GH genes too, which were up to 0.06% with P <0.01 and P <0.001, respectively. In animals with genotypes AA and AB of CSN3 gene, an increase in protein mass fraction in milk was 0.05% with P <0.01 and P <0.05, respectively. The protein content in the milk of cows with a homozygous TT genotype of TG5 g gene changed to a similar value, however, the obtained increase was not significant.

In general, it should be noted that there was a positive dynamics in the content of fat and protein mass fractions in milk of dairy cows during research period, which was largely determined by the genotype of particular genes.

Obviously, changes in the content of such important indicators as the fat and protein mass fraction in the milk have to affect its energy value (Figure 4).

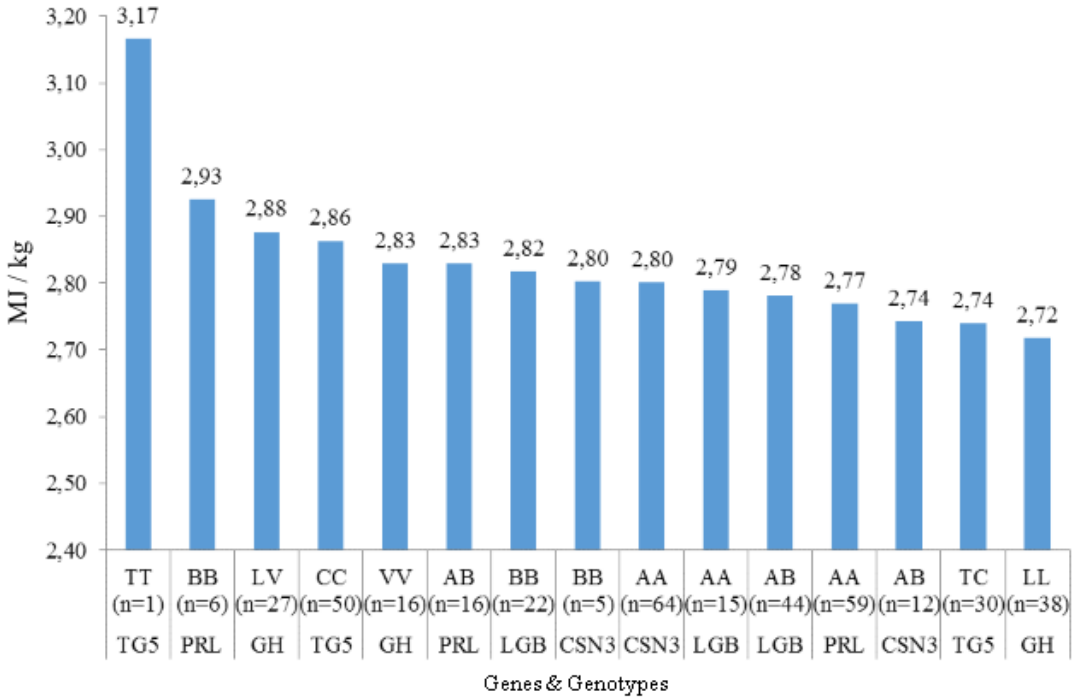


Figure 4. The energy value of milk from dairy cows

In the context of genes studied, as to CSN3 gene, the highest energy value was at milk obtained from animals with homozygous genotypes AA and BB (2.80 MJ/kg). As to LGB and PRL genes, the highest energy value was found in milk of homozygous cows with BB genotypes (2.82 and 2.93 MJ / kg). As to GH gene, the highest energy value was detected at milk obtained from animals with the heterozygous LV genotype (2.88 MJ/kg), and as to TG5 gene – at animals with the TT homozygous genotype (3.17 MJ / kg).

3.1.Discussion

We found that the highest increase in milk productivity is in dairy cows with a homozygous AA genotype of CSN3 gene and it is in accordance with data obtained by Chasovshchikova with coauthors (Chasovshchikova et al, 2017) in their studies. However, in a population of dairy cows in Hungary, Vági and Baranyi ((Vági & Baranyi, 2000) revealed that animals with heterozygous genotype AB show the highest rate in milk production. We suppose that this fact depends on the fact that they used first lactation animals in their studies and it affected on the results obtained (Vági & Baranyi, 2000).

Also, our data which shows that the presence of the BB genotype of PRL gene in dairy cows determined the highest level of milk productivity in animals that confirms previously obtained data by Nekrasov with coauthors (Nekrasov et al, 2017).

It is necessary to note that Di Gregorio with coauthors (Di Gregorio et al, 2017) associate allele B of the LGB gene in general and the BB genotype in particular with the highest values of milk yield, which is fully in accordance with our results with the same level of reliability($P < 0.01$). Metin Kiyici with coauthors (Metin Kiyici et al, 2019) considers GH gene as one of the promising candidates for assessing the potential milk production of dairy cow. In addition, according to Molee with coauthors (Molee et al, 2015), the LGB + GH gene model may be the most suitable for breeding to improve the milk production of cows. Sabour and Lin (Sabour & Lin, 1996), in turn, found that the V allele of the GH gene was preferable in terms of increasing the milk yield obtained from cows. On the contrary, Shariflou with coauthors (Shariflou et al, 2000) showed greater contribution of L allele to increase of milk yield in cows. Lucy with coauthors (Lucy et al, 1993) came to the same opinion in relation to the milk productivity of Holstein in

USA, while the influence of the V allele was most pronounced in the Jersey cattle breed.

Some other studies showed an increase in milk yield in cows with the LL genotype of the GH gene, but only in animals with the first lactation (Akyuz et al, 2015). On the other hand, Kovács with coauthors (Kovács et al, 2006) and Balogh with coauthors (Balogh et al, 2008) reported a greater contribution of the LV genotype in formation of milk productivity level. It should be noted that the absence of any connection between the genotype and the level of milk productivity was indicated by Hradecka with coauthors (Hradecka et al, 2008), while in our studies, the highest increase in milk productivity was found in animals with the LL genotype of GH gene.

It is interesting to note that, according to Zinnatova and Zinnatov (Zinnatova et al, 2014), the highest milk productivity was detected in animals with the CC genotype of TG5 gene. In our studies, the most increase in milk productivity was characteristic to animals with the TT genotype. However, due to the low frequency of its genotype, these data require additional refinement in further studies.

On the one hand, in a number of articles it was shown that different genotypes of CSN3 and LGB genes have a pronounced effect on the physicochemical milk composition (Schennink et al, 2008; Ozdemir et al, 2018; Neamt et al, 2017). On the other hand, Duifhuis-Rivera with coauthors (Duifhuis-Rivera et al, 2014) and Dogru (Dogru et al, 2015) were unable to confirm the presence of any of correlations mentioned above. At the same time a number of authors (Ozdemir et al, 2018; Van den Berg et al, 1992; Boland, & Hill, 2001; Wedholm et al, 2006; Heck et al, 2009) found that the B allele of CSN3 and LGB genes is associated with a higher protein mass fraction in cow milk. However, in our studies, as was reported above, animals with A allele of CSN3 gene (homozygous genotype AA) and LGB gene (homozygous genotype AA and heterozygous genotype AB) has the largest protein mass fraction in milk.

In addition, the presence of the A allele of CSN3 gene (homozygous AA and heterozygous AB genotypes) caused the maximum increase in protein mass fraction in milk during the study period, while for the LGB gene the most pronounced dynamics of this indicator was found in animals with B allele (homozygous genotype BB). Ambiguous data on the effect of PRL alleles on milk composition were obtained by Staiger with coauthors (Staiger et al, 2010), although our data showed that animal carriers of B allele showed not only the maximum protein content in milk, but also its most pronounced increase throughout entire research period. The influence of GH gene genotype on the protein content in cow's milk was reported by Chung with coauthors (Chung et al, 1996) and Dybus (Dybus, 2002). Moreover, according to their data, animals with homozygous LL genotype were higher in this indicator. We have confirmed the patterns they obtained, but it is it is necessary to note that animals with this genotype were characterized not only by the highest protein content, but also its most pronounced increase during the experiment. In addition, in our studies, dairy cows with VV homozygous genotype were characterized by high protein content. By the opinion of Zinnatova and Zinnatov (Zinnatova et al, 2014), individuals with the CC homozygous genotype of TG5 gene are characterized by higher milk protein production, which contradicts the our data, however, taking into account the low frequency of occurrence of animals with TT homozygous genotype, the results presented by them seemed to be most convincing.

According to results of Neamt with coauthors (Neamt et al, 2017) and Gurses with coauthors (Gurses et al, 2018), dairy cows with the AA genotype of CSN3 gene are characterized by the highest milk fat yield. In our studies, this pattern was confirmed, but, at the same time, we found the greatest influence of the homozygous BB genotype on the intensity of changes in the content of fat mass fraction in milk. In addition, Neamt with coauthors (Neamt et al, 2017) showed that the presence of AB heterozygous genotype of LGB gene in animals causes the highest fat content in milk of dairy cows, but we found that this genotype mainly affects the intensity of changes in milk fat content, while the highest milk fat content is more characteristic to animals with the homozygous BB

genotype. According to Patel and Chauhan (Patel & Chauhan, 2017), the presence of B alleles of PRL gene in animals can determine their highest milk fat content. In general, our results confirm this statement, however, if the maximum milk fat content was more typical for animals with the homozygous BB genotype, the most pronounced change in level of fat content in milk was for animals with the AB genotype.

The presence of a significant relationship between the fat content in cow's milk and the genotype of GH gene was reported by Hradecka with coauthors (Hradecka et al, 2008) and Khatami with coauthors (Khatami et al, 2005). We clarified that LV genotype determines only the content of the fat mass fraction in milk and VV genotype determines the intensity of changes in fat mass fraction in it. The homozygous TT genotype of TG5 gene according to Zinnatov with coauthors (Zinnatov et al, 2017) determines the highest milk production of dairy cows, which fact is also confirmed by us. At the same time in animals with CC homozygous genotype dynamics of fat mass fraction level in milk was found to be more pronounced.

Changes in the energy value of milk as a result of various feed products using and changes in the diet structure were reported in studies of Gafner with coauthors (Gafner et al, 2017). Our data on milk energy value are in accordance with values of this indicator established by these authors in general. Changes in the milk energy value in the context of polymorphism of genes of economically valuable traits was reported in a study by Safina (Safina, 2018), however, currently it is not possible to evaluate results obtained because of the difference in the set of marker genes studied, so additional study of this issue is required.

4.Conclusions

So, based on the data obtained we can conclude that the dynamics of milk production of dairy cows is for the most part determined by the alleles of the genes of economically valuable traits. The presence of B allele and especially BB homozygous genotype of PRL gene in studied population caused a maximum increase in their milk productivity up to 19.7%, however, this result was not reliable, and therefore it requires further investigation. The maximum statistically significant increase in dairy cows' productivity was found (15.0%, $P < 0.001$) was found in the presence of the L allele of GH gene and at homozygous LL genotype with balanced feeding. Statistically significant increase in milk productivity were also observed in animal which have alleles A and B with homozygous genotypes AA and BB for the CSN3 and LGB genes, respectively.

The maximum increase in the fat mass fraction in milk (0.22%) was found in animals with the AB genotype for the PRL gene, while the statistically significant increase in milk fat content depends on the presence of the C allele and the CC homozygous genotype of TG5 gene (0.15%, $P < 0.05$). The maximum increase in the protein mass fraction in milk in animals with the B allele and the BB homozygous genotype for the PRL gene was observed, which was to 0.11%. However, this change was not statistically significant. The maximum statistically significant increase in milk yield was found in dairy cows with B and L alleles and homozygous BB and LL genotypes for LGB and GH genes, which were up to 0.06% with $P < 0.01$ and $P < 0.001$, respectively.

Analysis of milks' energy value showed that A and B alleles and homozygous genotypes AA and BB of CSN3 gene and homozygous BB genotypes of LGB and PRL genes, as well as the T allele and TT homozygous genotype of TG5 gene, are associated with milk production with highest energy value, while as to GH gene it was found that this indicator was the highest in animals with heterozygous LV genotype. However, due to the lack of statistically significant changes in the energy value of milk, the data obtained required further clarification.

5.References

Akyuz, B., Agaoglu, O.K., Akcay, A., & Agaoglu, A.R. (2015) Effects of DGAT1 and GH1 polymorphism on milk yield in Holstein cows reared in Turkey. *Slovenian Veterinary Research*, 52, 185 – 191.

Balogh, O., Szepes, O., Kovacs, K., Kulcsar, M., Reiczigel, J., Alcazar, J.A., Keresztes, M., Febel, H., Bartyik, J., Fekete, S.Gy., Fesus, L., & Huszenicza, Gy. (2008) Interrelationships of growth hormone AluI polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. *Veterinarni Medicina*, 53(11), 604 – 616.

Boland, M., & Hill, J. (2001) Genetic selection to increase cheese yield: The Kaikoura experience. *Australian Journal of Dairy Technology*, 56, 171 – 176.

Chasovshchikova, M.A., Sheveleva, O.M., Svjazhenina, M.A., Tatarkina, N.I., Satkeeva, A.B., Bakharev, A.A., Ponomareva, E.A., & Koshchaev, A.G. (2017) Relationship between the genetic variants of kappa-casein and prolactin and the productive-biological characteristics of cows of the black-motley breed. *Journal of Pharmaceutical Sciences and Research,* 9(7), 1038 – 1044.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel J., & Doreau, M. (2007) Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology*, 109, 828 – 855.

Chung, E.R. Rhim T.J., & Han S.K. (1996) Associations between PCR-RFLP markers of GH and PRL genes and production traits. *Korean Journal of Animal Science*, 38, 321 – 336.

Di Gregorio, P., Di Grigoli, A., Di Trana, A., Alabiso, M., Maniaci, G., Rando, A., Valluzzi, C., Finizio, D., & Bonanno, A. (2017) Effects of different genotypes at the CSN3 and LGB loci on milk and cheese-making characteristics of the bovine cinisara breed. *International Dairy Journal*, 71, 1 – 5.

Dogru, U. (2015) β-Lactoglobulin genetic variants in Brown Swiss dairy cattle and their association with milk yield and quality traits. *Journal of Animal and Plant Sciences*, 25(2), 595 – 598.

Duifhuis-Rivera, Lemus-Flores T. C., Ayala-Valdovinos M. Á., Sánchez -Chiprés D. R., Galindo-García J., Mejía-Martínez K. & González-Covarrubias E. (2014) Polymorphisms in beta and kappa casein are not associated with milk production in two highly technified populations of Holstein cattle in México. *Journal of Animal and Plant Sciences,* 24(5), 1316 – 1321.

Dybus, A. (2002) Associations between Leu/Val polymorphism of growth hormone gene and milk production traits in Black and White cattle. *Archiv fur Tierzucht*, 45, 421 – 428.

Dybus, A., Swatowska I., Czerniawska-Piatkowska E., Grzesiak W., Wojcik J., Rzewucka E., & Zych, S. (2004) PIT1-HinfI gene polymorphism and its association with milk production traits in polish Black and White cattle. *Archiv fur Tierzucht*, 6, 557 – 563.

Furaeva N.S. (2013) Current state of pedigree base of a cattle of the Yaroslavl breed and prospect of its development. *Herald of Agroindustrial complex of Upper Volga region,* (1(21)), 21 – 30. (in Russian).

Gafner, V.D., Gorelik, O.V., & Bykova, O.A. (2017). Milk yields and quality obtained from cows fed rations supplemented with triticale. *News of the Orenburg State Agrarian University*, (4 (66)), 171 – 174. (in Russian).

Giniyatullin Sh.Sh. (2016) Cow feeding according to lactation periods and measures to increase milk yields. *Russian electronic scientific journal,* (1(19)), 263 – 279. (in Russian).

Gurses, M., Yuce, H. Onalan Etem, E. & Patir, B. (2018). Polymorphisms of kappa-casein gene and their effects on milk production traits in Holstein, Jersey and Brown Swiss cattle. *Animal Production Science*, 58, 778 – 784.

Heck, J.M.L., Schennink A., Valenberg H.J.F., Bovenhuis H., Visker M.H.P.W., van Arendonk J.A.M., & van Hooijdonk A.C.M. (2009) Effects of milk protein variants on the protein composition of bovine milk. *Journal of Dairy Science,* 92, 1192 – 1202.

Hradecka, E., Citek, J., Panicke, L., Rehout, V., & Hanusova, L. (2008) The relation of GH1, GHR and DGAT1 polymorphisms with estimated breeding values for milk production traits of German Holstein sires. *Czech Journal of Animal Science*, 53, 238 – 245.

Khatami S. R., Lazebny O. E., Maksimenko V. F., Sulimova G. E. (2005) Association of DNA polymorphisms of the growth hormone and prolactin genes with milk productivity in Yaroslavl and Black-and-White cattle. *Russian Journal of Genetics*, 41(2), 167–173.

Kovács, K., Völgyi-Csík J., Zsolnai A., Györkös I., & Fésüs L. (2006) Associations between the AluI polymorphism of growth hormone gene and production and reproduction traits in a hungarian holstein-friesian bull dam population. *Archives Animal Breeding*, 49(3): 236 – 249.

Lucy, M.C. Hauser, S.D., Eppard, P.J., Krivi, G.G., Clark, J.H., Bauman, D.E., & Collier, R.J. (1993) Variants of somatotropin in cattle: gene frequencies in mayor dairy breeds and associated milk production. *Domestic Animal Endocrinology*, 10, 325 – 333.

Maslyuk, A.N., & Tokareva, M.A. (2018). Optimization efficiency of protein and carbohydrate nutrition of highly productive cows. *Animal Husbandry and Fodder Production*, 101(4), 164-171. (in Russian).

Metin Kiyici, J., Arslan, K., Akyuz, B., Kaliber, M., Akse,l E.G., & Çinar, M.U. (2019) Relationships between polymorphisms of growth hormone, leptin and myogenic factor 5 genes with some milk yield traits in holstein dairy cows. *International Journal of Dairy Technology*, 72(1), 1 – 7.

Molee, A., Poompramun, C., & Mernkrathoke, P. (2015) Effect of casein genes - beta-LGB, DGAT1, GH, and LHR - on milk production and milk composition traits in crossbred Holsteins. *Genetics and Molecular Research*, 14(1), 2561 – 2571.

Neamt, R.I. Saplacan G., Acatincai S., Cziszter LT., Gavojdian D., & Ilie D.E. (2017) The influence of CSN3 and LGB polymorphism on milk production and chemical composition in Romanian Simmental cattle. *Acta Biochimica Polonica*, 64(3), 493 – 497.

Nekrasov, D.K. Kolganov, A.E., Kalashnikova, L.A., & Semashkin, A.V. (2017) The relationship of polymorphic variants of genes of the prolactin, growth hormone and kappa-casein with milk productivity of Yaroslavl breed cattle. *Agrarnyj vestnik Verhnevolzh'ja. [Agrarian Journal of the Upper Volga Region]*, (1(18)), 40-48 (in Russian).

Ozdemir, M,. Kopuzlu, S., Topal, M., & Bilgin O.C. (2018) Relationships between milk protein polymorphisms and production traits in cattle: a systematic review and meta-analysis. *Archives Animal Breeding*, 61(2), 197 – 206.

Patel, J.B. & Chauhan , J.B. (2017) Polymorphism of the prolactin gene and its relationship with milk production in gir and kankrej cattle. *Journal of Natural Science, Biology and Medicine*, 8, 167 – 170.

Perišić, P., Skalicki, Z., & Bogdanović, V. (2011).The state in the sector of milk production in European Union and in our country. *Biotechnology in Animal Husbandry*, 27(3), 315-327.

Sabour, M.P., & Lin, C.Y. (1996) Association of bGH genetic variants with milk production traits in Holstein cattle. *Animal Genetics*, 27(Suppl.2), 105.

Safina, N. (2018). Characterization of biological efficiency and full value of milk productivity in holstein heifers with different leptin (LEP) genotypes. *Vestnik of Kursk State Agricultural Academy*, (4), 131 – 134. (in Russian).

Schennink, A., Heck J.M.L., Bovenhuis H., Visker M.H.P.W., van Valenberg H.J.F., & van Arendonk J.A.M. (2008) Milk fatty acid unsaturation: Genetic parameters and effects of stearoyl-CoA desaturase (SCD1) and acyl CoA: diacylglycerol acyltransferase 1 (DGAT1). *Journal of Dairy Science*, 91, 2135 – 2143.

Shariflou, M.R., Moran, C., & Nicholas F.W. (2000) Association of Leu127 variant of the bovine growth hormone (bGH) gene with increased yield of milk, fat, and protein in Australian Holstein Friesians. *Australian Journal of Agricultural Research*, 51, 515 – 522.

Singh, U., Deb, R., Alyethodi, R. R., Alex, R., Kumar, S., Chakraborty, S., Dhama, K., & Sharma, A. (2014). Molecular markers and their applications in cattle genetic research: A

review. *Biomarkers and Genomic Medicine,* 6(2), 49–58.

Staiger, E.A. Thonney M.L., Buchanan J.W., Rogers E.R., Oltenacu P.A., & Mateescu R.G. (2010) Effect of prolactin, β-lactoglobulin, and κ-casein genotype on milk yield in East Friesian sheep. *Journal of Dairy Science*, 93(4), 1736 – 1742.

Turenkova E., & Vasilieva O. (2014) Feeding is a key factor for long productive life of a dairy cow. *Farm Animals*, (2(6)), 98 – 108.

Vági, J., & Baranyi, M. (2000) Association between milk protein genotypes and milk production and fertility in Hungarian Holstein Friesian, Hungarian Flekvieh and Hungarofries herds. *Állattenyésztés és Takarmányozás*, 49, 107 – 119.

Van den Berg, G., Escher J.T.M., De Koning P.J., & Bovenhuis H. (1992) Genetic polymorphism of κ-casein and β-lactoglobulin in relation to milk composition and processing properties. *Nederlands melk en Zuiveltijdschrift*, 46, 145 – 168.

Viale, E., Tiezzi, F., Maretto, F., De Marchi, M., Penasa, M., & Cassandro, M. (2017). Association of candidate gene polymorphisms with milk technological traits, yield, composition, and somatic cell score in Italian Holstein-Friesian sires. *Journal of Dairy Science,* 100(9): 7271–7281.

Wedholm, A., Larsen, L.B., Lindmark-Månsson, H., Karlsson, A.H., & Andrén A. (2006) Effect of protein composition on the cheesemaking properties of milk from individual dairy cows. *Journal of Dairy Science*, 89: 3296 – 3305.

Zhang L.P., Gan Q.F., Hou G.Y., Gao H.J., Li J.Y. & Xu S.Z. (2015) Investigation of TG gene variants and their effects on growth, carcass composition, and meat quality traits in Chinese steers. *Genetics and Molecular Research,* 14 (2): 5320-5326

Zinnatov F.F., Shamsova A.R., Zinnatova F.F., Akhmetov T.M, & Safiullina A.R. (2017). Interrelation of polymorphism of lipid metabolism genes (LEP, TG5) with milk production of cattle. *Scientific notes of Kazan State Academy of Veterinary Medicine named after N.E. Bauman*, 231 (3): 72 – 75. (in Russian).

Zinnatova, F.F., & Zinnatov, F.F. (2014) Role of lipid metabolism genes (DGAT1, TG5) in improving economically valuable traits cattle. *Scientific notes of Kazan State Academy of Veterinary Medicine named after N.E. Bauman*, 219(3): 164 – 168. (in Russian).

Acknowledgment

The work is a part of a state assignment: “Mobilizing the genetic resources of plants and animals, creating innovations that ensure the production of biologically valuable food products with maximum safety for human health and the environment”. Registration number: AAAA-A18-118031390148-1.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECT OF SODIUM CHLORIDE ON FAT OXIDATION IN THE PRESENCE OF HEME PIGMENTS

E.K.Tunieva [1*], A.A. Semenova [1], A.N. Ivankin [2], V.V. Nasonova [1], A.N. Nikolaeva [1]

[1]V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, 109316, Moscow, Talalikhina str., 26,

[2]Bauman Moscow State Technical University, Moscow, Russia

**e.tunieva@fncps.ru*

https://doi.org/10.34302/2019.11.4.10

Article history:
Received:
29 April 2019
Accepted:
29 November 2019

Keywords:
Back fat;
Fatty acid composition;
Heme iron;
Myoglobin;
Salt

ABSTRACT

The effects of salt on oxidative changes in fat and heme pigments were evaluated. The back fat was salted with sodium chloride in the presence of the hemoglobin. The indicators of the hydrolytic and oxidative spoilage were investigated in the samples of back fat from White Large pigs at two years of age. The proportions of heme and non-heme iron, as well as the content of metmyoglobin were determined in the samples of m. *Longissimus dorsi* from two-year-old female Large White pigs. Back fat salting did not significantly affect the acid value of back fat. Addition of salt in amounts of 3.5 % and 5.0 % initiated the oxidative changes. Addition of sodium chloride initiated fatty acid oxidation in the presence of the heme pigments. The content of unsaturated acids decreased by 22.8 % when 5.0 % of salt were added. The proportion of heme iron increased by 6.5 % relative to free iron when 5.0 % of sodium chloride were added. The positive correlation between the metmyoglobin content and the thiobarbituric acid value was found with the correlation coefficient of 0.98.

1.Introduction

For several decades, scientists have been studying the effect of meat salting on the oxidative changes. The data available from the scientific technical literature about the pro- and anti-oxidative effects of salt do not allow formulation of the unified view on the mechanism of the sodium chloride effect on fat oxidation. For example, according to some data, sodium chloride has the anti-oxidative effect (Andres *et al.*, 2004) or does not influence the fat chemical properties (Calligaris and Nicoli, 2006). On the contrary, several researchers found the pro-oxidative effect of sodium chloride (Gheisari *et al.*, 2010; Rhee and Ziprin, 2001; Jin *et al.*, 2012). Sarraga *et al.* (2002) established that addition of sodium chloride inhibited the activity of the antioxidant enzyme, glutathione peroxidase, but on the other hand salting facilitated a decrease in the thiobarbituric acid value. There are data about interrelations between oxidation of lipids and myoglobin; oxidation of one of these components leads to formation of chemical compounds that can catalyze oxidation of another (Faustman *et al.*, 2010; Min and Ahn, 2005; Min *et al.*, 2010). Many hypotheses explain the chemistry of the oxidative processes under the impact of sodium chloride. Among the mare the action of the reactive chloride ion on lipids or modification of protein heme that catalyzes lipid oxidation (Ladikos and Lougovois, 1990), interaction of the heme iron ions with the chlorine ion, displacement of the iron ion from

the protein macromolecule under an impact of the sodium ion (Zanardi *et al.*, 2010), *etc*. Incoherence of data on the salt effect on the chemical changes in fats, apparently, can be explained by differences in selection of research samples, when different meat types with different content of heme pigments as well as different types and parameters of meat technological processing were mainly used. With this experiment setting, the number of factors influencing the processes of hydrolysis and oxidation upon salting significantly increases and can be a consequence of only indirect effect of sodium chloride on fats. It is worth noting that among these factors are the dose of sodium chloride, the presence of the heme pigments, heat treatment and so on (Jin *et al.*, 2010; Cobos *et al.*, 2008).

Despite different explanations of the mechanism of salt action on chemical changes in fats, the majority of researches link the pro-oxidative effect of sodium chloride with its impact on heme iron. Nevertheless, an iron type and a mechanism of its influence on the oxidative processes still leave many questions unanswered. Therefore, the aim of this study was to investigate the relationship between of the fat and heme pigments oxidation under the impact of sodium chloride.

2. Materials and methods

2.1. Materials

2.1.1. Back fat samples

The indicators of the hydrolytic and oxidative spoilage were determined in the samples of back fat from White Large pigs at two years of age. Taking into consideration the technological doses of sodium chloride in the recipes of sausages and products from meat, a range of sodium chloride concentrations from 2.0 to 5.0 % was chosen for the experiment. For homogeneous salt distribution, back fat was minced in a grinder through a plate with a hole diameter of 2-3 mm and salted with salt in amounts of 0.0, 2.0, 3.5 and 5.0 % in the presence of the hemoglobin solution (160g/l) in an amount of 1.25 % of back fat weight as this dose ensures the concentration of heme iron in back fat equal to the concentration of heme iron in myoglobin contained in 100 g of pork. After salting, back fat was vacuumed and cooked in a water bath at a temperature of 80 °C, then chilled and stored at a temperature of 4±2 °C for 3 days.

2.1.2 Meat samples

The proportions of heme and non-heme iron, as well as the content of metmyoglobin were determined in the samples of m. *Longissimus dorsi* from two-year-old female Large White pigs. Meat was minced in a grinder through a plate with a hole diameter of 2-3 mm and salted with table salt in amounts of 0.0, 2.0, 3.5 and 5.0 %. The prepared samples were held at a temperature of 4±2°C for 24 hours, after which they were packed under vacuum and subjected to heat treatment until reaching a temperature of 72±2°C.

2.2 Methods

The acid value was determined by the method based on titration of free fatty acids in the ether-alcohol solution of fat with the aqueous solution of alkaline. 20 g of the sample were ground in a mortar with 40 g of anhydrous sodium sulfate until smooth, the mixture was extracted for 5 min with 100 ml of chloroform in a laboratory shaker, then filtered through a paper filter. 10 ml of the filtrate was mixed with 10 ml of ethanol and 2 drops of a 1% aqueous-alcoholic solution of phenolphthalein and titrated with a 0.1 M sodium hydroxide solution until a faint pink color was observed that was stable for 30 sec. The acid number X, mg KOH / g of fat contained in the sample was calculated by the formula: $X = 5.61 \cdot V \cdot K / m$, where V is the volume of a 0.1 M solution of potassium hydroxide used for titration, ml; K is the correction factor to the potassium hydroxide solution; 5.61 – the amount of potassium hydroxide contained in 1 ml of a 0.1 M solution; m is the mass of fat in the sample, determined gravimetrically in the filtrate, g.

Peroxide value by the method based on oxidation of iodhydric acid with peroxides contained in fat with the following titration of

released iodine with sodium thiosulphate. For this, 50 g of the sample were ground in a porcelain mortar with 100 g of anhydrous sodium sulfate to a homogeneous mass, extracted with 150 ml of chloroform in a laboratory shaker for 5 min and filtered through a paper filter. 10 ml of a fat-containing extract was mixed with 10 ml of glacial acetic acid and 1 ml of a 50% freshly prepared potassium iodide solution, the flask was immediately closed, the contents were stirred, and left for 5 min in a dark place at room temperature 22 °C. Then, 100 ml of distilled water was poured into the flask, thoroughly mixed, 1 ml of a 1% starch solution was added. The liberated iodine was titrated with a 0.01 M sodium thiosulfate solution until a milky white color, stable for 5 s. The peroxide value *X*, mmol of active oxygen / kg of fat (mmol O_2/kg) contained in the sample, was calculated by the formula $X = (V_1–V_2) \cdot C \cdot K \cdot 1000 / m$, where V_1 is the volume of sodium thiosulfate solution used in the analysis, ml; V_2 is the volume of sodium thiosulfate solution used in the control determination, ml; *C* is the concentration of the used sodium thiosulfate solution, mol / L; *K* is the correction factor for the titer of sodium thiosulfate solution; 1000 – coefficient taking into account the conversion of the measurement result in mmol / kg; *m* is the mass of a portion of fat, determined gravimetrically in the extract, g.

Determination of the thiobarbituric acid value was carried out by the method based on the development of stained substances as a result of interaction of fat oxidation products with 2-thiobarbituric acid and measurement of the color intensity on a spectrophotometer (Zhuravskaya *et al.*, 1985). 50 g of the sample were homogenized with 50 ml of distilled water, 47.5 ml of water and 2.5 ml of a 3 M hydrochloric acid solution were added, the mixture was placed in a distillation apparatus, and distillation was distilled off to collect 50 ml of distillate. 5 ml of the obtained distillate was mixed with 5 ml of a 0.02 M solution of thiobarbituric acid and kept for 35 minutes at a temperature of 100 ° C. In parallel, control was carried out with 5 ml of distilled water instead of distillate. The solutions were cooled for 10 min to a temperature of 25 °C and the absorbance was measured at a wavelength of 535 ± 10 nm. Thiobarbituric number *X*, mg malondialdehyde per kg of product, was calculated by the formula: $X = D \cdot 7.8$, where *D* is the optical density of the solution; 7.8 - empirical coefficient.

The fatty acid composition was determined using a flame ionization detector by the method of gas chromatography (Ivankin *et al.*, 2016). For this, a 10-g weighed portion of a sample was treated for 3–24 h with a mixture of 10 mL of chloroform and 10 mL of methanol by the modified Folch method in the presence of a 1% KCl solution to dissolve the lipid component; the extract was filtered through paper and evaporated to dryness. Then, 0.01 g of the residue was mixed with 3 mL of a 15% solution of acetyl chloride in methanol; the mixture was incubated for 2 h at 100°C; and the pH of the mixture was adjusted to 5.0–6.0 by adding a potassium hydroxide solution in methanol. Three milliliters of a saturated NaCl aqueous solution and 3 mL of hexane were added to the resulting mixture. The solution was allowed to stand for several minutes, and 0.2 mL of the clear hexane layer containing fatty acid methylesters was sampled for analysis. The fatty acid composition was determined using a 7890A gas chromatograph with a HP-Innowax capillary column (0.2 mm in diameter, 30 m in length, and with the thickness of the stationary phase layer of 0.33 μm) and a flame ionization detector (FID). Conditions of chromatography using a HP-Innowax capillary column with a FID: the temperature in the column oven was increased from 100 to 260 °C at a rate of 10°/min; the injector temperature was 250 °C; and the detector temperature was 300 °C. Nitrogen was used as a carrier gas with the flow rate of 20 mL/min; the hydrogen flow rate was 35 mL/min; the injection sample volume was 1 μL; and the split flow ratio was 1 : 100. The total analysis time was 30 min. The amount of an analyte was assessed by comparing its peak area with the peak area of an internal standard. Calculation of the content of individual fatty acids according to

an automatic program, taking into account graduation by standard substances.

Determination of iron in the samples was carried out by acid mineralization under pressure using 10 ml HNO_3 in the MARS 6 system. After mineralization, the solutions are transferred to a 50 ml flask and brought to the mark with distilled water. Analytical determination of iron concentration was carried out on the atomic absorption spectrophotometer with electrothermal atomization Agilent 280Z. Measurements were carried out using hollow cathode lamps at a wavelength of 248.3 nm, slit width 0.2 nm, with background correction. The measurement parameters were selected according to the specified parameters of the equipment used.

Heme iron was determined by the acidified acetone extraction method (Hornsey, 1956) with modifications. Meat (5 g) was transferred to a 50 ml Polypropylene centrifuge tube and 10.0 ml of acidified acetone (95.7 % acetone; 2.4% HCl) was added. The suspension was homogenized for 30 S at 13.500 rpm using Ultra Turrax T25, which was then washed 3 times with 3.0 ml of acidified acetone. The final concentration in the soluble phase of the suspension was 80% acetone and 2.0 % HCl. The suspension was quickly stirred and kept on ice for 1 hour. Insoluble substances were previously precipitated by centrifugation (1 hour, 0 ^oC, 10 000 g) and 5 ml of supernatant was filtered through a Minisart RC 15 filter. The filtrate absorption was measured at 640 nm and the iron heme content was calculated. All filtered samples were visually checked for turbidity before measurement. The turbid samples were filtered again before measurement.

Absorption spectra of MetMb, DeoMb and OxyMb solutions were obtained using a Varian Cary Bio-50 spectrophotometer (USA) with a 1 cm path length cuvette from 650 nm to 475 nm.

2.3 Statistical analysis

Each experiment was carried out in three replic ations. Data are reported as mean values with standard deviation. The statistical significance of differences between indicators was assessed using the Student's t-test.P values less than 0.05 were considered statistically significant.

3. Results anddiscussions

3.1. Fat hydrolysis and oxidation

Meat and meat products are a complex multi-component system, which significantly complicates acquisition of reliable data about an effect of sodium chloride on fat oxidation in the presence of many biochemical compounds of animal raw materials and different technological factors, among which are the heme pigments and temperature factor, which can affect the anti- and pro-oxidative activity of salt. In this connection, the indicators of the hydrolytic and oxidative spoilage of salted back fat in the absence and presence of the heme pigments after heat treatment were studied.

Addition of table salt in the presence of heme pigments did not significantly influence the hydrolytic changes in back fat – the acid value ($p>0.05$) (Fig. 1). Back fat salting with sodium chloride in an amount of 3.5 % facilitated an increase in the peroxide value by 37.6 % ($p<0.05$), the further increase in the salt dose up to 5.0 % initiated the growth of the peroxide value by 73.2 % ($p<0.05$) compared to unsalted back fat (Fig. 2). The similar trend was observed in the measurement of the thiobarbituric acid value. Addition of sodium chloride in amounts of 3.5 % and 5.0 % led to an increase in the thiobarbituric acid value by 43.9 % ($p<0.05$) and 80.7 % ($p<0.05$), respectively (Fig. 3).

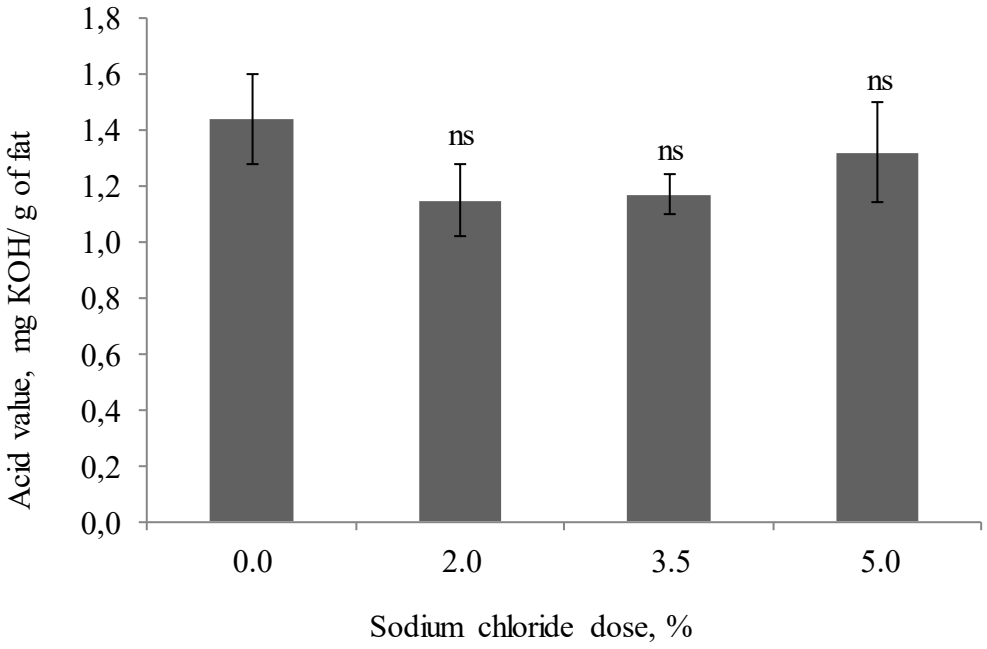


Figure 1. Effect of sodium chloride on the acid value in back fat in the presence of heme pigments
[ns]not significant at p≥0.05 in comparison with unsalted sample.

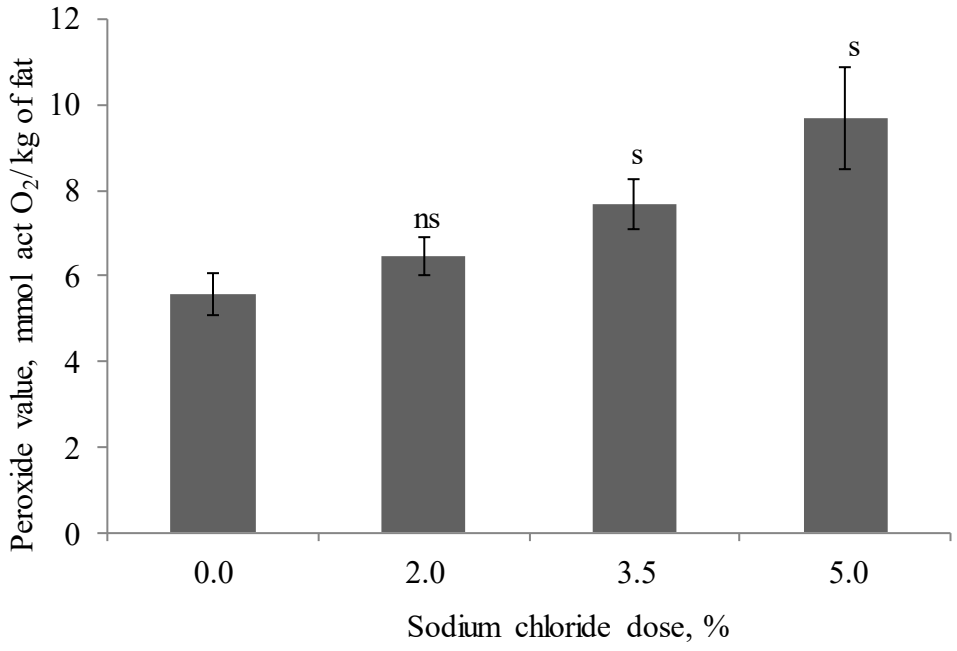


Figure 2. Effect of sodium chloride on the peroxide value in back fat in the presence of heme pigments
[ns]not significant at p≥0.05; [s] significant at p<0.05 in comparison with unsalted sample.

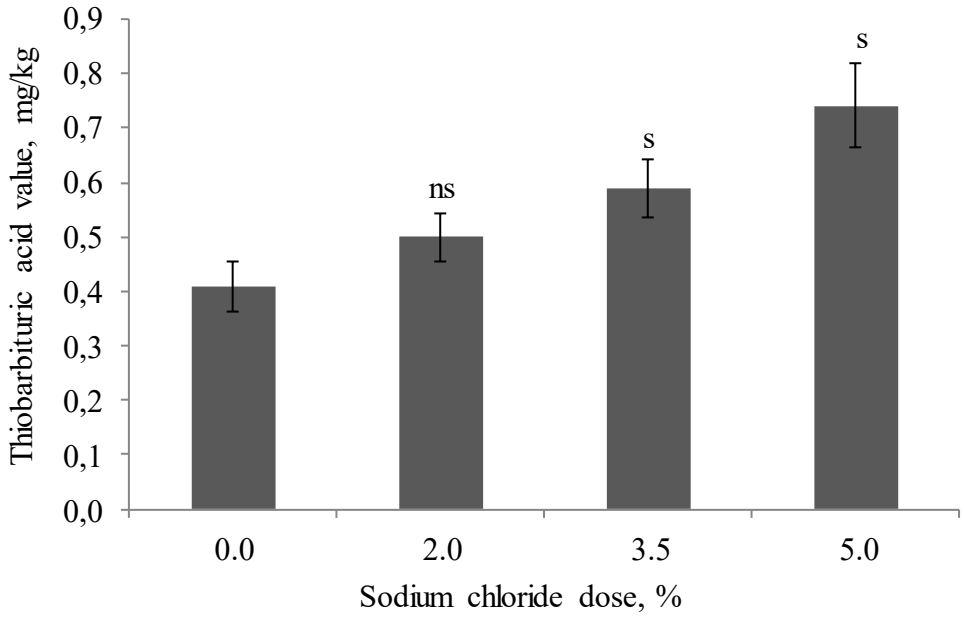


Figure 3. Effect of sodium chloride on the thiobarbituric acid value in back fat in the presence of heme pigments

[ns]not significant at $p \geq 0.05$; [s] significant at $p < 0.05$ in comparison with unsalted sample.

The studies on the effect of different salt doses on back fat oxidation in the absence of hemoglobin demonstrated that addition of salt into the back fat composition did not result in an increase in the indicators of the hydrolytic and oxidative spoilage. On the contrary, addition of 2.0% of salt caused a decrease in the oxidation product (Tunieva *et al.*, 2017).The antioxidative effect of small doses of salt can be explained by an effect of sodium chloride on a decrease in the water activity, which retards fat oxidation, and by the absence of the heme pigments in back fat. Compared to these data, the presence of heme initiated the oxidative changes in unsalted back fat by 2.2 times ($p<0.05$) for the peroxide value and 1.5 times ($p<0.05$) for the thiobarbituric acid value.

Therefore, the presence of the heme pigments changes the character of the table salt effect on lipid oxidation initiating chemical changes in fat in the presence of sodium chloride. The obtained data correspond to the results of the study on the volatiles composition of salted back fat, which suggest that in the presence of the heme pigments, addition of even small amounts of sodium chloride (2.0 %) initiates the oxidative processes compared to unsalted back fat (Tunieva and Ivankin, 2015).

Addition of table salt in the presence of heme pigments did not significantly influence the hydrolytic changes in back fat – the acid value ($p>0.05$). Back fat salting with sodium chloride in an amount of 3.5 % facilitated an increase in the peroxide value by 37.6 % ($p<0.05$), the further increase in the salt dose up to 5.0 % initiated the growth of the peroxide value by 73.2 % ($p<0.05$)compared to unsalted back fat. The similar trend was observed in the measurement of the thiobarbituric acid value. Addition of sodium chloride in amounts of 3.5 % and 5.0 % led to an increase in the thiobarbituric acid value by 43.9 % ($p<0.05$) and 80.7 % ($p<0.05$), respectively.

The studies on the effect of different salt doses on back fat oxidation in the absence of hemoglobin demonstrated that addition of salt into the back fat composition did not result in an increase in the indicators of the hydrolytic and oxidative spoilage. On the contrary, addition of 2.0% ofsalt caused a decrease in the oxidation

product (Tunieva *et al.*, 2017).The antioxidative effect of small doses of salt can be explained by an effect of sodium chloride on a decrease in the water activity, which retards fat oxidation, and by the absence of the heme pigments in back fat. Compared to these data, the presence of heme initiated the oxidative changes in unsalted back fat by 2.2 times ($p<0.05$) for the peroxide value and 1.5 times ($p<0.05$) for the thiobarbituric acid value.

Therefore, the presence of the heme pigments changes the character of the salt effect on lipid oxidation initiating chemical changes in fat in the presence of sodium chloride. The obtained data correspond to the results of the study on the volatiles composition of salted back fat, which suggest that in the presence of the heme pigments, addition of even small amounts of sodium chloride (2,0 %) initiates the oxidative processes compared to unsalted back fat (Tunieva and Ivankin, 2015).

3.2. Fatty acid composition

Back fat salting did not significantly influence the changes in the fatty acid composition in the absence of heme pigments ($p>0.05$). Addition of the hemoglobin solution led to changes in the character of the table salt effect on the fatty acid composition of back fat after heat treatment (Fig. 4). Addition of sodium chloride initiated oxidation of fatty acids. The quantity of unsaturated acids in the sample salted with 5.0 % of table salt decreased by 22.8 % relative to their content in the sample of unsalted back fat ($p<0.05$).

Therefore, the presence of the heme pigments initiated the oxidative changes in lipids during salting. The acceleration of the oxidative processes in the presence of salt is a consequence of the indirect pro-oxidative effect of sodium chloride linked with its impact on the heme pigments.

3.3. Heme and non-heme iron

To establish the mechanism of the heme pigment involvement in the development of the oxidative processes within the framework of the experiment, an effect of salt on the ratio of heme and non-heme iron was studied. The available data on the salt effect on the heme pigments during heat treatment are ambiguous. For example, the research of Kristensen and Purslow (2001) indicates an increase in the proportion of heme iron relative to free iron during heat treatment in the pork samples salted with sodium chloride. It is necessary to note that this positive trend was established only for heat treated salted meat as the studies on the effect of table salt on the meat heme pigments before cooking indicated the absence of significant differences in the content of heme and non-heme iron. On the contrary, Min *et al*. (2010) found that addition of sodium chloride to meat products from beef led to degradation of myoglobin, which facilitated an increase in the concentration of free iron in meat.

The results of our studies on determination of heme and non-heme iron indicate an increase in the proportion of heme iron relative to free iron by 6.5% as a result of salting upon addition of sodium chloride in an amount of 5.0 % ($p<0.05$) (Fig. 5).It is obvious that addition of sodium chloride stabilizes the heme pigments and prevents their destruction during heat treatment. Therefore, the negative correlation between heat denaturation of the heme pigments and oxidative changes was found. This effect can be explained differently: the lower catalyzing effect of free iron compared to heme iron and a role played by a form of iron oxidation, not by an iron type. The obtained results correspond to the study of Johns *et al*. (1989) who established that heme iron had a greater pro-oxidative effect compared to free iron.

Several studies give evidence of the relationship of heme and non-heme iron with fat oxidation. Rhee and Ziprin (1987) found that the heme iron concentration was more significant than an amount of non-heme iron when predicting lipid oxidation in meat. Other studies (Baron and Andersen, 2002; Tichivangana and Morrissey, 1985) demonstrated the stronger catalyzing effect of non-heme iron on meat product oxidation compared to heme iron.

Taking into consideration such ambiguous results of available studies and the fact that heme iron can also be present in the oxidized forms of myoglobin, we studied the changes in the proportion of metmyoglobin relative to the total content of the heme pigments depending on addition of sodium chloride (Fig.6).

The results showed that the ratio of metmyoglobin to the total content of the myoglobin forms increased with an increase in the salt dose. Therefore, the positive correlation was established between the metmyoglobin content and the thiobarbituric acid value (correlation coefficient 0.98%). The obtained results are consistent with other studies that established the relationship between oxidation of myoglobin and meat lipids (Rhee and Ziprin, 2001; Andersen and Skibsted, 1991; Yin and Faustman, 1993).

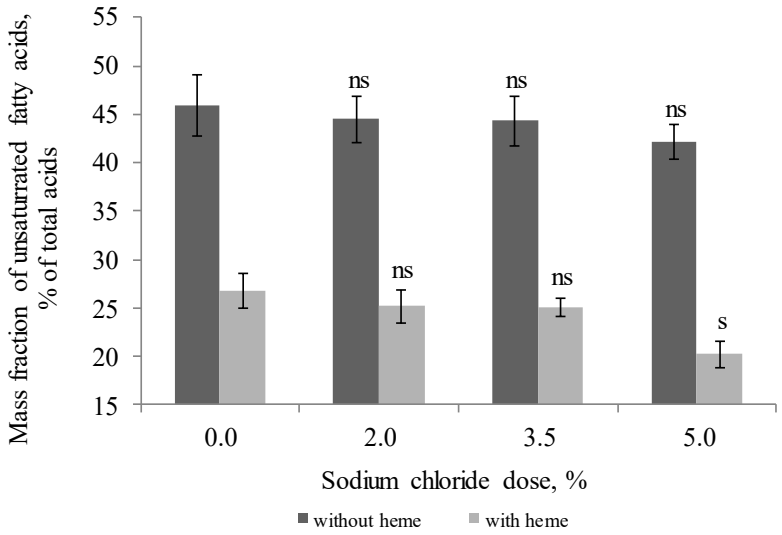


Figure 4. Dynamics of changes in the mass fraction of unsaturated fatty acids in back fat depending on the sodium chloride concentration

Averages in the same line do not differ significantly (ns) at $p \geq 0.05$or differ (s) at $p < 0.05$ from unsalted sample.

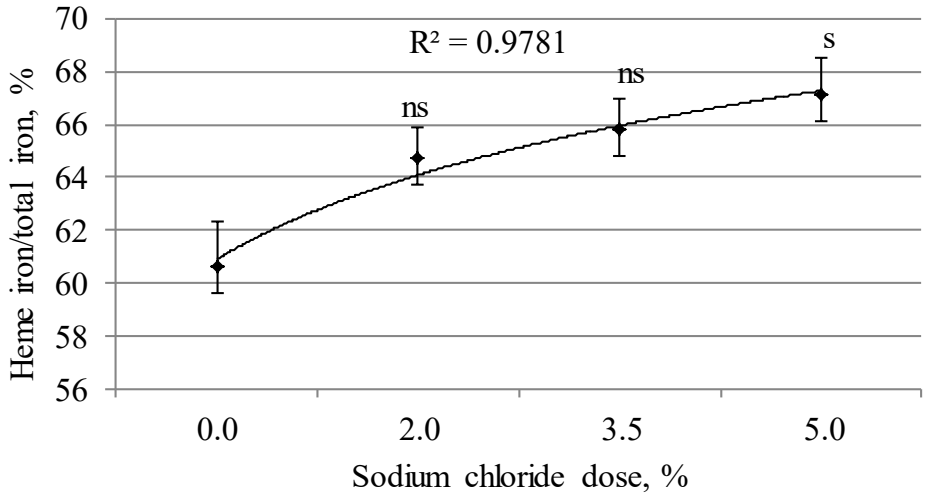


Figure 5. Changes in the proportion of heme iron relative to total iron depending on the sodium chloride concentration

[ns]not significant at p≥0.05; [s] significant at p<0.05 in comparison with unsalted sample

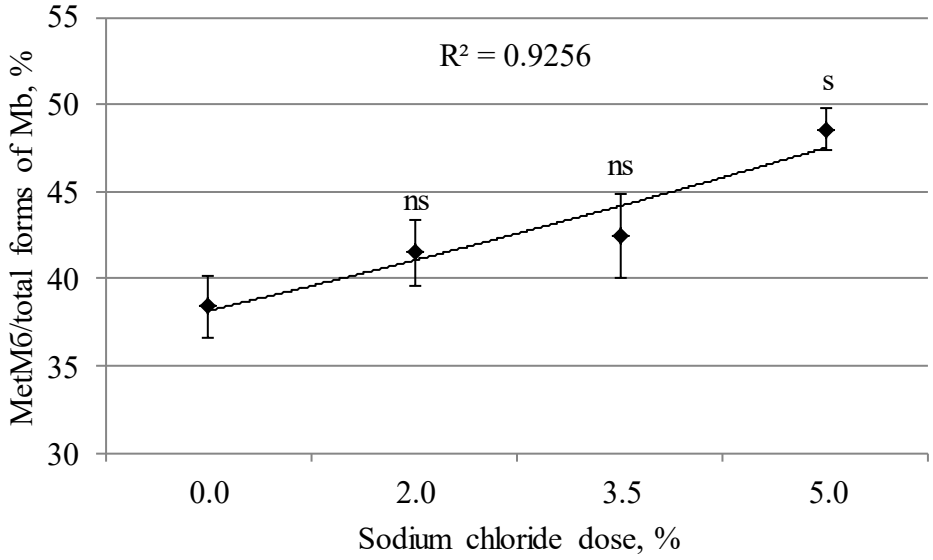


Figure 6. Changes in the myoglobin content depending on the sodium chloride concentration (Mb - myoglobin)

[ns]not significant at p≥0.05; [s] significant at p<0.05 in comparison with unsalted sample.

4. Conclusions

The results of the study make a contribution to substantiation of the sodium chloride effect on the oxidative changes. It was established that addition of sodium chloride increased the heme pigment stability to high temperature and concurrently initiated the oxidative changes in lipids. In this connection, it was suggested that heme iron had the higher catalyzing effect on fat oxidation compared to free iron. The obtained effect can be explained by the salt impact on the development of the myoglobin oxidized forms, which catalyzed lipid oxidation.

5.References

Andersen, H.J., Skibsted, L.H. (1991). Oxidative stability of frozen pork patties.Effect of light and added salt.*Journal of Food Science*, 56, 1182-4.

Andres, A.I., Cava, R., Ventanas, J., Muriel, E., Ruiz, J. (2004). Lipid oxidative changes throughout the ripening of dry-cured Iberian hams with different salt contents and processing conditions. *Food Chemistry*, 84, 375–81.

Baron, C.P., Andersen, H.J. (2002). Myoglobin-induced lipid oxidation. A review. *Journal of Agricultural and Food Chemistry*, 50, 3887–3897.

Calligaris, S,Nicoli, Mc. (2006). Effect of selected ions from lyotropic series on lipid oxidation rate. *Food Chemistry*, 94, 130-4.

Cobos, A.,Veiga, A., Diaz, O. (2008). Chemical and lipid composition of deboned pieces of dry-cured pork forelegs as affected by desalting and boiling: The effects of vacuum packaging. *Food Chemistry*, 106, 951–6.

Faustman, C., Sun, Q., Mancini, R., Suman, S.P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science, 86, 86–94.

Gheisari, H.R., Moller, J.K.S., Adams, C.E., Skibsted, L.H. (2010). Sodium Chloride or Heme Protein Induced Lipid Oxidation in Raw, Minced Chicken Meat and Beef. *Czech Journal of Food Sciences*, 28, 364–75.

Jin, G., He, L., Zhang, J., Yu, X., Wang, J., Huang, F. (2012).Effects of temperature and NaCl percentage on lipid oxidation in

pork muscle and exploration of the controlling method using response surface methodology (RSM).*Food Chemistry*,131, 817–25.

Jin, G., Zhang, J., Yu, X., Lei, Y., Wang, J. (2010). Lipolysis and lipid oxidation in bacon during curing and drying-ripening. *Food Chemistry***,** 123, 465–71.

Johns, A.M., Birkinshaw, L.H., Ledward, D.A. (1989). Catalysts of lipid oxidation in meat products. *Meat Science*, 25, 209–20.

Hornsey, H.C. (1956). The color of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. *Journal of the Science of Food and Agriculture*, 7, 534-540.

Ivankin, A.N., Oliferenko, G.L., Kulikovskii, A.V., Chernukha, I.M., Semenova, A.A., Spiridonov, K.I., Nasonova, V.V. (2016). Determination of unsaturated fatty acids with a migrating double bond in complex biological matrices by gas chromatography with flame ionization and mass spectrometry detection. *Journal of Analytical Chemistry*, 71(11), 1131-7.

Kristensen, L., Purslow, P.P. (2001). The effect of processing temperature and addition of mono- and di-valent salts on the heme-nonheme-iron ratio in meat. *Food Chemistry*, 73, 433-9.

Ladikos, D., Lougovois, V. (1990). Lipid Oxidation in Muscle Foods - a Review. *Food Chemistry***,** 35, 295-314.

Min, B., Ahn, D.U. (2005). Mechanism of lipid peroxidation in meat and meat products—a review. *Food Science and Biotechnology*, 14, 152–63.

Min, B., Cordray, J.C., Ahn, D.U. (2010). Effect of NaCl, myoglobin, Fe(II), and Fe(III) on lipid oxidation of raw and cooked chicken breast and beef loin. *Journal of Agricultural and FoodChemistry*, 58, 600-5.

Rhee, K.I., Ziprin, Y.A. (1987). Lipid oxidation in retail beef, pork and chicken muscles as affected by concentrations of heme pigments and non-heme iron and microsomal enzymic lipid peroxidation activity. *Journal of Food Biochemistry*, 11, 1-15.

Rhee, K.S., Ziprin, Y.A. (2001). Pro-oxidative effects of NaCl in microbial growth-controlled and uncontrolled beef and chicken. *Meat Science*, 57, 105–12.

Sarraga, C., Carreras, I., Garcia Regueiro, J.A. (2002). Influence of meat quality and NaCl percentage on glutathione peroxidase activity and values for acid-reactive substances of raw and dry-cured *Longissimus dorsi. Meat Science*, 62, 503–7.

Tichivangana, J.Z., Morrissey, P.A. (1985). Metmyoglobin and inorganic metals as pro-oxidants in raw and cooked muscle systems. *Meat Science*, 15, 107–16.

Tunieva, E.K., Nasonova, V.V., Stanovova, I.A., Spiridonov, K.I., Kurzova, A.A. (2017). Effect of salting on back fat hydrolysis and oxidation, *IOP Conf. Series: Earth and Environmental Science,* 85, 012047.

Tunieva, E.K., Ivankin, A.N. (2015). Influence of sodium chloride on the composition of volatile substances formed during the oxidation of lard. *Vsyo o myase*, 2, 27-30.

Yin, M.C., Faustman, C. (1993). The influence of temperature, pH and phospholipid composition upon the stability of myoglobin and phospholipids: A liposome model. *Journal of Agricultural and Food Chemistry*, 41, 853–7.

Zanardi, E., Ghidini, S., Conter, M., Lanieri, A. (2010). Mineral composition of Italian salami and effect of NaCl partial replacement on compositional, physico-chemical and sensory parameters. *Meat Science*, 86, 742-7.

Zhuravskaya, N. C., Alekhina, L. T., Otryashenkova, L. M. (1985). Research and quality control of meat and meat products. Agropromizdat, Moscow, 79-82.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

DETERMINATION OF TWO COLOR AGENTS IN HARD BOILED CANDY BY LASER-BASED PHOTOACOUSTIC SPECTROSCOPY AND COLORIMETRY

Mihály Kovács[1], Ottó Dóka[1]*, David Richfield[2]

[1]Department of Physics and Chemistry. Faculty of Mechanical Engineering, Informatics and Electrical Engineering. Széchenyi István University. H-9026 Győr, Egyetem sq. 1., Hungary
[2]Südzucker AG Zentralabteilung Forschung, Entwicklung, Services (ZAFES), Wormser Str. 11, D-67283 Obrigheim, Germany
**doka.otto@sze.hu*

https://doi.org/10.34302/2019.11.4.11

Article history:
Received:
15 August 2019
Accepted:
29 November 2019

Keywords:
Anthocyanin (E163);
Beta carotene (E160a);
Colored hard-boiled candy;
Laser-based photoacoustic spectroscopy;
Colorimetry.

ABSTRACT

Determination of color agents was performed by laser-based photoacoustic spectroscopy (LPAS) and colorimetry in hard boiled candies containing two different colorants (anthocyanin and beta carotene). LPAS and colorimetry are fast and direct methods which do not require any chemicals or complicated sample preparation such as extraction. Both LPAS and colorimetry were shown to be suitable for quick determination of anthocyanin and beta carotene content in hard boiled candies. The determination of anthocyanin content in colored hard-boiled candies is possible by LPAS at 532 nm or by colorimetry using ΔE*, while the beta carotene content can be measured by LPAS at 473 nm or by colorimetry using the Hue color index.

1.Introduction

Color is a key product attribute of foods and food supplements, and the colorants that have been approved by regulatory authorities for use in foods cover a wide range of hues. These colorants can be synthetic or natural in origin, with the natural colors including pure dyes and pigments, extracts, and coloring foodstuffs. The quality and dosage of food dyes are strictly regulated.

Many food producers have started to substitute artificial colorants with natural alternatives to achieve "clean label" in alignment with consumer preferences, due to concerns about possible health effects of artificial colorants (Burrows, 2009; Sloan, 2011; Shahid *et al.*, 2013; Cassiday, 2017; Bateman *et al.*, 2004; Mccann *et al.*, 2007).

The consumption of natural colorants has increased by 10-15% annually in recent years, a trend that is expected to continue (Cortez *et al.*, 2017).

The most important natural food colorants contain colored molecules from the chemical families of anthocyanins, carotenoids, chlorophylls, and betalains (Rodriguez-Amaya, 2016).

The anthocyanins (E163) form the largest group of water-soluble natural dyes, with more than 600 known (Prior and Wu, 2012). Their molecular structure changes reversibly depending on pH, due to multiple protonation reactions (Torskangerpoll and Andersen, 2005; Wrolstad and Culver, 2012; Rakic *et al.*, 2015) which lead to color changes. Depending on their chemical structure, anthocyanins can be pink, red, violet, blue or cyan (Carocho and Morales, 2015; Cui *et al.*, 2016). They can also undergo irreversible changes due to temperature or exposure to light, or due to reactions with

oxidants, metal ions, proteins or flavonoids, leading to changes in or loss of color (Rodriguez-Amaya, 2016). Anthocyanin stability can be increased by encapsulation (Zaidel *et al.*, 2014).

Carotenoids are lipophilic colorants with yellow to red colors. The four major sources of beta carotene (E160a) are plants, algae, fungi and chemical synthesis (Mortensen, 2006).

Chlorophylls are the major photosynthetic pigments of plants, and are widely used as green food colorants, both in native and chemically modified forms (Humphrey, 2004).

The betalains, including betanin, are a class of natural pigments that occur in beetroot red (E162) (EFSA, 2015).

HPLC with UV/VIS or photodiode array detection (DAD) is the standard and most widely used analytical method for quantification of anthocyanins and beta carotene, due to its resolution, sensitivity and selectivity (Stintzing *et al.*, 2006; Yoshioka and Ichihashi, 2008; Li *et al.*, 2016; Su *et al.*, 2016; Pedro *et al.*, 2016). Simultaneous determination of mixtures of up to 40 colorants is feasible with HPLC-DAD, by scanning the eluate at suitably chosen wavelengths (Yoshioka and Ichihashi, 2008; Prado and Godoy, 2007; Minioti *et al.*, 2007; Shen *et al.*, 2014).

Besides HPLC, several alternative analytical methods are used for quantification, for example spectrophotometry (Sayar and Özdemir, 1998; Liang *et al.*, 2011; Islam *et al.*, 2016), capillary electrophoresis (Watanabe and Terabe, 2000), and colorimetry (Stintzing *et al.*, 2006; Liang *et al.*, 2011).

Optical methods like derivative spectrophotometry are generally not considered to be suitable for quantification in multi-color mixtures without mathematical processing of the data (Yoshioka and Ichihashi, 2008; Sayar and Özdemir, 1998).

Laser-based photoacoustic spectroscopy (LPAS) is a technique where a sample is illuminated with a monochromatic laser, with an intensity that is modulated at a fixed frequency. When the wavelength of the laser coincides with an absorption band of an analyte in the sample, the radiation absorbed by the sample is rapidly converted to heat (Dumitras *et al.*, 2007). In the case of gaseous samples, the changes in pressure caused by this oscillating temperature change can be directly detected by a microphone, but in solid samples, the acoustic wave is generated within the solid sample, and propagates to the gaseous phase (Ball, 2006). Thermal waves also cause a periodic heating and cooling of the contacting layer of the surrounding gas. Both effects generate acoustic waves, which are detected by a microphone as a photoacoustic signal. The generated PA signal depends on optical and thermal parameters of the sample and the contacting gas.

The aim of this study was to explore the feasibility of laser-based photoacoustic spectroscopy (LPAS) and colorimetry for quantification of E163 and E160a in multiple colored hard-boiled candy samples, comparing the results with those achievable through colorimetry. A secondary target of the investigation was whether the effect of the two food colorants at the analytical wavelengths was independent.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Ten solid hard-boiled candy calibration samples were used for colorimetry and LPAS measurements (Table 1). The samples were prepared according to a standard industrial process: corn syrup, granular sugar (sucrose), and water were cooked at atmospheric pressure to 143 °C, after which further moisture was removed under reduced pressure (absolute pressure: 0.2 bar). The same amount of citric acid and flavor premix was added to each sample (titratable acidity calculated as citric acid: 0.26% m/m), and different amounts of colorants were added. The resulting candy mass was then mixed and cooled on a temperature-controlled table (temperature 20°C), yielding a hard candy (in a glassy state of matter).

Two series of samples were prepared. Series A contains varying amounts of anthocyanin grape-extract (Chr. Hansen; AC 12 WSP,

anthocyanin content 100%) with no other color added. The anthocyanin content of these samples was 0.00 (blank sample), 5.06, 8.14, 10.65, 14.75 and 17.42 mg/g. In series B, two colorants were applied, namely the above mentioned grape extract and liquid beta carotene colorant (Naturex Natural carotene COOF3221; beta-carotene content 5%). The amount of anthocyanin was fixed (9.50±1.20 mg/g), and the beta carotene contents were as follows: 0.00 (blank sample from beta carotene point of view), 0.16, 0.22, 0.37 and 0.42 mg/g.

Table 1. The ten investigated hard boiled candy samples and their colorant content. Series A means samples 5 to 10 and while series B samples 1-4 and 7.

Sample number	Anthocyanin content (mg/g)	Beta carotene content (mg/g)
1	9.65	0.16
2	9.06	0.22
3	10.10	0.37
4	8.42	0.42
5	5.06	0.00
6	8.14	0.00
7	10.65	0.00
8	14.75	0.00
9	17.42	0.00
10	0.00	0.00

The samples for the LPAS measurement were crushed in a mortar to get relatively fine powders, and the colorimetric measurements were done with uncrushed as well as crushed (powdered) samples.

2.2. Methods

2.2.1. Photoacoustic spectrophotometry

The home-made PA spectrometer used in this study comprised a modulator, a photoacoustic cell and either a 473 nm diode laser (Changchun New Industries Optoelectronics, MBL-III-473-50) or a 532 nm diode laser (Roithner, GLP-III-532-30).

The laser beam was mechanically chopped at a frequency of 17 Hz, collected by a quartz lens and focused into the PA cell. Radiation entered the PA cell through a quartz window 12.7 mm in diameter. A cylindrical polished stainless-steel sample holder chamber was used, with diameter 50 mm and height 20 mm, with an engraved hemispherical hole for the samples on the top, 5 mm deep and 10 mm in diameter. The volume of sample required to fill the sample holder was approximately 0.15 cm^3

A 3 mm long stainless-steel capillary tube with inner diameter 500 μm was used to connect a 4.2×4.75 mm electret microphone (Sennheiser KE 4-211-2) with the sample holder of the cell. The sensitivity of the microphone was 10 mV/Pa at 1000 Hz. The PA signal was processed by a dual phase lock-in amplifier (Stanford SR530) with 3 s time constant coupled to the computer. To determine the PA signals three independent analyses were performed where each single measurement represents 256 successive readings of the lock-in signal, and the data points are the averages of three consecutive independent measurements.

2.2.2. Colorimetry

The colorimetric indices were measured by a MiniScan XE Plus (HunterLab) colorimeter with a CIE D65 xenon lamp as light source with a 45/0° measurement geometry.

The results of colorimetry can be defined as direct CIELab (L*, a*, b*) and derived (Hue, C*, ΔE*, 2-lg(L*), lg(a*+100), lg(b*+100)) color indices respectively.

The L* index is the lightness value of the sample on the 0-100 scale where 0 is black, and 100 is white. The a* index expresses the position in the color space of a color on the green-red axis and b* on the blue-yellow axis. Negative a* is green and positive a* is red while negative b* is blue and positive b* is yellow.

Hue index (h_{ab}°) is the rotational degree of a color vector from the positive a* axis in the CIELab color space. Chroma (C*) is a quantitative attribute of colorfulness and used to determine the distance between a point in the color space and a grey color with the same lightness. The total color difference (ΔE*) represents the distance between two colors in the color space. We selected as reference point in the color space the L*= 93.24, a*=-0.82 and

b*=0.07 values which refer to the calibration white tile to the colorimeter.

CIELab indices of the candy samples (Series A and Series B) were measured, both crushed as in the sample preparation for the LPAS, and uncrushed. L*, a* and b* indices were measured directly, whereas standard derived color indices (C*, hue, ΔE*) were calculated, and three new indices calculated (2-lg(L*), lg(a*+100), lg(b*+100)).

Five independent measurements were performed on each sample, and the mean of the measured values was used. Statistical analysis was done with Microsoft Excel 2013.

3.Results and discussions

The PAS signals of the ten crushed candy samples, normalized to the output power of the lasers, are shown for series A (samples with only anthocyanin) and series B (sample with anthocyanin and beta carotene mixture) in Figure 1 and 2 respectively.

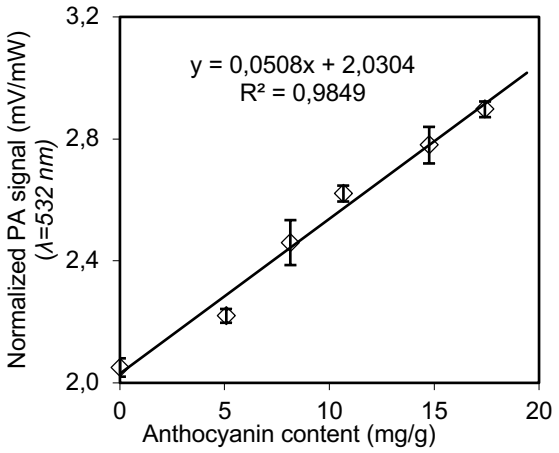


Figure 1. Calibration line between the anthocyanin content and the normalized PA signal measured on powdered, solid candy samples. The anthocyanin contents were between 0 and 17.42 mg/g and the samples do not contain β-carotene (mean±SD, n=3).

In both cases the normalized PA signal depends linearly on the color agent content of the samples, with good determination coefficients. Measuring the PA signal at 532 nm on the samples of series B (those containing anthocyanin) the obtained signal and the anthocyanin content do not differ significantly. This latter statement is confirmed by t-test ($p<0.05$) examined on the data.

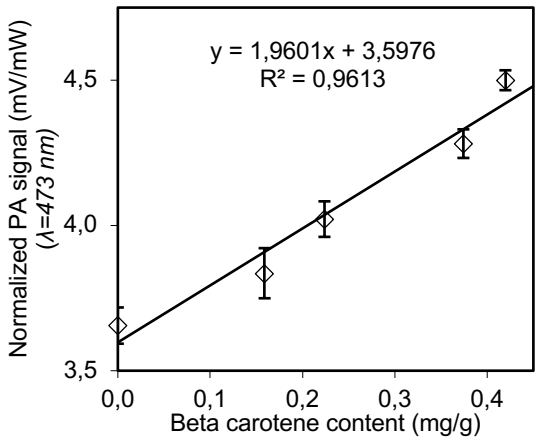


Figure 2. Calibration line between the beta carotene content and the normalized PA signal using the powdered, solid candy samples. The beta carotene contents were between 0 and 0.42 mg/g while the anthocyanin content of the samples was 9.50±1.20 mg/g (mean±SD, n=3).

Table 2. The calculated results of the comparison on the basis of the determination coefficients between color indices and anthocyanin and beta carotene content. Values in the second and third column refer to samples series A (anthocyanin content only), while values in the fourth and fifth column refer to samples series B (beta carotene content with constant amount of anthocyanin). Five independent measurements were carried out on the uncrushed solid and crushed samples as well.

	Anthocyanin content		Beta carotene content	
Color indices	R^2		R^2	
	uncrushed	powdered	uncrushed	powdered
L*	0.8829	**0.9427**	0.2242	0.2502
a*	0.5877	0.8396	0.3117	0.4591
b*	0.7101	0.8376	**0.9253**	**0.9575**
ΔE*	**0.9967**	**0.9507**	0.1981	0.0106
Hue	0.4048	0.3329	**0.9550**	**0.9763**
C*	0.2073	0.8780	0.3901	0.8114
2-lg(L*)	**0.9426**	**0.9497**	0.2102	0.2502
lg(a*+100)	0.5847	0.8321	0.3142	0.4594
lg(b*+100)	0.7169	0.8427	**0.9289**	**0.9610**

The second and third columns in Table 2 show the determination coefficients of linear regression for anthocyanin content (Series A). In the case of ΔE* for the uncrushed samples, the regression is not linear but quadratic. In uncrushed form the 2-lg(L*) and ΔE* indices, while in powdered form the L*, ΔE*, and 2-lg(L*) indices show the best correlation.

As an example, Figure 3 shows the correlation between ΔE* index and the anthocyanin content for crushed and uncrushed samples. The quadratic regression gives better correlation (R^2 = 0.9967) but does not yield a monovalent function above 16,5 mg/g anthocyanin content. The measured L* indices show that the crushing significantly darkens the samples. Values of L* are 50-70% lower for crushed samples.

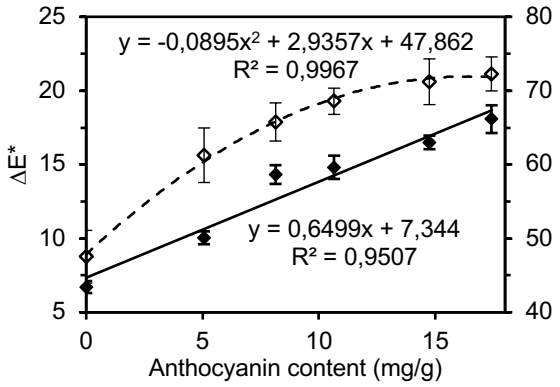


Figure 3. Calibration curves between ΔE* color indices and anthocyanin content for uncrushed (◊) and crushed (♦) samples series A (the samples contain anthocyanin only). The second Y-axis belongs to the uncrushed samples. The values represent the average of five independent measurements and the standard deviations.

Determination coefficients for linear regression between colorimetric indices and beta carotene content (Series B) are shown also in Table 2 (fourth and fifth columns). The best correlation was achieved for parameter b*, hue and lg(b*+100) color indices, while the worst one was ΔE* in both forms. As an example, Figure 4 shows the correlation between hue index and beta carotene content for uncrushed and crushed samples. In both cases the correlation is linear with determination coefficients (R^2) of 0.955 and 0.9763, respectively.

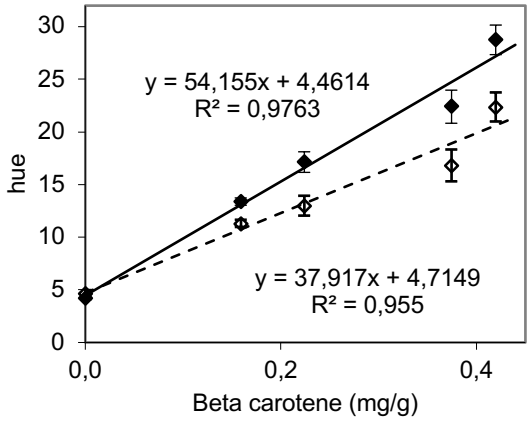


Figure 4. Calibration lines between hue indices and beta carotene content for uncrushed (◊) and crushed (♦) samples series B (the samples contain anthocyanin too). The values represent the average of five independent measurements and the standard deviations.

Comparison of the applied methods shows that anthocyanin and beta carotene content in hard boiled candy samples can be determined using both LPAS and colorimetry. A linear relationship was found between the colorant content and the PA signal in all cases, whereas this was only true for some of the CIELab indices. We consider only those correlations that have a determination coefficient higher than 0.9.

Accordingly, the anthocyanin content of the hard-boiled candy samples can be measured in uncrushed samples by colorimetry using the ΔE* and 2-lg(L*) color indices, while in powdered form, L*, ΔE*, and 2-lg(L*) or by LPAS at 532 nm.

The beta carotene colorant content of the uncrushed and powdered hard boiled candy samples can be determined by colorimetry using b*, hue, or lg(b*+100) color indices, or by LPAS at 473 nm.

The best results from measurement of the anthocyanin colorant content in hard boiled candies with both anthocyanin and beta carotene were by LPAS at 532 nm and by colorimetry using ΔE*. In both cases the determination

coefficients were good for anthocyanin, while poor for beta carotene.

The beta carotene content in anthocyanin - beta carotene colored candies potentially can be determined using the Hue colorimetrical index.

PA spectroscopy and colorimetry both offer some advantages compared with the conventional methods. No chemicals or special tools are required for preparation, and the whole analysis is significantly less time-consuming than with conventional methods. The results from LPAS require the same level of mathematical analysis as is needed in the interpretation of results from derivative spectrophotometry (Yoshioka and Ichihashi, 2008).

Although colorimeters are more widely available in industry, the results were shown to be less linear than those obtained with LPAS and were strongly affected by the physical condition of the sample.

4. Conclusions

The anthocyanin and beta carotene content in hard boiled candy samples can be determined using the applied methods: LPAS and colorimetry.

After validation of both methods, however, they could potentially be used as quality control methods to determine dosage of color agents in food supplement or confectionery production.

5.References

Ball, D.W. (2006). Photoacoustic spectroscopy. *Spectroscopy*, 21(9), 14-6.

Bateman, B., Warner, J.O., Hutchinson, E., Dean, T., Rowlandson, P., Gant, C., Grundy, J., Fitzgerald, C., Stevenson, J. (2004). The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Archives of Disease in Childhoo.*, 89(6), 506-11.

Burrows, A.J.D. (2009). Palette of Our Palates: A Brief History of Food Coloring and Its Regulation. *Comprehensive Reviews in Food Science and Food Safety*, 8(4), 394-08.

Carocho, M., Morales, P., Ferreira, I.C.F.R. (2015). Natural food additives: Quo vadis? *Trends in Food Science & Technology*, 45(2), 284-95.

Cassiday, L. (2017). Clean label: The next generation. *INFORM International News on Fats, Oils, and Related Materials*, 28(8), 6-10.

Cortez, R., Luna-Vital, D.A., Margulis, D., Mejla, E.G.D. (2017). Natural Pigments: Stabilization Methods of Anthocyanins for Food Applications. *Comprehensive Reviews in Food Science and Food Safety* 16(1), 180-98.

Cui, B., Hu, Z., Zhang, Y., Hu, J., Yin, W., Feng, Y., Xie, Q., Chen, G. (2016). Anthocyanins and flavonols are responsible for purple color of Lablab purpureus (L.) sweet pods. *Plant Physiology and Biochemistry*, 103, 183-90.

Dumitras, D.C., Dutu, D.C., Matei, C., Magureanu, A.M., Petrus, M., Popa, C. (2007). Laser photoacoustic spectroscopy: principles, instrumentation, and characterization. *Journal of Optoelectronics and Advanced Materials*, 9(12), 3655-701.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). (2015). Scientific Opinion on the re-evaluation of beetroot red (E 162) as a food additive. *EFSA Journal*, 13(12), 4318.

Humphrey, A.M. (2004). Chlorophyll as a Color and Functional Ingredient. *Journal of Food Science*, 69(5), 422-5.

Islam, S.N., Nusrat, T., Begum, P., Ahsan, M. (2016). Carotenoids and b-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency. *Food Chemistry*, 199, 628-1.

Li, D., Meng, X., Li, B. (2016). Profiling of anthocyanins from blueberries produced in China using HPLC-DAD-MS and exploratory analysis by principal component analysis. *Journal of Food Composition and Analysis*, 47, 1-7.

Liang, Z., Sang, M., Fan, P., Wu, B., Wang, L., Yang, S., Li, S. (2011). CIELAB Coordinates in Response to Berry Skin

Anthocyanins and Their Composition in Vitis. *Journal of Food Science*, 76(3), 490-7.

Mccann, D., Barrett, A., Cooper, C., Crumpler, D., Dalen, L., Grimshaw, K., Kitchin, E., Lok, K., Porteous, L., Prince, E., Sonuga-Barke, E., Warner, J.O., Stevenson, J. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community a randomized, double-blinded, placebo-controlled trial. *The Lancet*, 370(9598), 1560-7.

Minioti, K.S., Sakellariou, C.F., Nikolaos, S., Thomaidis, N.S. (2007). Determination of 13 synthetic food colorants in water-soluble foods by reversed-phase high-performance liquid chromatography coupled with diode-array detector. *Analytica Chimica Acta,* 583(1), 103-10.

Mortensen, A. (2006). Carotenoids and other pigments as natural colorants. *Pure and Applied Chemistry*, 78(8), 1477-91.

Pedro, A.C., Granato, D., Rosso, N.D. (2016). Extraction of anthocyanins and polyphenols from black rice (Oryza sativa L.) by modeling and assessing their reversibility and stability. *Food Chemistry*, 191, 12-20.

Prado, M.A., Godoy, H.T. (2007). Synthetic dyes are much used in processed foods. *Quim Nova*, 30(2), 268-73.

Prior, R.L., Wu, X. (2012). Analysis Methods of Anthocyanins In: Xu, Z., Howard, L.R. (Eds.) Analysis of Antioxidant-Rich Phytochemicals. *Howard Wiley-Blackwell*, 149.

Rakic, V.P., Skrt, M.A., Miljkovic, M.N., Kostic, D.A., Sokolovic, D.T., Ulrih, N.E.P. (2015). Effects of pH on the stability of cyanidin and cyanidin 3-O-β-glucopyranoside in aqueous solution. *Hemijska industrija*, 69(5), 511-22.

Rodriguez-Amaya, D.B. (2016). Natural food pigments and colorants. *Current Opinion in Food Science*, 7, 20-6.

Sayar, S., Özdemir, Y. (1998). First-derivative spectrophotometric determination of Ponceau 4R, Sunset Yellow and Tartrazine in confectionery products. *Food Chemistry*, 61(3), 367-2.

Shahid, M., Islam, S.U., Mohammad, F. (2013). Recent advancements in natural dye applications: a review. *Journal of Cleaner Production*, 53, 310-31.

Shen, Y., Zhang, X., Prinyawiwatkul, W., Xu, Z. (2014). Simultaneous determination of red and yellow artificial food colourants and carotenoid pigments in food products. *Food Chemistry*, 157, 553-8.

Sloan, A.E. (2011). Navigating the Natural Marketplace. *Food Technology*, 65(7), 24-3.

Stintzing, F.C., Trichterborn, J., Carle, R. (2006). Characterisation of anthocyanin–betalain mixtures for food colouring by chromatic and HPLC-DAD-MS analyses. *Food Chemistry*, 94(2), 296-09.

Su, X., Xu, J., Rhodes, D., Shen, Y., Song, W., Katz, B., Tomich, J., Wang, W. (2016). Identification and quantification of anthocyanins in transgenic purple tomato. *Food Chemistry*, 202, 184-8.

Torskangerpoll, K., Andersen, Ø.M. (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. *Food Chemistry*, 89(3), 427-40.

Watanabe, T., Terabe, S. (2000). Analysis of natural food pigments by capillary electrophoresis. *Journal of Chromatography A*, 880(1-2), 311-22.

Wrolstad, R.E., Culver, C.A. 2012. Alternatives to Those Artificial FD&C Food Colorants. *Annual Review of Food Science and Technology*, 3(1), 59-77.

Yoshioka, N., Ichihashi, K. (2008). Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array detection. *Talanta*, 74(5), 1408-13.

Zaidel, D.N.A., Sahat, N.S., Jusoh, Y.M.M., Muhamad, I.I. (2014). Encapsulation of Anthocyanin from Roselle and Red Cabbage for Stabilization of Water-in-Oil Emulsion. *Agriculture and Agricultural Science Procedia*, 2, 82-9.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

INFLUENCE OF FREEZING METHOD ON COLOR CHANGE AND ANTIOXIDANT ACTIVITY IN CHERRY FRUIT

Olena Vasylyshyna [1*], Yevhenii Postolenko[2]

[1] *Faculty of Engineering and Technology, Uman National University of Horticulture, Uman city, Institutska 1, 20305, Ukraine,*

[2] *Department of plant protection and analytical measurements, Research Station of Pomology L.P. Simirenko IS NAAS, Simirenko, 9,. Mlyev-1, Gorodyshchensky district, Cherkasy region, 19512, Ukraine*

**elenamila@i.ua*

https://doi.org/10.34302/2019.11.4.12

Article history:
Received:
15 August 2018
Accepted:
29 September 2019

Keywords:
Frozen cherry fruits;
Tanning and coloring agents;
Ascorbic acid;
Antioxidant activity.

ABSTRACT

Showing preprocessing cherry fruit of varieties Shpanka and Lotovka by sugar solution with the addition of chitosan or ascorutin. For this studies conducted over the years 2016−2017 with the fruits of cherry varieties Shpanka and Lotovka. Prepare fruit included: sorting, inspection, washing, hold 30 minutes in solutions of 20% sugar ascorutin 4% or 20% of the sugar with the addition of 1% chitosan, remove moisture, freezing at −25°C, packing in plastic bags of 0.5 kg and storage at −18° C. By taking control of raw fruit cherries packed in plastic bags. The content of tannins and colorants in cherry fruits is a varietal feature and after freezing in the fruits of the cherry varieties of Shpanka and Lotovka is reduced by 22 and 29%. Whereas in pre-treated fruits cherries by 20% sugar solutions with addition of 4% ascorutin or 1% chitosan decreased to 10 and 12%. The number of ascorbic acid in fruits, respectively, decreased to 23 and 38%. In addition, the smallest losses were for fruits treated with 20% sugar solution with the addition of 1% chitosan. According to the research, preservation of quality and biological value of frozen fruit cherry preprocessing contributes 20% sugar solution with the addition of 1% chitosan. The color indicator is a varietal feature. Antioxidant activity during freezing did not change significantly. It has been established that antioxidant activity correlates with the content of tannins and colorants and correlates with the content of ascorbic acid inverted.

1.Introduction

Cherries are a very attractive fruit to consumers, for their taste and colour attributes, as well as for their wealth of nutrients (Serra *et al.*, 2011).

The fruit has dark red colour and high dry matter, approximately 27.3 %, specific sweet and sour aroma derived from volatile compounds such as alcohols, carbonyls, esters and terpenes (Levaj *et al.*, 2010) and therefore is valuable raw material in food industry.

Regarding their phytochemical composition, sour cherries are rich source of polyphenol compounds which strongly influence the quality and nutritional value of the fruits and contributing to their sensorial attributes (Zoric *et al.*, 2016).

Moreover, cherries are a good source of natural antioxidant substances, namely polyphenols, which are reported to have many health benefits.

Cherry polyphenols include flavonoids: anthocyanins, flavan-3-ds and flavonols

hydroxychric acids and hydroxybenzoic acids (Goncalves *et al.*, 2004). Among these compounds, especial interest has been focused on anthocyanins, which are the polyphenols responsible for the red skin and flesh colour of fruits, due to their strong antioxidant and anti-inflammatory activities (Blando *et al.*, 2004; Wang *et al.*, 1999; Serra *et al*., 2011).

The predominant anthocyanins in cherry are cyanidin-3-rutinoside and cyanidin-3-glucoside. The major polyphenols in sweet cherry are anthocyanins followed by the hydroxycinnamic acid's. Thus, at the last sampling date, the highest levels of H-TAA were found in 'Sonata' and 'Cristalina' (130 mg/100 g) and the lowest in 'Brooks' (69.67±2.50 mg/100g) (Dıaz-Mula *et al.*, 2009).

Total anthocyanins of sweet cherries are between 30 (cv. Black Gold) and 79 (cv. Cristalina)mg cyanidin-3-glucoside equivalents (CGE)/100g, whereas total anthocyanins of sour cherries were between 45 (cv.Balaton) and 109 (cv. Sumadinka) mg CGE/100g (Ferretti *et al.*, 2010).

Total anthocyanin content ranged from 82 to 297 mg/100g for dark cherries and from 2 to 41 mg/100 g for the light coloured cherries (Goncalves *et al.*, 2007).

The evaluation of antioxidant activity, performed by ORAC and TEAC assays, revealed a relatively high antioxidant capacity for the fruit extracts (from 1145 to 2592 μmol TE/100 g FW) and a lower one for the callus extract (688 μmol TE/100 g FW) (Blando *et al.*, 2004).

Several studies showed that cherry anthocyanins, and especially cyanidins, have potential to inhibit tumour growth, slow cardiovascular diseases and retard the aging process (Serra *et al.*, 2011).

The correlation between antioxidant activity and phenolic compounds has been also found in several studies comparing a wide range of fruits cherry (Dıaz-Mula *et al.*, 2009; Vasylyshyna, 2017).

The chromatic parameters L, a, b, chroma and hue angle correlated negatively ($P < 0.001$) with the total anthocyanins levels, but not with total phenols ($P > 0.05$). Therefore, for cherries for human consumption, it seems important to have a simple and non-destructive technique for anthocyanins content determination, and in this way easily and quickly assess and monitor cherry quality on a large number of cherries (Goncalves *et al.*, 2007).

Colour is one of the most important indicators of maturity and quality of fresh, stored, and processed cherries. In cherries, colour is mainly influenced by the concentration and distribution of different anthocyanins in the skin (Gao & Mazza, 1995; Pedisic *et al.*, 2009) as well as pH and levels and types of colourless phenolics in the fruits and other factors such as light, temperature, oxygen, metal ions and enzymes (Goncalves *et al.*, 2007).

Fresh sweet cherries represent an important, but fragile, commodity in the agricultural export market. The harvesting season is very short, and cold storage is used to stretch the supply period in the season. However, the effects of different storage conditions on cherry quality, including colour development, is not well studied (Esti *et al.*, 2002; Szymczak *et al.*, 2003; Goncalves *et al.*, 2007).

The freezing process triggers the formation of ice in cellular fruits, which increases the volume of the fruit and damages the integrity of the cell, leading to fruit structure breakdown. Large drip loss found in the thawed product (Han *et al.*, 2004) will have a major effect on the appearance of the product. Another adverse consequence of freezing is that nonaqueous constituents become concentrated in the unfrozen phase. Thus, besides lowering reaction rate by lowering temperature, freeze-concentration can increase reaction rates, resulting in decreased anthocyanin and ascorbic acid contents in frozen stored of fruits (Sahari *et al*., 2004; Ngo *et al*., 2007).

According to Scibisz et all., (2007) measurements of the antioxidant activity and bioactive compounds contents of blueberries showed there were no significant differences between fresh and frozen fruits. Also in the works of Begon a de Ancos *et all*., (2000) at the end of long-term frozen storage (12 months), no

significant change of total phenolic content extracted was observed, but significant decreases of 14−21% in ellagic acid and of 33-55% in vitamin C were quantified.

Consequently, we have assigned task to study the change of color and antioxidant activity in cherry fruit in different ways of freezing.

2. Materials and methods

2.1. Materials

Studies conducted over the years 2016−2017 with the fruits of cherry variety Shpanka and Lotovka. Prepare fruit included: sorting, inspection, washing, hold 30 minutes in solutions of 20% sugar ascorutin 4% or 20% of the sugar with the addition of 1% chitosan, remove moisture, freezing at −25 °C, packing in plastic bags of 0.5 kg and storage at −18° C. By taking control of raw fruit cherries packed in plastic bags.

Fresh fruits and after six months of dry freezing determine soluble substances – refractometer (PAL-3 (ATAGO), Japan). Ascorbic acid was determined using the modified Tillman's method. Ascorbic acid was titrated with 2.6-dichloroindophenol under acid conditions (Naichenko, 2001). Tanning and coloring substances - by Neubauer and Leventhal (Naichenko, 2001), titrated with potassium permanganate (0.1n $KMnO_4$).

2.1.1.Antioxidant capacity. Antioxidant activity - by FRAP (Khasanov et all., 2004).

Measurements were performed on the millivoltmeter (MP 511 Lab pH Meter "Ulab", China) (mV). FRAP values were expressed as mmol 100g of dry matter, as mean value ± standard deviation (N = 3 replicates).

2.1.2.Colour analysis. Colour analysis was performed using a colorimeter (KFK-2, Russia) at by 30 mm thick plate. Three measurements were made at different points of the samples, and this procedure was repeated three times to get the average values.

Statistical analysis. The data were statistically processed using a two factor analysis of variance (ANOVA) method at significance level $P < 0.05$ on the PC program Statistica. A Fisher correlation analysis including all the parameters was also performed.

3.Results and discussions

As can be seen from Table 1, the content of dry soluble substances in fresh fruit of the cherry varieties of Shpanka averaged over two years of researching was 16.9%, while for fruit cherries variety of Lotovka – 15.2%, which is 1.7% lower, which is obvious due to the features of the variety.

As shown in Figure 1, after six months of frozen pre-processed fruit cherries in polyethylene bags, the content of dry soluble substances increased to 11–12%.

This is obviously due to the preliminary treatment of cherry fruit in sugar solutions with the addition of ascorutin or chitosan and the passage of osmotic processes.

The content of tannins and colorants in fresh fruit cherries averaged over two years of research was 0.67%, while for the fruits of the Lotovka variety, it was slightly less – 0.51%. After six months of freezing in the control version, their content decreased to 22–29%. While for fruit cherries, pre-treated with a sugar solution with chitosan addition, it decreased to 10–12%, and in processed solution of sugar with the addition of chitosan remained at the level of fresh cherry fruit. Obviously, the preservation of the content of tannins and colorants promoted to the before freezing. What is evidenced by the research results of Scibisz (2007) end Ngo et al. (2007) that the content of phenolic substances during freezing is reduced to 20–50%.

The content of ascorbic acid in the fruit of the cherry varieties of Shpanka variety was 19.8 mg/100g and Lotovka – 24.2 mg/100g. After six months of storage its contents decreased by 23 and 37.5%. This is confirmed by the results of the research of Begon a de Ancos (2000) end Ngo et al. (2007) on the reduction of the ascorbic acid content after freezing to 55%. Compared to fresh fruit cherries in pre-processed fruit cherry sugar solution with the addition of chitosan after freezing, the content of ascorbic acid remained at the level of fresh cherry fruit.

The content of tannins and colorants and ascorbic acid determines the antioxidant activity of the fruits and depends on the characteristics of the variety. For fruits, the cherry varieties of Shpanka are 38 mmoles / dm^3, and Lotovka − 23 mmoles/dm^3. After six months of freezing, antioxidant activity decreased to 15 and 18%. Whereas in the cultivated fruit, the cherry varieties of Shpanka and Lotovka with sugar solutions with the addition of ascorutin or chitosan remained at the level of fresh cherry fruit. The data obtained from studies are confirmed by Scibisz (2007) end Ngo et al. (2007) that freezing of fruits does not reduce their antioxidant activity.

Since color is one of the most important indicators of the maturity and quality of the fruits Pedišić (2009) end Gonçalves et al. (2007) was studied the change in the content of optical density and the world-propagation coefficient after freezing.

Table 1. The content of some components of the chemical composition cherry fruit varieties Shpanka and Lotovka

Varieties	Years	Dry soluble substances, %	Tanning and coloring, substances, %	Ascorbic acid, mg / 100g	Light transmit - tance ratio	Optical density, %	Antioxi-dant activity, mmol / dm^3
Shpanka	2016	16.1±0.2	0.67±0.03	17.6±0.2	27±2	0.62±0.22	48±2
	2017	17.6±0.2	0.67±0.02	22.0±0.3	29±1	0.54±0.24	28±3
	Average	16.9±0.2	0.67±0.02	19.8±0.2	28±2	0.58±0.23	38±2
Lotovka	2016	14.7±0.2	0.60±0.01	22.0±0.2	34±1	0.52±0.26	26±1
	2017	15.7±0.3	0.42±0.02	26.4±0.1	36±2	0.44±0.22	17±2
	Average	15.2±0.2	0.51±0.02	24.2±0.2	35±2	0.48±0.24	23±2
LSD_{05}		0.7	0.7	0.7	5.8	0.68	6.7

As can be seen from Figure 1, the change in the content of the optical density for fruit of the cherry varieties of Shpanka is 0.58, and Lotovka – 0.48.

After six months of freezing, it dropped to 48% and 14% accordingly, which is apparently due to the varietal peculiarity. After freezing of fruits cherry treated with sugar solution with the addition of ascorutin or chitosan, the optical density of the fruit of the cherry of the Spanka variety decreased to 53% and 75%, respectively, for the Lotovka variety to 83 and 79%. Similar results were obtained in the study of Gonçalves (2007).

As the color index is estimated by the world-propagation coefficient in the fruit of the Lotovka variety it was 28%, and Spanka 35%. After freezing, it increased to 11% and 46%, and in processed cherry fruit to 1.8 and 2.6 times, which is obviously due to the transfer of anthocyanins to cellular fruit juice and more intense coloration of cherry fruit. As the color is negatively correlated with the total content of anthocyanins Gonçalves (2007).

Processing of the data obtained by statistical methods of analysis allows us to show the regularity of numerical characteristics. Correlation indicators were established between the data obtained (Table 2, Figure 2). In particular, installed a strong and inverse correlation between the antioxidant activity and the content of ascorbic acid ($r = -0.72 \pm 0.05$), antioxidant activity and the content of tannins and colorants ($r = 0.54 \pm 0.05$), optical density and coefficient of light transmission ($r = -0.89 \pm 0.05$), dry soluble substances and tannins and colorants ($r = -0.78 \pm 0.05$).

Figure 2 shows the regression equation for these dependencies.

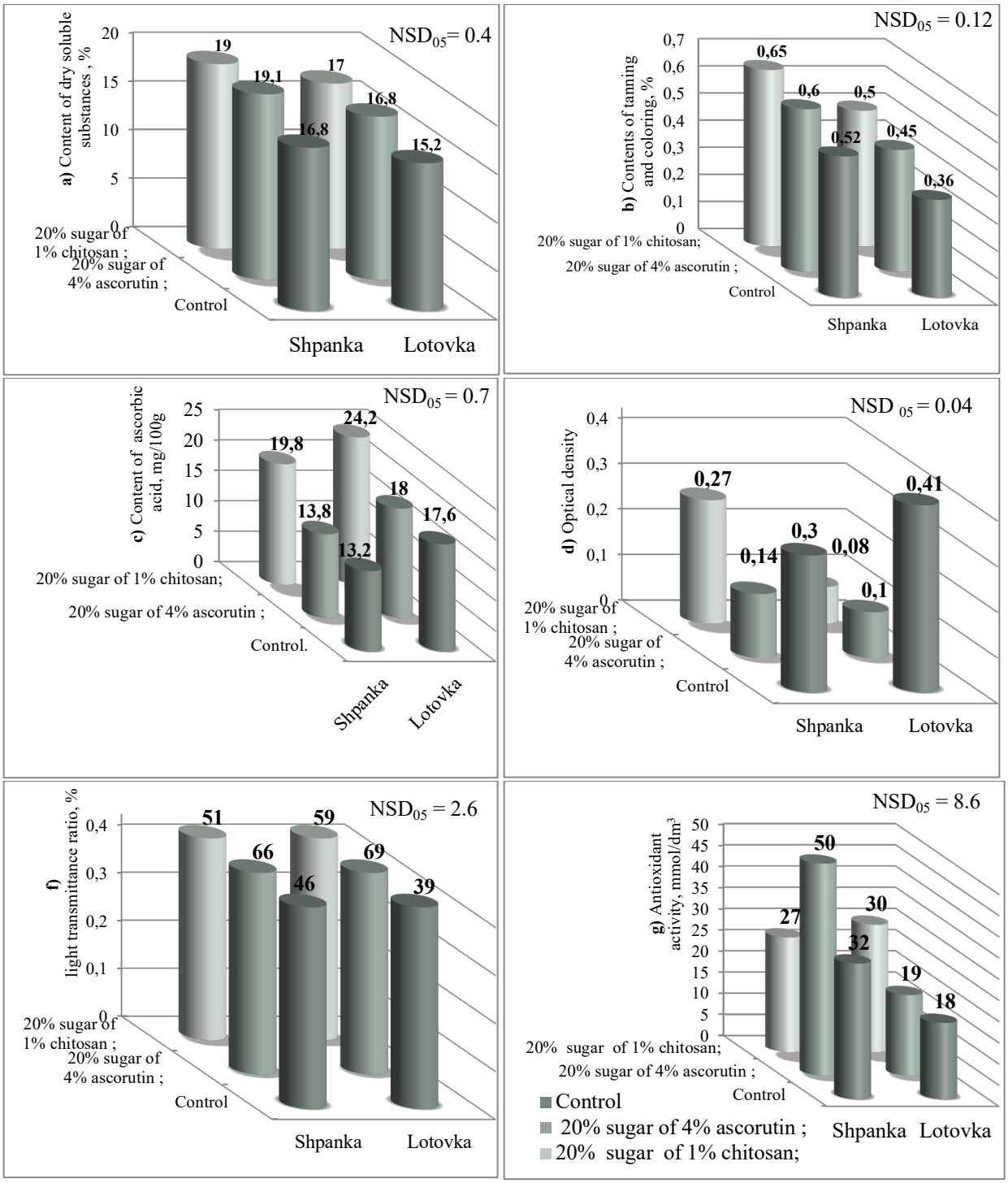


Figure 1. Contents: a) dry soluble substances; b) tanning and coloring; c) ascorbic acid; d) optical density; f) light transmittance ratio; g) antioxidant activity of fruits cherries varieties Shpanka and Lotovka

Table 2. The matrix of pairwise correlations between certain indicators of chemical composition and ability moisture-containing of frozen fruit cherries

Indicator	Dry soluble substances	Tanning and coloring substances	Ascorbic acid	Light transmittance ratio	Optical density	Antioxidant activity
Dry soluble substances	1	**0.78**	−0.10	0.46	−0.37	**0.61**
Tanning and coloring substances	**0.78**	1	−0.22	0.27	−0.22	**0.54**
Ascorbic acid	−0.10	−0.22	1	0.08	−0.39	**−0.72**
Light transmittance ratio	0.46	0.27	0.08	1	**−0.89**	0.46
Optical density	−0.37	−0.22	−0.39	**−0.89**	1	−0.17
Antioxidant activity	**0.61**	**0.54**	**−0.72**	0.46	-0.17	1

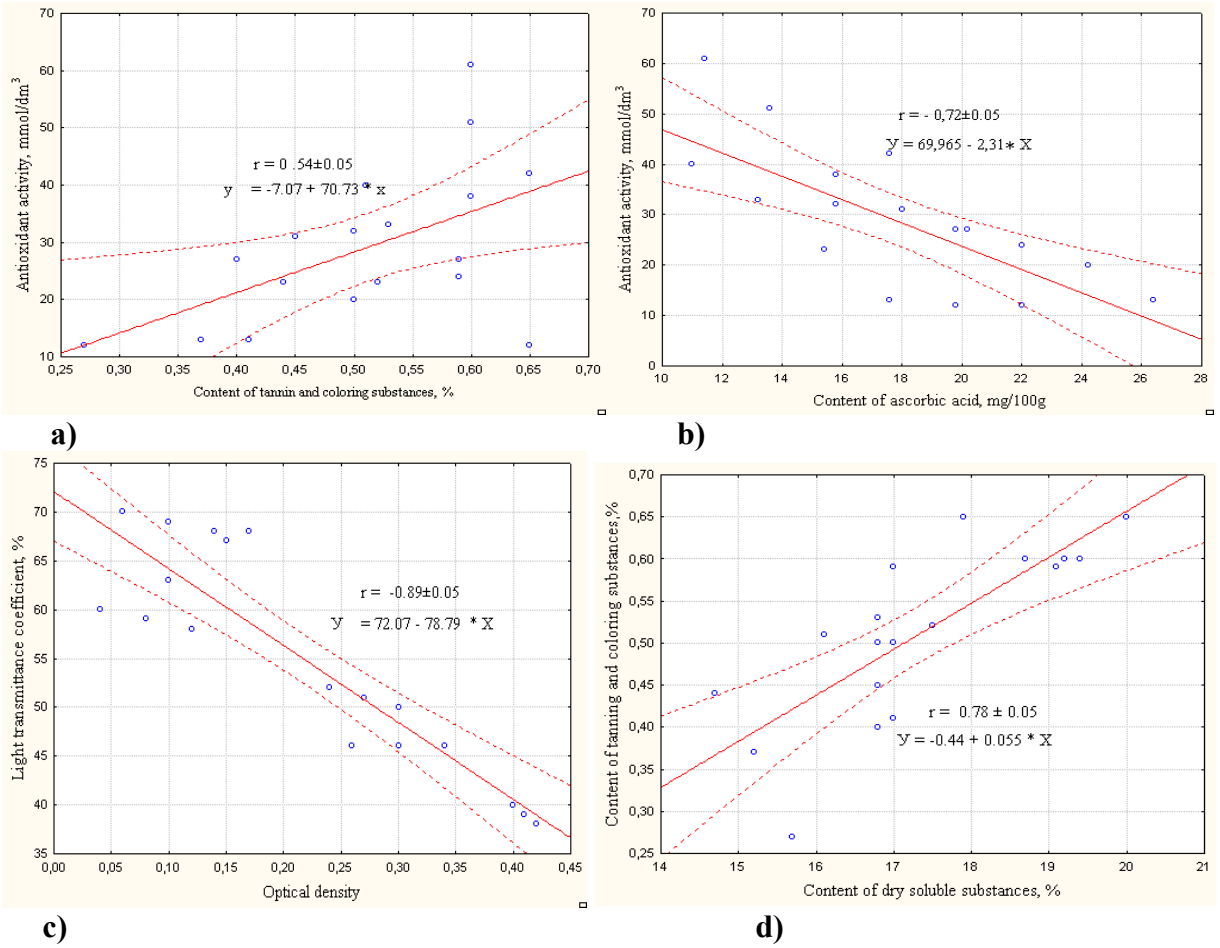


Figure 2. Regression equation and correlation between a) antioxidant activity and content: tanning and coloring agents and b) ascorbic acid, c) light transmittance ratio and optical density; d)content of soluble substances and tanning and coloring substances of fruit cherries varieties Shpanka and Lotovka

4.Conclusions

The content of tannins and colorants in cherry fruits is a varietal feature and after freezing in the fruits of the cherry varieties of Shpanka and Lotovka is reduced by 22 and 29%. Whereas in pre-treated fruits cherries by 20% sugar solutions with addition of 4% ascorutin or 1% chitosan decreased to 10 and 12%. The number of ascorbic acid in fruits, respectively, decreased to 23 and 38%. In addition, the smallest losses were for fruits treated with 20% sugar solution with the addition of 1% chitosan.

Antioxidant activity during freezing did not change significantly. It has been established that antioxidant activity correlates with the content of tannins and colorants and correlates with the content of ascorbic acid inverted.

The color indicator is a varietal feature. The optical density of the fruits of the cherry after the freezing negatively correlated and depended on the coefficient of light transmission.

5.References

Begon a de Ancos, Gonzalez, E. M., Pilar Cano, M. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal Agricultural and Food Chemistry*, 48, 4565−4570.

Blando, F., Gerardi, C., Nicoletti, I. (2004). Sour cherry (Prunus cerasus L.) anthocyanins as ingredients for functional foods. *Journal of Biomedicine and Biotechnology*, 5, 253–258.

Dıaz-Mula, H.M., Castillo, S., Martınez-Romero, D., Valero, D., Zapata, P.J., Guille'n, F., Serrano, M. (2009). Sensory, nutritive and functional properties of sweet cherry as affected by cultivar and ripening stage. *Food Science and Technology International*, 15(6), 535−543.

Esti, M., Cinquanta, L., Sinesio, F., Moneta, E., Di Matteo, M. (2002). Physicochemical and sensory fruit characteristics of two sweet cherry cultivars after cool storage. *Food Chemistry*, 76, 399–405.

Ferretti, G., Bacchetti, T., Belleggia, A., Neri, D. (2010). Cherry antioxidants: from farm to table. *Molecules*, 15, 6993−7005.

Gao, L., Mazza, G. (1995) Characterization, quantification and distribution of anthocyanins and colourless phenolics in sweet cherry. *Journal of Agricultural and Food Chemistry*, 43, 343–346.

Goncalves, B., Silva, A.P., Moutinho-Pereira, J., Bacelar, E., Rosa, E., Meyer, A.S. (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (Prunus avium L.). *Food Chemistry*, 103, 976–984.

Gonçalves, B., Landbo, A.K., Knudsen, D., Silva, A.P., Pereira, J.M., Rosa, E. (2004). Effect of ripeness and postharvest storage on the phenolic profiles of cherries (Prunus avium L.). *Journal of Agricultural and Food Chemistry*, 52, 523–530.

Han, C., Zhao, Y., Leonard, S.W., Traber, M.G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria ananassa) and raspberries (Rubus ideaus). *Postharvest Biology and Technology,* 33(1), 67–78.

Khasanov, V.V., Ryzhova, G.L., Maltseva, E.V. (2004). Methods for the determination of antioxidants. *Chemistry of Plant Raw Material*, 3, 63−75.

Levaj, B., Dragovic-Uzelac, V., Delonga, K., Kovacevic Ganic, K., Banovic, M., Bursac Kovacevic, D. (2010). Polyphenols and volatiles in fruits of two sour cherry cultivars, some berry fruits and their jams. *Food Technology and Biotechnology*, 48(4), 538–547.

Naichenko, V.M. (2001). Practicum on the technology of storage and processing of fruit and vegetables. (pp.158–162), Kyiv: FADA Ltd.

Ngo, T., Wrolstad, R.E., Zhao, Y. (2007). Color quality of Oregon strawberries − impact of genotype, composition, and processing. *Journal of food science*, 72(1), 25−32.

Pedisic, S., Levaj, B., Dragovic-Uzelac, V., Skevin, D., Skendrovic Babojelic, M.

(2009). Color parameters and total anthocyanins of sour cherries (Prunus cerasus L.) during ripening. *Agriculturae Conspectus Scientificus*, 74 (3), 259−262.

Sahari, M.A., Boostani, F.M., Hamidi, E.Z. (2004). Effect of low temperature on the ascorbic acid content and quality characteristics of frozen strawberry. *Food Chemistry*, 86(3), 357–363.

Scibisz, I., Mitec, M. (2007).The changes of antioxidant properties in highbush blueberries (Vaccinum corymbosum L.) during freezing and long term frozen storage. *Acta Scientiarum Polonorum Technologia Alimentaria*, 6(4), 75−82.

Serra, A.T., Duarte, R.O., Bronze, M.R., Duarte, C.M. (2011). Identification of bioactive response in traditional cherries from Portugal. *Food Chemistry*, 125, 318–325.

Szymczak, J.A., Rutkowski, K.P., Miszczak, A., Rozpara, E. (2003). Sensory evaluation of okordiao sweet cherry after storage. *Polish journal of food and nutrition sciences*, 12/53(3), 45−49.

Vasylyshyna E. (2017). Changes in antioxidant activity of cherry fruits and grapes during freezing. *Journal of food, agriculture & environment*, 15(2), 52−54.

Wang, H., Nair, M.G., Strasburg, G.M., Chang, Y.C., Booren, A.M., Gray, J.I. (1999). Antioxidant and anti-inflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *Journal of Natural Products*, 62, 294–296.

Zoric, Z., Pedisic, S., Kovacevic, D.B., Jezek, D., Dragovic-Uzelac, V. (2016). Impact of packaging material and storage conditions on polyphenol stability, colour and sensory characteristics of freeze-dried sour cherry (prunus cerasus var. Marasca). *Journal of Food Science and Technology*, 53(2), 1247–1258.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EVALUATION OF ANTIDIABETIC AND ANTICHOLESTEROL PROPERTIES OF BISCUIT PRODUCT WITH MANGROVE FRUIT FLOUR (MFF) SUBSTITUTION

Jariyah[1*], Endang Yektiningsih[2], Ulya Sarofa[1]

[1]*Department of Food Technology, Faculty of Engineering, University of Pembangunan Nasional "Veteran" Jawa Timur, Raya Rungkut Madya, Gunung Anyar, Surabaya 60294, East Java, Indonesia*

[2]*Department of Agribusiness, Faculty of Agriculture, University of Pembangunan Nasional "Veteran" Jawa Timur, Raya Rungkut Madya, Gunung Anyar, Surabaya 60294, East Java, Indonesia*

**jariyah.tp@upnjatim.ac.id*

https://doi.org/10.34302/2019.11.4.13

Article history:
Received:
15 January 2019
Accepted:
29 September 2019

Keywords:
Antidiabetic;
Anticholesterol;
Biscuit;
Bruguiera gymnorhiza;
Sonneratia caseolaris.

ABSTRACT

These fruits contain bioactive compounds and dietary fibers which is very potential for substitution to biscuit products. This study evaluated the antidiabetic and anticholesterol properties in the produced biscuits that were substituted with mangrove fruit flour (Pedada and Lindur fruits). This study used split plot design consisted of 2 factors. The first factor was divided into 5 groups (positive control group, negative control group, biscuit control group, Pedada biscuit group, and Lindur biscuit group), each group consisted of 4 rats and the second factor was blood taking time consisted of 5 time intervals (0, 1, 2, 3 and 4 weeks). The study parameters were rats' blood glucose, body weight, short chain fatty acids (SCFA), and lipid profiles. This study obtained that biscuits produced using mangrove fruit flour had antidiabetic properties since it could decrease rats' blood glucose and increased of body weight for 4 weeks. The biscuits feeding had no significant effect on acetic acid and butyric acid, but had significant effect on propionic acid level. Anticholesterol properties indicated that the biscuits could decrease of total cholesterol, low density, lipoprotein (LDL), triglyceride, and could increase of high density lipoprotein (HDL).

1.Introduction

This study is a continuation of previous study about physicochemical and organoleptical properties of biscuits that was subtituted with mangrove fruit flour (MFF). There were two types of mangrove fruit used as flour in this biscuit ingredients. The first type of mangrove fruit belonged to Pedada (*Sonneratia caseolaris*) then was called as pedada fruit flour (PFF) and the second type belonged to Lindur (*Bruguiera gymnorizha*) then was called as Lindur Fruit Flour (LFF).

The biscuit formulation was added with emulsifier Sodium Stearoyl Lactylate (SSL) 0.5%. The results of this study showed that biscuit preferred by the panelist was with substitution 20% of PFF (biscuit PFF) and 20% of LFF (biscuit LFF). The previous results showed that PFF and LFF contains dietary fibers and bioctive compounds, such as flavonoid, phenol, tannin (Jariyah *et al.,* 2015), vitamin C, and minerals (Jariyah *et al.,* 2014). Those compounds are good for health and can be used for substitution in biscuit products.

In order to find the functional properties of biscuit PFF and LFF, the antidiabetic and anticholesterol properties were evaluated to produce diabetic-friendly biscuit for diabetic

patients. The results of study by Jariyah *et al*. (2016) showed that biscuit with substitution 20% of PFF and 0.5% lecithin had effect to lower blood glucose level 7.63 mg/dL during 2 hours. Harijono *et al.* (2013) reported that biscuit contained of alginat had effect to lower blood glucose level 9.41%. Muhtadi *et al*. (2015) reported that *Citrus sinensis* also had antidiabetic and antihypercholesterolemic effect.

Besides diabetes, cardiovascular disease is the main cause of human mortality in developing countries which related to cholesterol level issues (Gaziano, 2007). Therefore, the biscuit in this study is expected to give a contribution as alternative functional food product from mangrove fruit, which can be consumed by diabetic patients and can decrease the cholesterol level.

2. Materials and methods

Mangrove fruits belonged to Pedada (*Sonneratia caseolaris*) and Lindur (*Bruguiera gymnorhiza*) were obtained from Wonorejo Village, Surabaya. The production of mangrove fruit flour referred to procedure from Jariyah *et al.* (2016). Wheat flour, margarine, Sodium Stearoyl Lactylate (SSL), eggs, sodium bicarbonate, glucose syrup, were obtained from Soponyono Market, Surabaya. The biscuits were produced in Laboratory of Food Processing Technology, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Indonesia.

Male wistar rats were used to evaluate antidiabetic and anticholesterol properties. The rats were obtained from Laboratory of Food and Nutrition Study Centre, University of Gadjah Mada Yogyakarta. Twenty of rats were 2-3 months in age and 180-225 g in weight. This study got a license in Ethical Clearance from Brawijaya University, Malang, East Java, Indonesia.

2.1. Procedure of Biscuit Production

The process of biscuit production referred to the study from Jariyah *et al.* (2016) with modification from Sindhuja *et al.* (2005) and El-Sharnouby *et al.* (2012). The sugars, margarines, eggs, and glucose syrup were mixed and added by SSL 0.50% until homogen. Then added with sodium bicarbonat, salts, skim milk, MFF, and continued mixing to produce dough. The next process was to produce dough sheets with diameter 3-4 cm; thickness 7.5 mm. The dough sheets then were baked at 150^{o}C for 6-10 mins and cooled for 30-45 mins. Once finished the biscuits' antidiabetic and anticholesterol properties were analyzed.

2.2. Evaluation of Antidiabetic properties

Evaluation of antidiabetic properties was conducted with analyzed the decreasing of blood glucose level through in vivo experiment used 20 wistar rats Rattus novergicus, divided into 5 groups (each group consist of 4 rats) then the rats were adapted for a week. During adaptation phase the rats were fed (standard AIN-93M) and feed through *ad libitum* drinking. To generate a hyperglicemic state (diabetic), the rats were induced by alloxan 80 mg/kg body weight that dilluted in distilled water aquades. Each rat was injected by intraperitoneal injection as many as 2 ml/200g body weight. Diabetic state will be obtained if the blood glucose level of the rats reach >200 mg/dL, this evaluation results were noted as the result in week-0. For each group then were divided into several condition:

K1: The control-normal, were given fed standard AIN-93M
K2: Diabetic, were given fed standard AIN-93M
K3: Diabetic, were given fed biscuit control
K4: Diabetic, were given fed biscuit LFF
K5: Diabetic, were given fed biscuit PFF

Monitoring of body weight and blood glucose level were conducted every week for 4 weeks long. Blood drawing of the rats was conducted through retro orbital plexus for 1 ml. Then the blood sample was centrifuged at 4000 rpm for 15 mins at room temperature. The supernatant was collected and glucose serum level was measured by glucose oxidase

methode using spectrophotometer at λ 500 nm. After week-14, surgical procedure was conducted to collect the rats ceacum, then the ceacum was analyzed for short chain fatty acid level by using Gas Chromatography.

2.3. Evaluation of Anticholesterol properties

The evaluation of anticholesterol properties of biscuit was conducted by analysis of lipid profile using in vivo methods, with 20 wistar rats, divided into 5 group (each group consisted of 4 rats). These rats were adapted for a week and fed with standard AIN-93M and *ad libitum* of drinking. To generate hypercholesterol condition (total of cholesterol > 150 mg/dl), the rats were fed with high cholesterol feeding in the form of pellets which contained of 50 mL cooking oil, 10 g egg yolks, 0.1% propylthiouracyl (PTU). This feeding was given as 15 g/day, high cholesterol feeding was stopped after the rats reached hypercholesterol state, then the experimental feeding was given as:

K1: Control normal, were given fed standard AIN-93M
K2: Hypercholesterol, were given fed standard AIN-93M
K3: Hypercholesterol, were given fed biscuit control
K4: Hypercholesterol, were given fed biscuit LFF
K5: Hypercholesterol, were given fed biscuit PFF

Blood drawing procedure for cholesterol analysis were obtained every week for 4 weeks-long. The monitoring parameters consisted of lipid profile (total of cholesterol, LDL, HDL, triglyceride).

2.4. Data Analysis

Data were analyzed using split plot in time design, with SPSS software version 24.0 and Benferroni test (α= 5%).

3.Results and discussions

3.1. Antidiabetic Properties

Antidiabetic properties were detected from the decreasing decrease blood glucose level in rats. Analysis result showed that biscuit diet to experiment rats significantly different towards serum glucose level ($p<0.05$), Table 1. On the first week, blood glucose level for all groups of the rats increased up to 210.46 mg/dL after injected by alloxan, except group K1. Blood glucose level of diabetic rats (K2) did not show significant difference and increased up to 214.2 mg/dL (1.13%). This results caused by alloxan injection affected the damage of β pancreas cell, so insulin could not be produced again and caused permanent diabetic as reported by Szkudelski (2001).

The decreasing effect of blood glucose level in group fed with control biscuit (K3) up to 33.63 mg/dL (15.79%). Group with biscuit LFF feeding (K4) and PFF (K5) showed decreasing effect of blood glucose level up to 101.80 mg/dL (48.52%) and 109.91 mg/dL (52.22%). This results showed that dietary fibers and bioactive compounds of mangrove fruit flour had hypoglycemic effect, which shortened transit time in the intestine, so glucose absorption could be decreased and lowered the hyperglycemic state.

The decreasing effect of blood glucose also could be caused by bioactive compounds and dietary fibers from both types of mangrove fruits which could inhibit disaccharidase activity and extended the stomach emptying time. This mechanism caused glucose absorption slow and increased insuline sensitivity in peripheral tissues, so blood glucose level decreased. Result of study from Harijono *et al*. (2012) showed that feeding of water soluble polysaccharide from Gembili during 28 days could lower glucose level, then feeding of fiber from fenugreek (*Trigonella foenum-graecum*) could lower glucose level up to 20.27% for 4 weeks (Abdelatif *et al.,* 2012). The average of decreasing of blood glucose level between groups after 4 weeks showed in Fig.1

Table 1. Effect of biscuit on blood glucose levels and body weight of normal and diabetic rats

Week	Blood glucose levels (mg/dL)				
	K1	Diabetic +			
		K2	K3	K4	K5
0	65.48 ± 1.89[a]	211.82 ± 4.73[a]	212.87±4.37[a]	209.83±4.00[a]	210.46±3.37[a]
1	66.12 ± 1.88[a]	212.31 ± 4.49[b]	199.28±5.74[b]	197.21± 3.36[b]	195.04±2.68[b]
2	66.83 ± 1.78[a]	213.33 ± 4.39[b]	188.33±2.01[b]	160.60± 2.28[b]	158.76±2.06[c]
3	67.11 ± 1.88[a]	213.73± 4.55[b]	185.25± 2.93[b]	143.03± 1.53[d]	140.47±1.27[d]
4	67.71 ± 2.22[a]	214.21 ± 2.21[b]	179.24± 1.29[b]	108.03±4.18[c]	100.55±1.55[e]
	Body weight (g)				
Week	K1	Diabetic +			
		K2	K3	K4	K5
0	199.25±4.03[a]	190.00±3.56[a]	189.75±5.12[b]	190.75±3.50[c]	193.25±2.50[ab]
1	206.75±4.27[a]	187.00±3.56[b]	194.00±5.35[a]	193.75±3.86[c]	196.50±3.00[b]
2	215.25±4.19[a]	184.25±3.59[b]	199.00±5.60[a]	200.25±2.99[b]	202.75± 2.63[b]
3	223.25±4.99[a]	182.00±3.37[b]	205.75±5.56[a]	206.25±4.03[b]	209.25±3.30[a]
4	230.75±3.77[a]	178.00±3.16[b]	211.50±4.43[a]	213.00±3.16[a]	214.75±3.50[a]

*Different code indicated the differences in one column

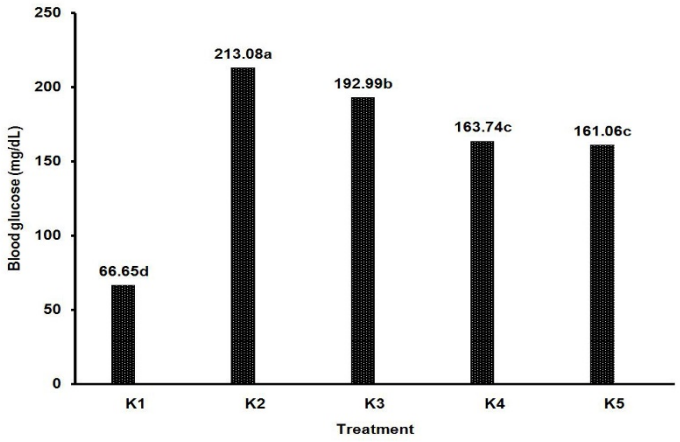


Figure 1. Changes of blood glucose of rats during 4 weeks feeding biscuit in diet

The decreasing of blood glucose level of group fed with biscuit control up to 20.09 mg/dL (9.43%) compared to diabetic group fed with standard AIN 93-M (K2). Group fed with biscuit LFF (K4) showed decreasing effect up to 49.34 mg/dL (21.16%), and group fed with biscuit PFF (K5) showed decreasing effect up to 52.02 mg/dL (24.41%). The decreasing effect on this study was lower than study reported by Morada *et al.* (2011) showed that feeding of extract of Sonneratia alba could lower glucose level up to 66.9%, while giving of fibers 50-150 mg/kg during 8 weeks could decrease glucose level up to 16-61%, also

pressed the glucose level elevation, extended the stomach emptying time and glucose difusion in the intestine (Moharib & El-Batran, 2008).

The decreasing of glucose level was assumed also caused by polifenol, flavonoid, saponin, and tannin compounds in mangrove fruit flour. Some researchers reported that bioactive compounds like saponin, flavonoid, triterpenoid, tannin, were known to lower glucose level (Yin *et al.,* 2004; Chandrika *et al.,* 2006; Smith & Adanlawo, 2014; Koneri *et al.,*2014; El- Barky *et al.,* 2017).

3.2.The Rats Body Weight

The alteration of the rats body weight on group K2 (diabetic) decreased up to 6.32%, Table 1. This was caused by β pancreas cell was damaged due to induction of alloxan, so insulin could not be produced and glucose could not enter cell tissues. Because of this mechanism, glycogenesis process was disrupted both in the muscle and liver, so glycogen production also decreased and followed by decrease of muscle mass that affect body weight. Sousa *et al.* (2015) reported that glycogen in the liver will increase as well as glycogenesis process increase.

The increase of body weight of group fed with biscuit control (K3) was less higher than biscuit LFF (K4) feeding amount 11.66%. This was caused by sour taste on biscuit PFF which can affect the appetite, then body weight of group fed with biscuit PFF (K5) increased up to 11.13%. The increase of body weight showed that bioactive compound and dietary fiber of mangrove fruit flour could enhance peripheral insulin sensitivity, so glucose could be absorbed into celland body weight increased. Dietary fiber could repair pancreatic function to produce insulin (Lattimer & Haub, 2010), so cell could acquire enough energy to store glucose in the muscle and the rats body weight would increase (Weyer *et al.,* 2001), the other hand diabetic caused protein glycation and affect body weight (Yin et al., 2004).

3.3. Short Chain Fatty Acid (SCFA)

The average level of SCFA acetic acid in the cecum of rats after 4 weeks was 20.25 to 24.90 mMol/L, butiric acid from 29.36 to 38.70 mMol/L, and propionic acid from 2.64 to 4.51 mMol/L. The result of analysis showed that feeding of biscuit from mangrove fruit flour was not significantly different towards acetic acid and butiric acid level ($p>0.05$), but significantly different ($p<0.05$) towards propionic acid level.

Fig. 2 showed that group of control (normal rats) and group of diabetic rats with feeding standard AIN 93M had the same profile, this was due to feeding of standard contained of fiber from CMC which could be degraded into SCFA. The highest level of propionic acid and butiric acid were found at group fed by biscuit PFF, LFF, and control. SCFA as the fermentation product will be absorbed in the intestine and transported into liver through enterohepatic circulation. It was a system which connected a tube between liver and intestine to help digestive process and used for metabolic substance by liver (Koh *et al.,* 2016).

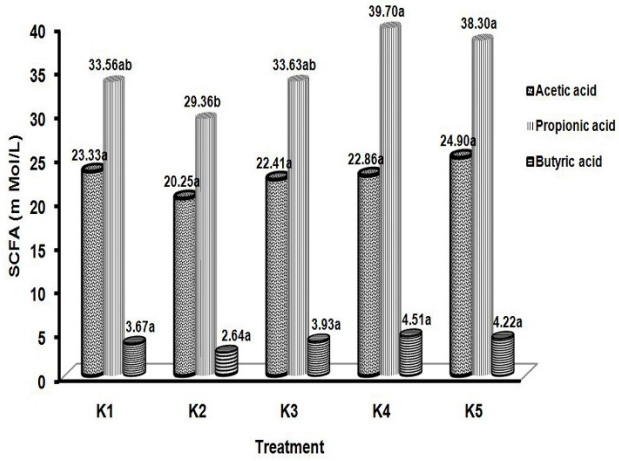


Figure 2. Profile of SCFA

The increase of SCFA production gave benefits in order to lower the glucose production by liver (Harijono, 2012). SCFA was metabolized at three parts of the body i.e., at colonic epithelial cell which used butiric as main substrate to maintain energy production; at liver cell which metabolized butiric residue

and propionic to be utilized in gluconeogenesis; and muscle cell which conducted acetic residue oxidation to produce energy. Soluble dietary fiber like pectine and guar gum generally fastly fermented in the colon (Topping & Clifton, 2001; Henningsson *et al.,* 2002), but insoluble dietary fiber like cellulose, hemicellulose, and lignin only have small effect on postprandial glucose (Dikeman & Fahey, 2006).

Table 2. Effect of biscuit on total cholesterol, LDL-C, levels of normal and hypercholesterol of rats

Week	Total Cholesterol levels (mg/dL)				
	K1	Hypercholesterol +			
		K2	K3	K4	K5
0	85.43 ± 1.71[a]	184.77 ± 4.52[a]	182.45±3.69[a]	182.45±2.06[a]	181.29±1.47[a]
1	87.42 ± 2.00[b]	186.24 ± 4.66[a]	159.73±2.79[b]	153.69± 1.45[b]	146.31±1.97[b]
2	87.77 ± 2.18[b]	186.50 ± 4.64[a]	151.28±1.92[b]	139.24± 3.18[b]	129.57±3.07[c]
3	88.91 ± 2.19[b]	187.25± 4.73[a]	132.95± 1.82[c]	118.05± 3.48[c]	110.43±2.19[d]
4	89.60 ± 2.23[b]	188.09 ± 4.73[a]	127.52± 1.65[d]	112.25±4.47[d]	104.87±2.53[e]
LDL –c (mg/dL)					
Week	**K1**	**Hypercholesterol+**			
		K2	**K3**	**K4**	**K5**
0	34.61 ± 1.60[a]	75.95 ± 1.73[a]	77.16 ± 2.08[a]	76.13 ± 1.27[a]	74.91 ±2.07[a]
1	35.11 ± 1.36[a]	76.77 ± 1.57[a]	68.44 ± 2.74[a]	63.83 ± 2.09[b]	54.97 ± 1.88[b]
2	35.86 ± 1.26[a]	75.52 ± 4.52[a]	60.17 ± 2.28[a]	56.72 ± 1.73[b]	50.86 ±1.89[c]
3	37.20 ± 2.14[a]	78.20 ± 3.87[a]	55.88 ± 2.90[b]	44.64 ± 2.15[c]	39.97 ± 1.53[d]
4	37.99 ± 2.11[a]	79.03 ± 4.11[a]	53.23 ± 2.77[c]	42.29 ± 1.55[c]	37.10 ± 1.59[e]

*Different code indicated the differences in one column

3.4. Anticholesterol Properties

Anticholesterol properties of biscuit was evaluated from lipid profile which consisted of total cholesterol, LDL, HDL, and triglyceride. The total cholesterol level of rats in hypercholesterolemic up to 184.77 mg/dL (53.12%) higher than the initial condition. The total cholesterol level of group K1 and K2 during 4 weeks did not significantly increase and tend to be stable (Table 2). Decreasing effect of total cholesterol in group K3 up to 54.93 mg/dL (30.10%), while the group K4 and K5 decreased up to 70.20 mg/dL (38.47%) and 76.42 mg/dL (42.15%). The lowest total cholesterol level was shown in group K5, the decreasing effect in this group is lower than previous study by giving orange peel extract 125 mg/kg body weight could lower total cholesterol level up to 54.77 mg/dL (Muhtadi *et al.,* 2015).

But this result was lower than giving of soluble fiber β-glucan 3.30% which could lower total cholesterol more than 33.00% (Dikeman & Fahey, 2006). The diet which consists of much dietary fiber causes extending absorption of food and carbohydrate in the intestine, so postprandial glucose level will decrease. This condition decreases insulin secretion that will affect inhibition of HMG Co-A reductase, so the synthesis of cholesterol will also decrease (Daubioul *et al.,* 2002).

The other factors which also play role in decreasing cholesterol were saponin, flavonoid, tannin instead of dietary fiber contained in mangrove fruit flour. Three factors could bind

bile acid in the intestine. Bile acid was produced from cholesterol and absorbed again by the intestine, inhibition of reabsorption of bile acid and throwing through feces could decrease cholesterol level in blood (Dasofunjo *et al.,* 2012).

The presence of phenol in mangrove fruit flour also played role to lower the total cholesterol. Phenol compound has proven could lower hypercholesterolemia (Bok *et al.,* 1999; Rehrah *et al.,* 2007) because phenol was able to inhibit modification of LDL oxidation that could cause atherosclerosis. The function of antioxidant promoted cholesterol eflux which mediated by HDL. Capacity of cholesterol eflux increase as presence of flowing HDL, where this condition depended on the length and saturation of fatty acid in HDL (Arora *et al.,*2000). The presence of phenol compound in mangrove fruit flour was able to lower total cholesterol. Phenol compound has proven could lower hypercholesterolemia The previous study showed that, when HDL was oxidized, HDL would loose its unsaturated fatty acids, so the lowering capacity of free cholesterol would also decrease. This had relation with decrease of HDL flow rate (Shehata & Soltan, 2012). The alteration of decreasing effect of cholesterol during 4 weeks between groups shown in Fig. 3.

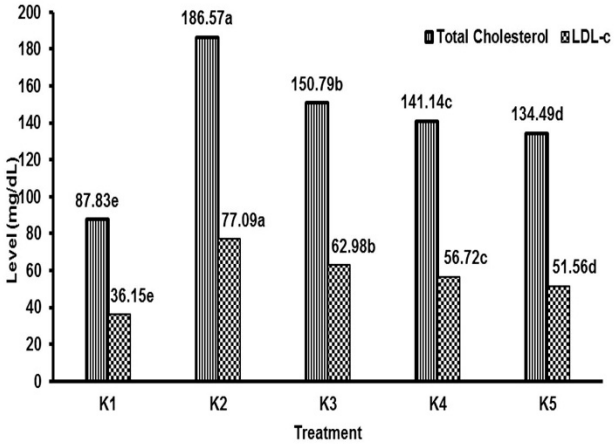


Figure 3. Changes of cholesterol total and LDL-c of rats during 4 weeks feeding biscuit in diet

Fig. 3 showed that group fed with biscuit control (K3) had decreasing effect on cholesterol up to 35.80 mg/dL (19.18%) rather than group K2 (negative control), while group fed with biscuit LFF (K4) and biscuit PFF (K5) had decreasing effect 45.43 mg/dL (24.35%) and 52.08 mg/dL (27.91%).

This decreasing effect was assumed that dietary fiber and bioactive compounds saponin, flavonoid, tannin in mangrove fruit flour had a role in decreasing cholesterol. Hasanah et al. (2016) proved that tannins could inhibit HMG Co-A reductase and acyl-Coenzym A Cholesterol acyltransferase (ACAT) which was enzyme for cholesterol synthesis, absorption, and its release to blood stream.

3.5. LDL-c

The average of LDL cholesterol (LDL-c) for every week shown in Table 2 and Fig. 3. The LDL-c in normal and hypercholesterolemia rats were given fed standard AIN 93-M during 4 weeks did not show significant difference, but the rats were given fed biscuit control (K3), biscuit LFF (K4), and biscuit PFF (K5) could lower LDL-c level significantly for each group 23.93 mg/dL (31.01%); 33.84 (44.45%) and 37.81 mg/dL (50.47%). This showed that soluble dietary fiber in mangrove fruit flour was able to lower LDL-c level, while insoluble dietary fiber (cellulose. hemocellulose, lignin) was not able to lower LDL-c level. The previous study stated that soluble dietary fiber of orange peel was able to lower LDL-c up to 53.47% [33]. Furthermore El- Khoury *et al.* (2012) reported that feeding of soluble dietary fiber from β-glucan amount 10 g/day during 5 weeks could lower LDL-c up to 14.30 mg/dL. All types of soluble dietary fiber such as psyllium, pectin, and guar gum had ability in decreasing LDL-c level (Brown *et al.,* 1999).

The mechanism of decreasing LDL-c level by dietary fiber is that dietary fiber can alter absorption and metabolism of bile acid; dietary fiber can modify absorption and metabolism of lipid; short chain fatty acid procuded in fermentation of dietary fiber can affect

metabolism of cholesterol and lipoprotein; and dietary fiber can alter insulin or other homones concentration and tissue sensitivity to hormone (Anderson *et al.,* 1999).

Soluble dietary fiber also extend stomach emptying process and bind bile acid, so bile acid level decrease. In result, the body naturally produce bile acid from cholesterol in the blood stream. The absorption of blood cholesterol cause VLDL level which was procuded will be in small amount. Because of LDL was synthetized from VLDL, so decreasing of VLDL also lower LDL-c level in blood (Eze *et al.,* 2014). According to Then et al. (2009) decreasing of cholesterol would lower remnant chilomicron. so the conversion of VLDL to LDL decreased.

3.6. HDL-c

The average level of HDL cholesterol (HDL-c) every week shown in Table 3. This table showed that HDL-c level in group of normal rats, and hypercholestrolemia rats during 4 weeks did not significant differences ($p>0.05$). But group fed with biscuit control (K3) the HDL-c level increased up to 20.51 mg/dL (45.07%), group fed with LFF (K4) up to 32.51 mg/dL (56.36%) and group fed with PFF (K5) up to 33.35 mg/dL (56.17%) . The increase of HDL was assumed that presence of dietary fiber and bioactive compound in mangrove fruit flour such as flavonoids, tannins, saponins, and phenol gave significant effect to increase HDL-c.

Table 3. Effect of biscuit on HDL-C and triglyceride levels of normal and hypercholesterol of rats

Week	Total Cholesterol levels (mg/dL)				
	K1	Hypercholesterol +			
		K2	K3	K4	K5
0	85.43 ± 1.71[a]	184.77 ± 4.52[a]	182.45±3.69[a]	182.45±2.06[a]	181.29±1.47[a]
1	87.42 ± 2.00[b]	186.24 ± 4.66[a]	159.73±2.79[b]	153.69± 1.45[b]	146.31±1.97[b]
2	87.77 ± 2.18[b]	186.50 ± 4.64[a]	151.28±1.92[b]	139.24± 3.18[b]	129.57±3.07[c]
3	88.91 ± 2.19[b]	187.25± 4.73[a]	132.95± 1.82[c]	118.05± 3.48[c]	110.43±2.19[d]
4	89.60 ± 2.23[b]	188.09 ± 4.73[a]	127.52± 1.65[d]	112.25±4.47[d]	104.87±2.53[e]
LDL –c (mg/dL)					
Week	**K1**	**Hypercholesterol+**			
		K2	**K3**	**K4**	**K5**
0	34.61 ± 1.60[a]	75.95 ± 1.73[a]	77.16 ± 2.08[a]	76.13 ± 1.27[a]	74.91 ±2.07[a]
1	35.11 ± 1.36[a]	76.77 ± 1.57[a]	68.44 ± 2.74[a]	63.83 ± 2.09[b]	54.97 ± 1.88[b]
2	35.86 ± 1.26[a]	75.52 ± 4.52[a]	60.17 ± 2.28[a]	56.72 ± 1.73[b]	50.86 ±1.89[c]
3	37.20 ± 2.14[a]	78.20 ± 3.87[a]	55.88 ± 2.90[b]	44.64 ± 2.15[c]	39.97 ± 1.53[d]
4	37.99 ± 2.11[a]	79.03 ± 4.11[a]	53.23 ± 2.77[c]	42.29 ± 1.55[c]	37.10 ± 1.59[e]

*Different code indicated the differences in one column

The increase of HDL-c level might be caused by increase of apolipoprotein A with undefined mechanism. Apolipoprotein (A) is one of protein molecule that support forming of HDL-c particles (Eze *et al.,* 2014). In Fig. 4 showed that the increase of HDL-c in group fed with biscuit control (K3) up to 10.69 mg/dL compared with group of hypercholesterolemic rats fed with standar AIN-93M. while group fed with biscuit LFF (K4) and grup fed with

biscuit PFF (K5) for each group increased up to 18.52 mg/dL and 21.84 mg/dL.

3.7. Triglyceride

The average of triglyceride level in group of normal rats. hypercholesterolemic rats, and group fed with biscuit control did not show significant differences, but group feeding of biscuit LFF (K4) and PFF (K5) could lower triglyceride significantly every week, Table 3. The decreasing of triglyceride of group K4 and K5 for each up to 27.93 mg/dL (21.89%); and 42.02 mg/dL (32.35%).

The mechanism of decreasing triglyceride was assumed that affected by level of soluble dietary fiber (pectin) from mangrove fruit flour and short chain fatty acid from dietary fiber fermentation in the rats colon. Pectin could inhibit absorption lipid in the intestine, so triglyceride and cholesterol in blood would decrease. In the gastrointestinal tract, pectin binds bile acid and excretes it together with feces. Dietary fiber also binds bile acid so it can not re-enter to enterohepatic cycle and increases bile acid excretion in fecal with various mechanism such as bile acid binding, gel forming, and micelle form binding (Dhingra *et al.,*2012). If the excretion of bile acid increases, cholesterol and triglyceride absorption will be disrupted and triglyceride serum level will decrease.

Fig. 4 showed that decreasing of triglyceride after consuming biscuit from mangrove fruit flour during 4 weeks could not reach level nearest to triglyceride from group of normal rats.

Research from Abdelbaky *et al.* (2009) showed that triglyceride decreased up to 48.26% and could reach triglyceride level nearest to group of control (normal). The decreasing of triglyceride by soluble dietary fiber occured inconsistently, soluble dietary fiber from barley was known could lower triglyceride level (Talati *et al.,* 2009), while other soluble dietary fibers like psyllium, oat, and guar gum could lower total cholesterol but not followed by decreasing of triglyceride significantly (Slavin *et al.,* 2009). In group of rats fed with biscuit control, triglyceride lowered up to 11.29 mg/dL (8.66%) compared with group of hypercholesterolemic rats (K2), while group fed with biscuit LFF (K4) and PFF (K5), for each group was 19.97 mg/ dL (15.28%) and 25.98 mg/dL (19.93%).

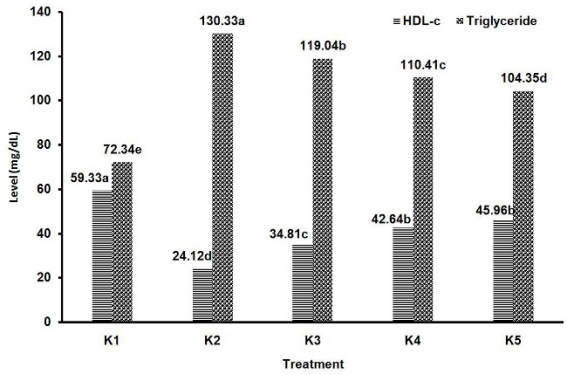


Figure 4. Changes of LDL-c and triglyceride of rats during 4 weeks feeding biscuit in diet

4. Conclusions

The mangrove fruit flour-substituted biscuits has antidiabetic properties which able to decrease blood glucose level in rats up to 101.80 mg/dL for biscuit LFF and 109.91 mg/dL for biscuit PFF. These biscuits also had anticholesterol properties which able to lower total cholesterol up to 70.20 mg/dL for biscuit LFF and 76.42 mg/dL for biscuit PFF. LDL-c lowered up to 33.84 mg/dL for biscuit LFF and 37.81 mg/dL for biscuit PFF. While HDL-c increased up to 32.51 mg/dL for biscuit LFF and 33.35 mg/dL for biscuit PFF. Triglyceride lowered up to 27.93 mg/ dL and 42.02 mg/dL for each biscuit LFF and biscuit PFF. The SCFA in cecum of rats after 4 weeks as followed 20.25 mMol/L to 24.90 mMol/L for acetic acid and 29.36 mMol/L to 39.70 mMol/L for butiric acid, 2.64 mMol/L to 4.51 mMol/L for propionic acid.

5. References

Abdelatif, A. M., Ibrahim, M. Y., Mahmoud, A. S., (2012). Antidiabetic effects of fenugreek (*Trigonella foenum-graecum*) seeds in the domestic rabbit (*Oryctolagus*

cuniculus). *Research Journal of Medicinal Plant,* 6(6), 449-455.

Abdelbaky, M. S., Elmehiry, H. F., Ali, N. K. M. (2009). Effect of some citrus peels on hypercholesterolemic rats. *Proceedings of the 1st International and 4th Arab Annual Scientific Conference* on: Academic Acreditation for Higher Spesific Education Institutions and Programs in Egypt and Arab World Reality and Expectation, 2009, 1627-1639.

Anderson, J. W., Deakins, D. A., Bridges, S. R. (1999). Soluble Fiber, Hypocholesterolemic Effects and Proposed Mechanism. In D. Kritchevsky, C. Bonfield, J. W. Anderson (Ed), Dieatry Fiber: Chemistry, Physiology, and Health Effects. (pp. 339-358), New York: Plenum Press.

Arora, A., Byrem, T. M., Nair, M. G., Strasburg, G. M. (2000). Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Archives of biochemistry and biophysics,* 373(1), 102-109.

Bok, S. H., Lee, S. H., Park, Y. B., Bae, K. H., Son, K. H., Jeong, T. S., Choi, M. S. (1999). Plasma and hepatic cholesterol and hepatic activities of 3-Hydroxy-3-Methyl-Glutaryl-CoA reductase and acyl coA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *The Journal of nutrition,* 129(6), 1182-1185, 1999.

Brown, L., Rosner, B., W. Willett, W., Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. *The American journal of clinical nutrition,* 69(1), 30-42.

Chandrika, U. G., Wedage, W. S., Wickramasinghe, S. M. D. N., Fernando, W. S. (2006) Hypoglycaemic action of the flavonoid fraction of *Artocarpus heterophyllus* leaf. *African Journal of Traditional, Complementary and Alternative Medicines*, 3(2), 42-50.

Dasofunjo, K., Nwodo, F. O. C., Ipav, S. S., Ngu, T. A. (2012). The effect of ethanolic leaf extract of *Piliostigma thonningii* on serum lipid profile of male wistar albino rats. *Journal of Natural Product and Plant Resources,* 2 (6), 665-669.

Daubioul, C., Rousseau, N., Demeure, R., Gallez, B., Taper, H., Declerck, B., N. Delzenne. (2002). Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. *The Journal of nutrition,* 132(5), 967-973, 2002.

Dhingra, D., Michael, M., Rajput, H., Patil, R. T. (2012) Dietary fiber in foods: a review. *Journal of food science and technology,* 49(3), 255-266.

Dikeman, C. L., Fahey, G. C. (2006). Viscosity as related to dietary fiber: a review. *Critical reviews in food science and nutrition,* 46(8), 649-663.

El- Barky, A. R., Hussein, S. A., Alm-Eldeen, A. A., Hafez, Y. A., Mohamed, T. M. (2017). Saponins and their potential role in diabetes mellitus. *Review Diabetes Management,* 7(1), 148-158.

El- Khoury, D., Cuda, C., Luvohyy, B. L., Anderson, G. H. (2012). Beta glucan: health benefits in obesity and metabolic syndrome. *Journal of Nutrition and Metabolism,* 2012, 1-28.

El- Sharnouby, G. A., Aleid, S. M., Al-Otaibi, M. M. (2012). Nutritional Quality of Biscuit Supplemented with Wheat Bran and Date Palm Fruits (*Phoenix dactylifera* L.). *Food and Nutrition Science,* 3(3), 322-328.

Eze, O. F., Ani, J. C., Obasi, N. E. (2014). Dietary fibre potentials of some selected flours and their effect on blood glucose and serum cholesterol reduction. *European Journal of Food Science and Technology,* 2(2), 1-12.

Gaziano, T. A. (2007). Recuing the growing burden of cardiovascular disease in the developing world. *Health Affairs (Millwood),* 26(1), 13-24.

Harijono, Estiasih, T., Suharharum, W. B., Suwita, I. K. (2012). Effect of hypoglycemic water-slouble polysaccharides from *Dioscorea esculanta*. *Journal of Technology and Food Industry,* 23,1-8.

Hasanah, Q., Hasim, Faridah, D. N., Andrianto, D. (2016). Inhibition activity of HMG-CoA reductase by rice brain extract and its fractions as anticholesterolemia in vitro study. *Der Pharma Chemica,* 8(23), 1-5.

Henningsson, A. M., Björck, I. M., Nyman, E. M. (2002). Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats. *The Journal of nutrition,* 132(10), 3098-3104.

Jariyah, J., Widjanarko, S. B., Yunianta, Estiasih T., Sopade P. A. (2014). Pasting properties of mangrove fruit flour and starches mixtures. *International Food Research Journal,* 21(6), 2161-2167.

Jariyah, J., Widjanarko, S. B., Yunianta, Estiasih, T. (2015). Phytochemical and acute toxicity studies of ethanol extract from Pedada (*Sonneratia caseolaris*) fruit flour (PFF). *International Journal on Advanced Science, Engineering and Information Technology,* 5(2), 95-98.

Jariyah, J., Widjanarko, S. B., Yunianta, Estiasih, T. (2016). Quality evaluation of Wheat-Pedada Fruit Flour (PFF) biscuit with different emulsifiers. *Agriculture and Agricultural Science Procedia,* 9(2016),518 – 524.

Harijono, Estiasih, T., Sunarharum, W. B., Hartono, M. D. (2013). Hypoglecemic effect of biscuits containing water-soluble polysaccharides from wild yam (*Dioscorea hispida* Dennts) or lesser yam (*Dioscorea esculanta*) tubers and alginate. *International Food Research Journal,* 20(5), 2279-2285.

Koh, A., de Vadder, F., Kovatcheva-Datchary, P., Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell,*165(6), 1332-1345.

Koneri, R. B., Sammadar, S., Ramalah. (2014). Antidiabetic activity of a triterpenoid saponin isolated from *Momordica cymbalaria* Fenzl. *Indian journal of experimental biology,* 52(1),46-52.

Lattimer, J. M., Haub, M. D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients,* 2(12), 1266-1289.

Moharib, S. A., El-Batran. (2008). Hypoglycemic effect of diatery fibre in diabetic rats. *Research Journal of Agriculture and Biological Sciences,* 4(5), 455-461.

Morada, N. J., Metillo, E. G., Uy, M. M., Oclarit, J. M. (2011). Anti-diabetic polysaccharide from mangrove plant*, Sonneratia alba* Sm. *International Conference on Asia Agriculture and Animal*, 1(2011), 197-200.

Muhtadi, Haryoto, Azizah, T., Suhendi, A., Yen K. H. (2015). Antidiabetic and anti hypercholesterolemic activities of Citrus sinensis peel : in vivo study. *National Journal of Physiology, Pharmacy and Pharmacology,* 5(5), 382-385.

Rehrah, D., Ahmedna, M., Yu, J., Goktepe, I., Hurley, S., Anner, T., Rao, A. P. (2007) Enhanced cholesterol and triglyceride lowering effect of West African green tea. *Journal of the Science of Food and Agriculture,* 87(7), 1323-1329.

Shehata, M. M. S. M., Soltan, S. S. A. (2012). The effects of purslane and celery on hypercholesterolemic mice. *World Journal of Dairy and Food Sciences,* 7 (2*),* 212-221.

Sindhuja, A., Sudha, M. L., Rahim A. (2005). Effect of incorporation of amaranth flour on the quality of cookies. *European Food Research and Technology,* 221(2005), 597-601.

Slavin, J. L., Savarino, V., Paredes-Diaz, A., Fotopoulos, G. (2009). A review of the role of soluble fiber in health with specific reference to wheat dextrin. *The Journal of international medical research,* 37(1),1-17.

Smith, Y. R. A., Adanlawo, I. G. (2014). Hypoglycaemic effect of saponin from the root of *Garcinia kola* (Bitter Kola) on alloxan-induced diabetic rats. *Journal of Drug Delivery & Therapeutics,* 2(6), 9-12.

Sousa, R. V. R. B., Guedes, M. I. F., Marques, M. M. M., Viana, D. A., da Silva, I. N. G., Rodrigues, P. A. S., Vieira, I. G. P. (2015).

Hypogkycemic effect of new pectin isolated from *Passiflora glandulosa* Cav in alloxan-induced diabetic mice, *World Journal of Pharmacy and Pharmaceutical Sciences,* 4(1),1571 -1586.

Szkudelski, T. (2001). The mechanism of aloxan and streptozotocin action in β cells of the rat pancreas. *Physiological research / Academia Scientiarum Bohemoslovaca,* 50(6), 536-328.

Talati, R., Baker, W. L., Pabilonia, M. S., White, C. M., Coleman, C. I. (2009). The effects of Barley-derived soluble fiber on serum lipids. *Annals of family medicine,* 7(2), 157-163.

Then, A. H., Bardosono, S., Harahap, I. P. (2009). The effect of indigestible dextrin and phytosterol on serum LDL-cholesterol level on hypercholesterolemic subjects. *Medical Journal of Indonesia,* 18(2), 114-119.

Topping, D. L., Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological reviews,* 81(3),1031-1064.

Weyer, C., Tataranni, P. A., Bogardus, C., Pratley, R E. (2001). Insulin resistance and insulin secretory disfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. *Diabetes Care,* 24(1), 89-94.

Yin, X., Zhang, Y., Wu, H., Zhu, X., Zheng, X., Jiang, S., Zhuo, H., Shen, J., Li, L., Qiu, J. (2004). Protective effects of Astragalus saponin I on early stage of diabetic nephropathy in rats. *Journal of pharmacological sciences,* 95(2),256-266.

Acknowledgment

This study was a part of Leading Research of Universities, researchers would say thank you for the support of research funding from Indonesian Ministry of Research, Technology, and Higher Education in 2017.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CHARACTERISTICS AND APPLICATION OF SILVER NANOPARTICLES IN THE FOOD INDUSTRY - REVIEW

Anna Mikołajczuk-Szczyrba[1*], Marek Kieliszek[2], Liviu Giurgiulescu[3], Barbara Sokołowska[1]

[1]Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology, Department of Microbiology, 36 Rakowiecka St., 02-532 Warsaw, Poland;
[2]Institute of Food Sciences, Department of Food Biotechnology and Microbiology, Warsaw University of Life Sciences − SGGW, 159C Nowoursynowska St., 02-776 Warsaw, Poland;
[3]Chemistry-Biology Department, Technical University of Cluj Napoca, North Universitary Center of Baia Mare, 76 Victoriei St., Romania
**anna.mikolajczuk-szczyrba@ibprs.pl*

https://doi.org/10.34302/2019.11.4.14

Article history:
Received:
25 March 2019
Accepted:
29 November 2019

Keywords:
Nanotechnology;
Silver nanoparticles;
Food;
Antibacterial activity.

ABSTRACT

Nanocolloids are substances that are constantly gaining technological significance in various industries. Due to their unique properties, they are also used in a wide variety of fields, including medicine, biotechnology, and agriculture. It has been proven that nanocolloids act antagonistically on many different microorganisms. This article highlights some of the achievements and challenges associated with the use of silver nanocolloids in the food industry. Currently, research and development programs are focused on discovering new nanomaterials and nanostructures for novel applications. Thus, it could be understood that the prospects for the future are exciting, and more challenges are expected with the continuous increase in research on these substances.

1. Introduction

Nanotechnology is currently a rapidly growing, interdisciplinary field of science that allows us to obtain products with unique and innovative properties. According to the current definition, nanotechnology is a science that deals with the application, production, and processing of materials at the level of individual atoms and molecules (Singh, 2017; Peters, 2016).

Nanoparticles are objects that have at least one dimension in the range of 1–100 nm. They have the same chemical composition as their macro counterparts but differ in a number of characteristics. Even among the nanoparticles, the optical, magnetic, and thermodynamic properties, as well as the characteristics such as color, solubility, strength, diffusivity, and toxicity, vary (Singh, 2017). In addition, the ultrasmall dimensions and high surface-to-volume ratio of the nanoparticles enable them to acquire new physicochemical properties. Furthermore, the strongly developed specific surface of the nanoparticles affects their adsorption properties and antimicrobial activity (Peters, 2016).

Thanks to the unique properties, nanoparticles are used in medicine and many other industries (Quing, 2018; Fu, 2014). The possibilities of applications of nanoparticles in the agri-food sector cover many fields, including agriculture, food processing and packaging, and the production of dietary supplements (Singh, 2017; Dunkan, 2011). To date, few of the potential applications of nanoparticles have been implemented in the food industry, but many works are still at the research and development stage (Głód, 2014). The reason for

this is, among others, imprecise legal regulations associated with the use of nanoparticles in the field of food packaging and processing, as well as the lack of accurate toxicological data covering the impact of nanoparticles on the environment and human health. Despite this, the use of nanoparticles in the food industry has increased significantly over the last decade. The largest increase in nanoparticle applications has been recorded in the sector of packaging materials (He, 2016; Carbone, 2016). In the production of food packaging, nanomaterials, metal nanoparticles, and their oxides are most commonly used. An example would be titanium oxide nanoparticles that are used in polymer nanocomposites as a protective factor against the harmful effects of ultraviolet radiation (LIorens, 2012). Currently, the most active area of research in the packaging materials industry is the use of nanoparticles with biocidal properties. Nanoparticles that possess antimicrobial properties include some metals, such as Cu, Zn, Ti, and Ag. Among them, silver nanoparticles (Ag-NPs) deserve special attention as they exhibit the most effective biocidal activity against a wide range of microorganisms (Carbone, 2016).

2. Antibacterial activity of silver nanoparticles

Silver was already known in antiquity due to its antibacterial properties. To prevent diseases, the ancient Greeks covered silverware and placed silver coins in water jars to extend shelf life. For many years, silver compounds have been known to exert a basic protective function against numerous infections and diseases caused by microorganisms (Dunkan, 2011). The intensification of the phenomenon of antibiotic resistance observed for several years has again increased the importance of silver in the fight against microorganisms (Lemire, 2013). Compared with other antimicrobials, which are usually directed at specific groups of organisms, silver has a broad spectrum of antimicrobial activity (Quing, 2018).

The growing importance of silver as a bactericide and the development of nanotechnology have led to the initiation of research into the effectiveness of Ag-NPs in the fight against food pathogens. The results of experimental studies have shown the extremely strong antibacterial activity of Ag-NPs against many bacteria, including those that are food-spoiling and pathogenic to humans. It was confirmed that Ag-NPs have the ability to inactivate and inhibit the growth of numerous bacterial species, including, among others, *Escherichia coli*, *Salmonella enterica* Typhimurium, and *Listeria monocytogenes*. In addition, Ag-NPs have been shown to be toxic to some fungi (e.g. *Candida albicans*, *Aspergillus niger*) and viruses (e.g. HIV) (King, 2018; Dunkan, 2011).

Several publications describe the multifaceted action of Ag-NPs on bacterial cells. However, despite thorough analyses, the mechanism of action of Ag-NPs has not been clearly defined yet and remains a subject of research (Quing, 2018). The effect of nanoparticles on bacterial cells was first analyzed in a model *E. coli* species. Due to the action of Ag-NPs, pores (cavities) were formed on bacterial sheaths, which led to the loss of tightness of the wall-called membrane syndrome-and consequently to cell autolysis (Liao, 2019). Subsequent investigations, extended to other bacterial species, have allowed the identification of three basic mechanisms underlying the effect of Ag-NPs on bacterial cells: 1) direct binding to the cell membrane and impairment of its function; 2) interaction with cellular components (e.g. proteins, enzymes, DNA); and 3) induction of oxidative stress by inducing the production of reactive oxygen species (King, 2018; Lemire, 2013).

After anchoring in the cell membrane of bacteria, Ag-NPs cause changes in its structure and function. The electrostatic potential is modified, and the membrane permeability increases. In this way, cell components that are essential for the microorganisms are lost or there occurs an uncontrolled uptake of substances from the environment (Qing, 2018; Liao, 2019). In addition, through electrostatic interactions with the surface of cellular shields, including

membrane proteins, Ag-NPs can penetrate inside the bacterial cell. Once inside the cell, Ag-NPs react with numerous structures and biomolecules, leading to more extensive damage. Ag-NPs have a high affinity for thiol groups of proteins and cause their denaturation and inactivation. The result is impairment of the activity of important intracellular enzymes (Mihindukulasuriya, 2014). The indirect involvement of Ag-NPs in the inhibition of ATP synthesis as a result of interaction with the proteins participating in its production has also been proven (Qing, 2018). Ag-NPs can react with phosphorus and sulfur contained in DNA leading to serious damage to its structure (Liao, 2019). The mechanism of the biocidal action of Ag-NPs may also be induced by their interaction with ribosomes. Following the interaction, the translation process and protein synthesis are inhibited. Worth changing in the cells of Gram-negative bacteria exposed to Ag-NPs were changes in the signal transduction system. Ag-NPs have been shown to be responsible for the dephosphorylation of tyrosine residues of protein substrates resulting in the inhibition of signal tradition (Prabhu, 2012).

It should be emphasized that Ag-NPs are a source of silver ions that enhance the antibacterial effect of nanosilver. Silver ions released from Ag-NPs can bind to the functional groups of proteins and enzymes, causing serious effects on bacterial metabolism. In addition, it should be emphasized that Ag-NPs reduce the expression of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione, which play a key role in the antioxidation process (Qing, 2018). Silver ions are also involved in the production of reactive oxygen species, which in increased amounts cause, among others, protein oxidation and lipid peroxidation (He, 2016).

The antibacterial activity of Ag-NPs depends on many factors, including the properties of microorganisms, external conditions (environmental pH, temperature, salinity, presence of oxygen), as well as the size and shape of the nanoparticles themselves. It has been proven that the antibacterial potential of nanosilver increases with a decrease in the diameter of Ag-NPs. Smaller nanoparticles have a relatively larger surface for the release of silver ions and greater protein-binding efficiency and are also easier to pass through the pores in the bacterial membrane. The toxic effects of nanoparticles on microorganisms also depend on their shape: triangular-shaped nanoparticles have a stronger antibacterial effect than the elongated ones (Singh, 2015; LIorens, 2012).

3. Silver nanoparticles-application in the food industry

One of the goals set by the food industry is to ensure consumer safety and extend the shelf life of food products. The desire to accomplish the above tasks contributes to finding new solutions for promoting food safety and preventing the spread of pathogenic microorganisms transmitted through contaminated food (Table 2). Proper storage of food products is necessary to achieve these goals, and hence, packaging

materials play an important role in both the food and medical sectors (Olmos, 2018). Innovations in the packaging industry focus on the development of new, active materials that, in addition to providing the basic protective barrier against the negative environmental factors, are capable of maintaining and improving sensory quality and increasing the microbiological safety of the packaged product. In recent years, materials with antimicrobial properties obtained by incorporating Ag-NPs in the polymer structure have been of great interest among researchers (Olmos, 2018; Li, 2017). The active packaging component-nanosilver-interacts directly with food and/or the space around it, limiting or inhibiting the growth of pathogenic and food-spoiling microorganisms (Carbone, 2016; Sharma, 2017). The antibacterial potential of a material containing Ag-NPs is conditioned by many factors. In addition to the size of the nanosilver particles themselves, their concentration in the polymer matrix and the degree of agglomeration are important. To efficiently perform their antimicrobial function, Ag-NPs should be suitably small in size and well

dispersed in the polymer (without agglomeration) (Rhim, 2014; Lorens et al.,2012).

Table 1. Applications of nanoparticles in the food industry

Nanomaterial	Types of food	Tested microorganism	References
Polyethylene, AgNPs	Nuts	Bacteria, fungi	Tavakoli *et al.,* 2017
Low density polyethylene, AgNPs	Barberry	Bacteria	Valipoor Motlagh *et al.,* 2012
Low density polyethylene, AgNPs, TiO_2	Rice	*A. flavus*	Li *et al.,* 2017
Polyvinylpyrrolidone, AgNPs	Fresh asparagus	Psychotrophic bacteria, yeasts and molds	An *et al.,* 2008
Polyvinylchloride, AgNPs	Ground beef	*E. coli*, *S. aureus*	Mahdi *et al.,* 2012
Cellulose, AgNPs	Beef meat	Lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas*	Fernandez *et al.,* 2010a
Cellulose, AgNPs	Fresh-cut melon	Bacteria, yeasts	Fernandez *et al.,* 2010b
Hydroxypropylmethylcellulose, AgNPs	-	*E. coli*, *S. aureus*	de Moura *et al.,* 2012
Cellulose nanofibril, AgNPs	-	*S. aureus, E. coli* O157:H7	Yu *et al.,* 2019
Pullulan, AgNPs	Turkey meat	*L. monocytogenes*, *S. aureus*	Khalaf *et al.,* 2013
Starch, AgNPs	-	*S. aureus, E. coli, C. albicans*	Abreu *et al.,* 2015
Chitosan, gelatin, AgNPs	Red grapes	Fungi	Kumar *et al.,* 2018
Chitosan, Cellulose, AgNPs	-	*E. coli*, *S. aureus*	Li *et al.,* 2015
Agar, AgNPs	-	*E. coli* O157:H7, *L. monocytogenes*	Rhim *et al.,* 2014
Agar, banana powder, AgNPs	-	*E. coli*, *L. monocytogenes*	Orsuwan *et al.,* 2016

Depending on the material used, an antibacterial packaging can be a synthetic polymer or a biodegradable or edible coating. The latter is composed of natural polymers, usually polysaccharides, lipids, or animal and plant proteins (Shit, 2014). Among the most commonly used artificial polymers, polyethylene (PE), polyvinyl chloride (PVC), and polyvinylpyrrolidone (PVP) deserve a special mention (Carbone, 2016).

In the scientific literature, there are numerous studies on the use of Ag-NPs in packaging materials showing promising results. In one interesting study carried out by Tavakoli et al. (2017), the effect of PE-silver foils on extending the shelf life of four types of nuts (hazelnuts, walnuts, pistachios, and almonds) were examined. Studies have shown that the addition of Ag-NPs to PE packaging significantly reduces the growth of microorganisms. Positive effects were also observed in the case of mold, with a visible decrease in their numbers. Furthermore, the use of packaging made of Ag-NPs was shown to increase the shelf life of nuts by an average of 6 months.

In turn, Valipoor Motlagh et al. (2012) showed that low-density polyethylene (LDPE) films containing Ag-NPs play a significant role in maintaining the microbiological and sensory quality of dried barberry. LDPE foil made with nanosilver showed a strong antimicrobial activity by limiting the growth of bacteria and mold in the tested material. The fruit stored in LDPE foil containing Ag-NPs retained the fresh aroma and red color for about 2–3 weeks longer, compared to the fruit stored in the foil without the addition of Ag-NPs. Li et al. (2017) examined the activity of LDPE film containing Ag-NPs/TiO_2 against *Aspergillus flavus* during

rice storage. Microbiological analyses carried out after 35 days of product storage showed that LDPE/Ag-NPs/TiO_2 significantly inhibits mold growth and positively affects the physicochemical properties of rice. Studies were carried out in comparison with the rice stored in pure PE film, which showed no activity against *A. flavus*. Thus, the synthesized new LDPE/Ag-NPs/TiO_2 film seems to be a promising packaging material because contamination of rice with molds, and in particular *A. flavus*, is the main cause of product loss at the storage stage.

An et al. (2008) conducted a study in which they proved that storing fresh asparagus in the coatings made of PVP with the addition of Ag-NPs will extend their shelf life by approximately 10 days. It was found that PVP/Ag-NPs coatings significantly reduced the growth of psychrotrophic bacteria, yeast, and mold in the tested vegetable samples. In addition, asparagus samples stored in coatings containing Ag-NPs showed a smaller mass loss and lesser ascorbic acid compared to the control samples stored without the addition of nanosilver. It was also shown that vegetables protected with an Ag-NPs coating maintained a saturated green color longer, which was the result of slower chlorophyll degradation.

Mahdi et al. (2012) evaluated the impact of PVC-nano packaging Ag-NPs on the shelf life of beef minced meat stored at 4°C. Research results showed a marked improvement in the microbiological quality, primarily a reduction in the growth of *E. coli*. Inhibition of bacterial growth resulted in an extension of the shelf life of meat to 7 days, while the meat stored in traditional packaging deteriorated after 2 days.

In recent years, the use of natural biodegradable materials, which are an alternative to biologically degradable films, is gaining popularity. The use of ecological materials allows limiting the use of plastic films, which constitute a great burden to the natural environment due to long-term decomposition (Shit, 2014). In addition, some natural polymers (e.g. gelatin) can be obtained from the waste and by-products of the agri-food industry, for example, waste from the fishing industry. A number of researchers are using eco-friendly materials as carriers for Ag-NPs. The most commonly used biodegradable polymers for contact with food include cellulose, pullulan, starch, chitosan, and agar (Khalaf, 2013).

Fernandez et al. (2010a) assessed the ability of cellulosic absorbent pads containing Ag-NPs to inhibit microbial growth during the storage of pork. Microbiological analyses showed a significant reduction in the overall count of bacteria. In another work (Fernandez et al. 2010b), the same research team investigated the use of cellulose pads from Ag-NPs in storing fresh pieces of melon. Significant differences in appearance and microbiological quality were observed between the control samples stored without the addition of Ag-NPs and the fruits containing nanosilver rootstocks. The use of antibacterial pads effectively reduced the number of bacteria and yeast in the tested material, thus confirming the broad-spectrum biocidal activity of nanosilver. In addition, the presence of nanosilver rootstocks delayed the aging process of the fruit, probably by blocking the effects of ethylene (Duncan, 2011; Fernandez, 2010b).

De Moura et al. (2012) obtained an active packaging material based on hydroxypropyl methylcellulose and Ag-NPs, which in addition to exhibiting an effective antibacterial activity against *E. coli* and *Staphylococcus aureus*, improved the water vapor barrier properties and increased the mechanical strength of the nanocomposite. In addition, the authors determined the relationship between the size of Ag-NPs and their antibacterial activity. Based on the results of the research, it was confirmed that nanoparticles with a smaller diameter (41 nm) have a stronger bactericidal effect than the particles with a larger diameter (100 nm). Yu et al. (2019) tested the antibacterial activity of a film composed of cellulose nanofibril and Ag-NPs against food-pathogenic *E. coli* O157:H7 and *L. monocytogenes*. The results showed that the modified nanomaterial had effective antibacterial properties against the bacteria studied, with *E. coli* being more sensitive to nanosilver than *L. monocytogenes.*

Khalaf et al. (2013) investigated and compared the effectiveness of two pullulan coatings containing, respectively, Ag-NPs and zinc oxide nanoparticles in reducing the occurrence of *L. monocytogenes* and *S. aureus* in turkey meat. It was shown that pullulan composites enriched with Ag-NPs exerted a stronger antibacterial activity against the studied pathogens than the pullulan coatings containing zinc oxide.

Abreu et al. (2015) created a film based on starch and Ag-NPs. The resulting nanocomposite inhibited the growth of *S. aureus*, *E. coli*, and *C. albicans*.

Kumar et al. (2018) developed an innovative hybrid food film composed of two commonly used biopolymers, chitosan and gelatin, with the addition of Ag-NPs. The researchers evaluated the effectiveness of this nanocomposite in extending the shelf life of red grapes. After 14 days of storage at 37°C, the fruits protected in chitosan-gelatin-Ag-NPs hybrid foil were characterized by a fresh appearance and smooth surface, whereas grapes stored in a film made of PE and chitosan-gelatin hybrid without the addition of Ag-NPs had an unpleasant smell and showed mold formation on their surface.

Agar-based films supplemented with Ag-NPs also have good antimicrobial properties. Rhim et al. (2014) investigated the effect of silver-agar coatings on reducing the growth of two food-borne pathogenic bacteria: *E. coli* O157:H7 and *L. monocytogenes*. The authors proved that the control coatings without the addition of nanosilver did not show any antimicrobial activity. In contrast, the coatings supplemented with Ag-NPs exhibited antibacterial properties against the tested pathogens, with a stronger effect against *E. coli*. It was found that the antibacterial activity of the coatings depended on the concentration of Ag-NPs and the studied bacterial strain. The above result is consistent with the earlier reports revealing the stronger antibacterial activity of Ag-NPs against Gram-negative bacteria than Gram-positive ones. The difference in Ag-NPs activity is caused by the different structures of the wall membrane assembly of both types of bacteria.

4. Conclusions

The possibilities of applying nanotechnology in the food industry are extremely promising. In recent years, the use of antibacterial packaging materials made with Ag-NPs has gained importance. The use of Ag-NPs in polymers used for packaging offers many benefits including, among others, extended shelf life of food products and the prevention of the spread of food-borne pathogens. The use of Ag-NPs can also reduce the consumption of preservatives. In addition to the above advantages, the negative effects of using Ag-NPs should also be mentioned. The main threat is related to the possibility of migration of Ag-NPs from the packaging materials to food products. Thus, there is a real risk of uncontrolled and unconscious consumption of Ag-NPs. However, the toxic effects of the long-term ingestion of low but frequent doses of Ag-NPs have not yet been studied. Therefore, in order to be able to widely use Ag-NPs in packaging materials, further research is needed to determine every possible negative impact of these materials on human health and the environment.

5. References

Abreu, A S., Oliveira, M., de Sa, A., Rodrigues, R.M., Cerqueira, M.A., Machado, A.V. (2015). Antimicrobial nanostructured starch based films for packaging. *Carbohydrate Polymers,* 129, 127-134.

An, J., Zhang, M., Wang, S., Tang, J. (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *LWT – Food Science and Technology*, 41(6), 1100-1107.

Carbone, M., Donia, D.T., Sabbatella, G., Antiochia, R. (2016). Silver nanoparticles in polymeric matrices for fresh food packaging. *Journal of King Saud University – Science*, 28(4), 273-279.

De Moura, M.R., Mattoso, L.H., Zucolotto, V. (2012). Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles and their use as active food packaging. *Journal of Food Engineering*, 109(3), 520-524.

Duncan, T.V. (2011). Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *Journal of Colloid and Interface Science*, 363(1), 1-24.

Fernandez, A., Picouet, P., Lloret, E. (2010a). Cellulose-silver nanoparticle hybrid materials to control spoilage-related microflora in absorbent pads located in trays of fresh-cut melon. *International Journal of Food Microbiology*, 142(1-2), 222-228.

Fernandez, A., Picouet, P., Lloret, E. (2010b). Reduction of the spoilage-related microflora in absorbent pads by silver nanotechnology during modified atmosphere packaging of beef meat. *Journal of Food Protection*, 73(12), 2263-2269.

Fu, P.P., Xia, Q., Hwang, H.M., Ray, P.C., Yu, H. (2014). Mechanisms of nanotoxicity: generation of reactive oxygen species. *Journal of Food and Drug Analysis*, 22(1), 64-75.

He, X., Hwang, H.M. (2016). Nanotechnology in food science: Functionality, applicability, and safety assessment. *Journal of food and drug analysis*, 24(4), 671-681.

Khalaf, H.H., Sharoba, A.M., El-Tanahi, H.H., Morsy, M.K. (2013). Stability of antimicrobial activity of pullulan edible films incorporated with nanoparticles and essential oils and their impact on turkey deli meat quality. *Food Dairy Science*, 4, 557-573.

King, T., Osmond-McLeod, M.J., Duffy, L.L. (2018). Nanotechnology in the food sector and potential applications for the poultry industry. *Trends in Food Science & Technology*, 72, 62-73.

Kumar, S., Shukla, A., Baul, P P., Mitra, A., Halder, D. (2018). Biodegradable hybrid nanocomposites of chitosan/gelatin and silver nanoparticles for active food packaging applications. *Food Packaging and Shelf Life*, 16, 178-184.

Lemire, J.A., Harrison, J.J., Turner, R.J. (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nature Reviews Microbiology*,11(6), 371.

Li, L., Zhao, C., Zhang, Y., Yao, J., Yang, W., Hu, Q., Wang C., Cao, C. (2017). Effect of stable antimicrobial nano-silver packaging on inhibiting mildew and in storage of rice. *Food Chemistry*, 215, 477-482.

Liao, S., Zhang, Y., Pan, X., Zhu, F., Jiang, C., Liu, Q., Cheng, Z., Dai, G., Wu, G.,Wang, L., Chen, L. (2019). Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*. *International Journal of Nanomedicine*, 14, 1469.

Llorens, A., Lloret, E., Picouet, P.A., Trbojevich, R., Fernandez, A. (2012). Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends in Food Science & Technology*, 24(1), 19-29.

Mahdi, S. S., Vadood, R., Nourdahr, R. (2012). Study on the antimicrobial effect of nanosilver tray packaging of minced beef at refrigerator temperature. *Global Veterinaria*, 9(3), 284-289.

Mihindukulasuriya, S.D.F., Lim, L.T. (2014). Nanotechnology development in food packaging: A review. *Trends in Food Science & Technology*, 40(2), 149-167.

Valipoor Motlagh, N., Hamed Mosavian, M.T., Mortazavi, S. A. (2013). Effect of polyethylene packaging modified with silver particles on the microbial, sensory and appearance of dried barberry. *Packaging Technology and Science*, 26(1), 39-49.

Olmos, D., Pontes-Quero, G., Corral, A., González-Gaitano, G., González-Benito, J. (2018). Preparation and characterization of antimicrobial films based on LDPE/Ag nanoparticles with potential uses in food and health industries. *Nanomaterials*,8(2), 60.

Orsuwan, A., Shankar, S., Wang, L.F., Sothornvit, R., Rhim, J.W. (2016).

Preparation of antimicrobial agar/banana powder blend films reinforced with silver nanoparticles. *Food Hydrocolloids*, 60, 476-485.

Peters, R.J., Bouwmeester, H., Gottardo, S., Amenta, V., Arena, M., Brandhoff, P., Marvin, J.P.H., Mech, A., F.B., Moniz, Pseudo, L.Q., Rauscherde, H., Schoonjans, R., Undas, A.K., Vettori, V.M., Weigel, S., Aschberger, K. (2016). Nanomaterials for products and application in agriculture, feed and food. *Trends in Food Science & Technology*, 54, 155-164.

Prabhu, S., Poulose, E.K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters*, 2(1), 32.

Yun'an Qing, L.C., Li, R., Liu, G., Zhang, Y., Tang, X., Wang, J., Liu, H., Qin, Y. (2018). Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *International Journal of Nanomedicine*, 13, 3311–3327.

Rhim, J.W., Wang, L.F., Lee, Y., Hong, S.I. (2014). Preparation and characterization of bio-nanocomposite films of agar and silver nanoparticles: laser ablation method. *Carbohydrate Polymers*, 103, 456-465.

Sharma, C., Dhiman, R., Rokana, N., Panwar, H. (2017). Nanotechnology: an untapped resource for food packaging. *Frontiers in Microbiology*, *8*, 1735.

Shit, S.C., Shah, P.M. (2014). Edible polymers: Challenges and opportunities. *Journal of Polymers*, *2014*. Article ID 427259

Singh, R., Shedbalkar, U.U., Wadhwani, S.A., Chopade, B. A. (2015). Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. *Applied Microbiology and Biotechnology*, 99(11), 4579-4593.

Singh, T., Shukla, S., Kumar, P., Wahla, V., Bajpai, V.K., Rather, I.A. (2017). Application of nanotechnology in food science: perception and overview. *Frontiers in Microbiology*, 8, 1501.

Tavakoli, H., Rastegar, H., Taherian, M., Samadi, M., Rostami, H. (2017). The effect of nano-silver packaging in increasing the shelf life of nuts: An in vitro model. *Italian Journal of Food Safety*, 6(4), 6874.

Yu, Z., Wang, W., Kong, F., Lin, M., Mustapha, A. (2019). Cellulose nanofibril/silver nanoparticle composite as an active food packaging system and its toxicity to human colon cells. *International Journal of Biological Macromolecules*, 129, 887-894.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

BIOACTIVITY OF OLIVE OIL MILL WASTEWATER AGAINST GREY MOULD DISEASE

Ioannis K. Vagelas[1*], Liviu Giurgiulescu[2]

[1]University of Thessaly BIOPOLIS Larissa, 41110 Larissa, Greece
[2]Chemistry-Biology Department, Technical University of Cluj Napoca, North Universitary Center of Baia Mare, 76 Victoriei St., Romania
**vagelas@teilar.gr*

https://doi.org/10.34302/2019.11.4.15

Article history:
Received:
15 March 2019
Accepted:
10 December 2019

Keywords:
Olive oil mill wastewater; Antifungal activity; Post-harvest diseases.

ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) was investigated. Filtered and sterilized olive OMW was tested as growth inhibitor of Botrytis cinerea mycelium in vitro. The effect of filtered and sterilized olive OMW was also tested on strawberries fruits infected with the Botrytis cinerea in vivo.The results show that the filtered sterilized olive OMW inhibits the growth of Botrytis cinerea mycelium in vivo confirming the antifungal activity probably due to presence of the phenols phenol content of olive OMW solution.

1.Introduction

During olive oil extraction a large amount of solid and aqueous residues known as olive oil mill wastewaters (olive OMWs) are produced annually worldwide where the majority of it is produced in the Mediterranean basin. The uncontrolled disposal of olive OMW is becoming a serious environmental problem due to its high content in phenolic compounds: tannins and flavonoids (Gonzales *et al*. 1999; Hamdi, 1992). Some of these phenols are responsible for several biological effects, including antibiosis (Rodrvguez *et al.*, 1988) and phytotoxicity (Capasso *et al*., 1992). They also appear to be involved in the defense of plants against invading pathogens, including bacteria, fungi and viruses (Marsilio *et al.* 2001). The use of olive OMW for plant and harvested fruits protection against microorganism could be a solution for residues management and nature protection. The main objective on this study was to examine the post-harvest biological control of grey mould (*Botrytis cinerea* Pers.: Fr.) on fresh-market strawberries with olive OMW.

2.Materials and methods

2.1.In vitro assessment of antimicrobial *activity* of olive OMW on Botrytis cinerea mycelia

The antifungal effect of olive OMW solution was tested against Botrytis cinerea mycelia in vitro. Tests were made on PDA (Potato Dextrose Agar; DIFCO) in 9 cm Petri dishes. *Treatments (experiments) were PDA plates with a) olive* OMW added into the medium and autoclaved and b) a drop of *filter sterilized olive OMW* (using a syringe filler 0.2 μm) *added onto the agar surface. In the first experiment a 25ml of* olive OMW were added into 1l agar and further sterilized by autoclaving (121 ^{0}C for 20 min). In the second *experiment* a drop (50 μl) of filter sterilized olive OMW was added onto the centre of each plate. Fifteen agar plates per treatment were inoculated with a mycelium plug (5 mm in diameter) of the above fungus which was taken from the periphery of 7 days old fungal colonies. Mycelia plugs were placed onto the centre of each plate or next to the olive OMW drop. Equal plate numbers were used as control (without

olive OMW). Plates were incubated at 21ºC for six days and fungus mycelium growth was recorded.

2.2.Antimictobial activity of olive OMW against grey mould in vivo

Botrytis cinerea isolated from market strawberries was used for this experiment. Spores suspension was prepared by isolating spores of above *Botrytis* species, from 7 days old cultures. Three agar plates per fungus culture were used to collect spores. Spores were collected in 1l Erlenmeyer flask which contained distilled water by washing the agar surface with 3ml distilled water and filter the produced solution through sterilized muslin. In each flask spores suspension was adjusted at 10^6 spores/ml. A 50ml of olive OMW were added in each flask. Fresh-market strawberries were surface sterilized and soaked for 3 min in 1l beakers contained 500 ml of the above spore and olive OMW solution. *After that time* **f**ruits removed from the flasks, dried for 10 min in a laminar flow unit and incubated at 21ºC for 12 days. *Olive OMW was passed through Whatman f*ilter paper No 2 before added to each beaker. After the incubation time, the spores number of each strawberry fruits was counted by scraping fruits surface into 200ml *beaker* which contained 50ml *distill water. The spore number per treatment and per beaker was counted by optical microscope using a hematocytometer.* Also, after the incubation period, the mycelium (molt) formation of each strawberry fruit was recorded and mold formation was sorted in six classes (0-5, as reported by Vagelas et al. 2009), where 0 is equal to healthy fruits, 1=slightly mold fruits and 5=heavy mold fruits. The experiment had fourteen replicates and four treatments; strawberry fruits infected with spores and olive OMW and strawberries infected only with spores, treated only in olive OMW and treated only with sterilized water were used as control.

2.3.Statistical analysis

Data were analyzed using the Minitab statistical package. Analysis of variance was used to assess treatments or/and experiments effect.

3.Results and discussions

3.1.In vitro assessment of antimicrobial activity of olive OMW

The mycelia growth of *B. cinerea* significantly decreased ($p < 0.001$) when filtered sterilized olive OMW was added on the agar surface where *B. cinerea* mycelium was growing (Fig. 1). In details, there was a statistical significant difference between filtered sterilized olive OMW and control (untreated PDA and sterilized with olive OMW PDA), ($P<0.001$). The total phenols content (0.4%), found on filtered sterilized olive OMW could be an explanation of olive OMW antimicrobial activity resulted the strongly inhibition of fungus mycelia growth.

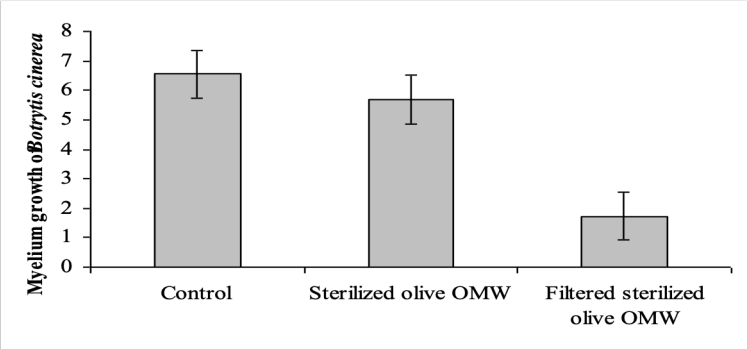


Figure 1. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on mycelium growth of *Botrytis cinerea*.

3.2.Antimictobial activity of olive OMW against grey mould in vivo

The olive OMW significantly reduced the number of *B. cinerea* (P<0.001) spores (conidia). The average spore's number was $3.4x10^6$ for strawberry fruit infected only with *B. cinerea* and $1,6x10^2$ conidia/strawberry fruit infected with *B. cinerea* and treated with olive OMW. Further, a high mold formation was recorded only in treatments with strawberry fruits treated with fungus conidia suspension (Fig. 2).

The filter sterilized olive OMW significantly inhibited the growth of *Botrytis cinerea* mycelia and showed only fungistatic activity against grey mould *in vitro* probably due to phenols content (Fig. 2). A phenol content of 0.4% was indentified *in vitro* present experiment.

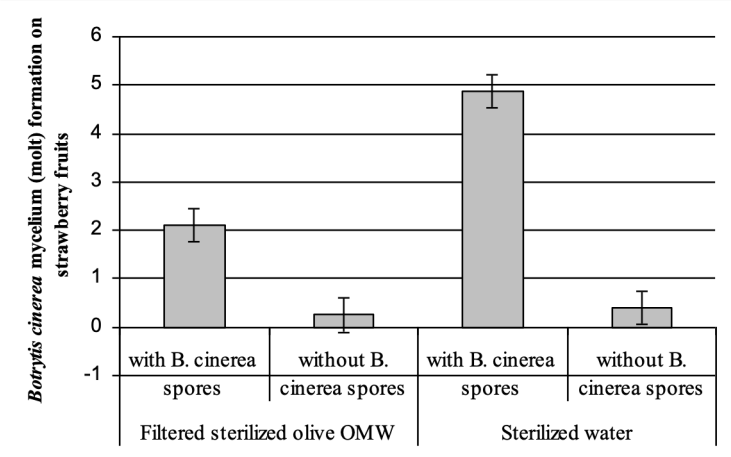


Figure 2. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on mycelium mold formation of *Botrytis cinerea* on strawberry fruits.

4.Conclusions

Olive oil mill wastewater (olive OMW) contains phytotoxic components capable of inhibiting the growth of microorganisms (Ramos-Cormenzana *et al.*, 1996) and plants (Martin et al., 2002). Olive OMW contains phenolic compounds (Ramos-Comenzana *et al*., 1995) polysaccharides, lipids, proteins, and a number of monocyclic and polymeric aromatic molecules (Ethaliotis *et al*. 1999) which might exhibit inhibition effects towards some specific microorganism populations. In the current study filter sterilised olive OMW significantly reduced the growth of *Botrytis cinerea*. According to D'Annibale *et al.* (2004) phenolic compounds are the main determinants of the phytotoxic effect of olive residues. Thus, the phenolics of olive OMW used in this experiment had negative effect on *Botrytis cinerea* mycelia *in vitro*. The used for olive OMW sterilization at 121 ^{0}C for 20 min probably removed or destroyed the phenolic compounds from olive OMW solution resulted a same or a better growth media for all tested fungi *in vitro*. Furthermore, the production of *B. cinerea* spores on fruits inhibited by olive OMW. We assume that the presence (0,4%) of phenolic compounds of olive OMW found in this study, suppresses fungus reproduction and possible could offer a protection on strawberry fruits from post-harvest diseases. Overall we believe that t*he olive OMW due to phenolics have antifungal activity and could possible used against fruit fungal pathogens for preventing post harvest diseases*.

5.References

D'Annibale, A., R. Casa, F. Pieruccetti, M. Ricci and R. Marabottini 2004. Lentinula edodes removes phenols from olive mill wastewater: impact on durum wheat (*Triticum durum* desf.) germinability. *Chemosphere* 54, 887–894.

Ethaliotis C., K. Papadopoulou, M. Kotsou, I. Mari and C. Balis 1999. Adaptation and

population dynamics of Aztobacter vinelandii during aerobic biological treatment of olive mill wastewater. *FEMS Microbiology Ecology,* 30, 301-311.

Gonzales, D.M., E. Moreno, Q.J. Sarmiento and R.A. Cormenzana 1999. Studies on antibacterial activity of wastewaters from olive oil mills (Alpechin): Inhibitory activity of phenolic and fatty acids. *Chemosphere* 20, 423-432.

Hamdi, M. 1992. Toxicity and Biodegradability of olive mill wastewaters in batch anaerobic digestion. *Applied Biochemistry and Biotechnology.* 37, 155-163.

Marsilio, V., C. Campestre and B. Lanza 2001. Phenolic compounds change during California-style ripe olive processing, *Food Chemistry,* 74, 55–60.

Niaounakis, M. and C.P. Halvadakis 2004. *Olive-Mill Waste Management: Literature review and Patent Survey.* Typothito – George Dardanos Publications, Athens, Greece.

Ramos-Comenzana A., M. Monteolica-Sanchez and M.J. Lopez 1995. Bioremediation of alpechin. *International Biodeterioration & Biodegradation – Journal,* 35, 249-268.

Rodrvguez, M.M., J. Pırez, A. Ramos-Cormenzana and J. Martvnez 1988. Effect of extracts obtained from olive oil mill waste on *Bacillus megaterium* ATCC 33085. *Journal of Applied Microbiology* 64, 219-222.

Vagelas, I., A. Papachatzis, H. Kalorizou and E. Wogiatzi. 2009. Biological control of Botrytis fruit rot (Gray mold) on strawberry and red pepper fruits by olive oil mill wastewater. *Biotechnology & Biotechnological Equipment*, 4, 1489-1491.

CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

THE EFFECT OF ANTIOXIDANT AND ANTIBACTERIAL LIQUID SMOKE NANOCAPSULES ON CATFISH FILLET (*Pangasius* sp.) DURING STORAGE AT ROOM TEMPERATURE AND COLD TEMPERATURE

Fronthea Swastawati[1*], Ahmad Ni'matullah Al-Baari[2], Eko Susanto[1], Lukita Purnamayati[1]

[1] Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, SH Tembalang, Semarang 50275 Central Java – Indonesia.

[2]Faculty of Animal and Agricultural Sciences, Diponegoro University, Jl. Prof. H. Soedarto, SH Tembalang, Semarang 50275 Central Java – Indonesia.

** fronthea_thp@yahoo.co.id.*

https://doi.org/10.34302/2019.11.4.16

Article history:
Received:
25 March 2018
Accepted:
20 November 2019

Keywords:
Catfish;
Nanoencapsulation;
Liquid smoke;
Antioxidant;
Antibacterial.

ABSTRACT

The purpose of this study was to determine the effect of antioxidant and antibacterial of liquid smoke nanocapsules on a catfish filet (*Pangasius* sp.). A combination of liquid smoke (corncob and coconut shell) were processed into nanocapsules using three encapsulan i.e: gum arabic, maltodextrin, and alginate with a ratio of 1/6: 4/6: 1/6 each. Liquid smoke nanocapsules was containing total phenolic content, carbonyl, and Radical Scavanging Activity, there were 3.682 mg GAE/g, 3.439%, and 91.348%, respectively. Liquid smoke nanocapsules was applied to the catfish and stored at room temperature (28ºC±2ºC) and cold temperature (5ºC). Observations were made on days 0, 2, 4, 6, 8, and 10 to parameter PV, TBA, TVBN and TPC. The results showed that liquid smoke nanocapsules could effectively inhibit the oxidation of fat catfish showed with PV and TBA acceptable. Liquid smoke nanocapsules was also capable of inhibiting the activity of microbes, indicated by the value of TVBN and TPC which were still below standard at all temperatures and long storage time.

1.Introduction

Catfish were easily damaged by the changes of fat content (oxidation process, lipoxygenase damage, etc), protein and microorganisms (Masniyom, 2011). This damage is indicated by peroxide numbers, TBA (Valdes *et al.*, 2015), TVBN (Tian *et al*., 2012; Castro, 2012) and TPC (Adilla *et al*., 2017) which increases during storage. Catfish have high nutrient content especially fat and protein. Catfish contain palmitic acid (24.05%), oleic acid (27.55%), and linoleic acid (7.63%). In addition, catfish also contains non essential amino acids, such as glutamate (3.33%) and essential amino acids, for example lysine (1.82%) (Nurilmala *et al*., 2015). The high content of fatty acids and amino acids of catfish, resulting in catfish being damaged continuously during cold storage temperatures (Abbas *et al*., 2005). Therefore, treatment were needed to inhibit catfish damage during storage.

Liquid smoke is one of the smoke condensation products in the form of liquid. Liquid smoke is widely used compared to traditional curing methods because it is easy to use and more economical. Liquid smoke also has several compounds such as phenol, acids and carbonyl that acts as an antibacterial and antioxidant (Saloko *et al*., 2014). Several studies has been done by other researcher using coconut shell liquid smoke to inhibit fish damage, such as tuna (Saloko *et al*., 2014) tilapia (Ariestya *et al*., 2016), and catfish (Swastawati, 2008). Other research elaborated the use of corncobs liquid smoke in tilapia (Youssef *et al*., 2015) and

milkfish (Swastawati *et al*., 2016); which shows the shelf life of tilapia fillet for 6 days at cold temperature storage (5ºC) (Ariestya *et al*., 2016). Coconut shell liquid smoke increased the shelf life of mackerel fishballs for 32 hours at room temperature storage (Zuraida *et al*., 2011). While corncob liquid smoke was able to extend the shelf life of stingrays for 3 days at room temperature storage (Swastawati *et al*., 2012) and tilapia meatballs for 15 days at cold temperature storage (4ºC) (Youssef *et al*., 2015). The existence of differences in the capability of coconut shell liquid smoke and corncobs liquid smoke increasing the shelf life of the product encourage the incorporation of these two liquid smokes in application of the product, which is expected to give effect in different shelf life at different storage temperatures. All the previous researcher were only use one raw material of liquid smoke. In this study, we apply combination of two raw materials i.e coconut shell and corncob (50:50) which is hope will give longer shelf life because these mixture of raw material were found to contain higher polyphenols (Anggraini *et al*., 2017; Swastawati *et al*., 2014; Lombok *et al*., 2014; Yuniningsih and Anggraini, 2013).

Polyphenols were volatile bioactive components of liquid smoke. In addition, polyphenols have low and unstable water solubility (Conte *et al*., 2016). Therefore, a system capable to improve the properties of polyphenols and maintaining polyphenols during storage was required. Nanoencapsulation technology changed liquid smoke in liquid form to a nano-sized powder (nanocapsules) of 1 to 2000 nm Etheridge *et al*., 2013) has an advantage in the delivery of bioactive components that were efficient in penetrating cells in desired products (Ezhilarasi *et al*., 2012). Many research were limited to coconut shell encapsulation (Saloko *et al*., 2014; Ariestya *et al*., 2016; Novianty *et al*., 2015; Ali *et al*., 2014; Saloko *et al*., 2012). Based on the above description, this study examined the effect of combination liquid smoke nanocapsules (coconut shell and corncob liquid smoke) on catfish fillet during storage of room temperature and cold temperature.

2. Materials and Methods

2.1. Materials

The materials used in this study were the corncob and coconut shell to produce liquid smoke. Each materials was processed into liquid smoke by pirolisator machine in laboratory of Fisheries and Marine Science Faculty, Diponegoro University, Semarang, Indonesia. Maltodextrin DE 10, arabic gum and Na-alginate were obtain from Multi Kimia Raya Semarang, Indonesia, meanwhile catfish were obtained from the local market in Semarang, Indonesia.

2.2. Nanoencapsulation of Liquid Smoke

Nanoencapsulation processed was carried out according to Saloko *et al*., (2013) with modification in core and coating materials. Coconut shell liquid smoke and corn cob liquid smoke was mixtured with ratio 1:1. Nanoencapsulation was processed by maltodextrin, gum arabic, and Na-alginate with a ratio of 1:4:1 was mixed with a combination of coconut shell and corncob liquid smoke. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. Supernatant was separated and filtered to obtain a solution of pure nanoparticles. The solution of nanoparticles was heated at 50°C in waterbath for 15 minutes and homogenized using a homogenizer at a speed of 4000 rpm for 2.5 minutes. The sample was dried with a spray dryer with inlet temperature about 130ºC, while the outlet temperature about 70°C. The nanocapsules was collected on a sealed bottle and stored at room temperature.

2.2.Characteristic of Liquid Smoke Nanocapsules

2.2.1. Analysis of Total Phenolic Content

A amount of 1 gram liquid smoke nanocapsules was diluted to a volume of 25 ml aquadest. 1 ml solution was diluted to 10 ml aquadest. Next 2.5 ml of it's solution was taken and diluted to 10 ml. After that, 1 ml solution

was put into a test tube and 1 ml saturated Na_2CO_3 (Merck, Germany) was added and left for 10 minutes at room temperature. Folin ciocalteu reagent (Sigma-Aldrich, USA) 0.5 ml and 7.5 ml of distilled water were added and homogenized by using a vortex for 30 minutes at room temperature. The absorbance of samples were measured at 760 nm wavelength. Phenolic content of samples was calculated as GAE in mg/g dry material (AOCS, 1990).

2.2.2. Analysis of Total Carbonyl

An amount of 1.6 mg of sample was diluted to 10 ml with carbonyl-free ethanol. 1 ml of solution was reacted with 2 ml solution of 2,4-dinitrophenyl-hydrazine (Sigma-Aldrich, USA) with a drop of concentrated hydrochloric acid in ethanol saturated. The mixture was heated in waterbath at temperature 50ºC for 30 min. About 5 ml alcoholic solution of potassium hydroxide (Merchk, Germany) were added when the mixture was cool. Then 2 ml of distilled water was added and measured with a spectrophotometer with a wavelength of 480 nm. Results were calculated by comparing it with the standard curve of acetaldehyde 2,4-dinitrophenylhydrazone (2,4-DNPH) and calculated equivalent of 13.7 ppm acetaldehyde (Sigma-Aldrich, USA) in the sample (Alice *et al*., 1961).

2.2.3. Radical Scavanging Activity

Radical Scavanging Activity (RSA) was measured by Li and Guo (2010) with modifications. Each sample was reacted with DPPH (Sigma-Aldrich, USA) 0.004 g/ml of ethanol. 0.1 ml of sample was added with 3.9 ml of DPPH and incubated at 28ºC for 30 minutes. Scavanging activity on DPPH radical was measured at 515 nm wavelength. Percent of RSA was measured according to the following equation:

$$\% \text{ RSA} = \{(Acontrol - Asample) \times A^{-1}\} \times 100\% \, control$$

2.2.4. PAH Analysis

Solid-Liquid Extraction

Two grams of freeze-dried fish fillet mixed with a mixture of the 20 ml standard solution with 13 PAH was equal to 0.5 µg.kg-1, considered as internal standards which were homogenized in 40 ml of cyclohexane/ethyl acetate (50:50; v/v) and it was shaked during 30 minutes. The solution was centrifuged at 5000 rpm for 30 min at 0°C. After being homogenized, the liquid part was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 6 ml of cyclohexane. PAH quantification was the result of the mean of measures carried out on three individual smoked fillets in the same conditions.

2.2.5. Scanning Electron Microscopy (SEM)

Morphology of liquid smoke nanocapsules was observed by using Scanning Electron Microscopy (FEI, Inspect S50). The sample was layered with gold and it was monitored by a magnification of 1,000 times at the voltage of 20 kV.

2.3. Application Liquid Smoke Nanocapsules on Catfish

Catfish fillet with a size of 25 x 15 x 1 cm with a weight of approximately 100 grams, was smeared with liquid smoke nanocapsules as much as 1% of the weight of the fillet. After that, catfish fillet was roasted at a temperature of 90°C for 4 hours. Smoke catfish fillet was stored at room temperature (28ºC±2ºC) and cold temperature (5ºC) for 10 days and analyzed every 2 days.

2.3.1. Peroxide Value (PV) Analysis

Peroxide value analysis was conducted by Memon *et al.,* (2010). The sample was dissolved in a mixture of chloroform (Merck, Germany) and glacial acetic acid (Merck, Germany) and added with a solution of potassium iodide (Merck, Germany). The mixture was finally titrated with sodium thiosulfate solution (Merck, Germany) 0.01 M with 1% starch indicator.

2.3.2. Thiobarbituric Acid (TBA) Analysis

TBA analysis was conducted by Molla *et al.,* (2015), 2 ml of 20% trichloroacetic acid (Merck, Germany) and 2 ml of 0.67% thiobarbituric acid (Fluka Chemika, Switzerland) was added to 1 ml of the sample solution. The mixture was heated at 100°C for 10 minutes in waterbath. The mixture was centrifuged at 3000 rpm for 20 minutes. Supernatant containing TBARS absorbance was measured at 532 nm wavelength using a spectrophotometer.

2.3.3. Total Volatil Base Nitrogen (TVBN) Analysis

Total Volatile Base Nitrogen (TVBN) was carried out according Indonesian National Standard 2354.8:2009 (BSN, 2009). Briefly, 25 g samples was weighed and mixed with 75 mL TCA (Merck, Germany) 7%. 1 ml filtrat was put in conway cup of outer chamber which had previously been added 1 mL K_2CO_3 (Merck, Germany). Another Conway cup of inner chamber was added 1 mL Boric acid and 2-3 drops of indicator (screen metal red) until the color was green. Blanko had been used 1 mL TCA 7%. Conway cup was incubated at 37°C until 2 hours. Conway cup in the inner chamber of blanko was titrated with HCl until the color was pink. Conway cup of samples titrated with boric acid until the color was equal with blanko.

2.3.4. Total Plate Count (TPC) Analysis

Total Plate Count (TPC) was obtained by Indonesian National Standard 2332.3:2015 (BSN, 2015). Fish samples were diluted into Butterfields Phosphat Buffered (Merck, Germany) with concentration of 10^4, 10^3, and 10^5. One milliliter of each sample solution was placed into petridisc containing plate count agar (PCA) (Merck, Germany). Petridisc containing samples was incubated with the opposite position at 35°C for 48 hours. The number of colony were calculated by hand tally counter for the amount 25-250.

3.Results and discussions

3.1.Characterization of Liquid Smoke Nanocapsules

The content of total phenols, total carbonyl, and RSA of liquid smoke nanocapsules in a row was consecutively 3.682 mg GAE/g, 3.439% and 91.348% (Table 1). Total phenolic content of liquid smoke nanocapsules was influenced by the total phenolic content of liquid smoke and the composition of the coating material. Based on Hardianto and Yunianta (2015) the total phenolic content of corn cob liquid smoke was lower than coconut shell liquid smoke.

Table 1. Characteristics of Liquid Smoke Nanocapsules

Characteristics	Results
Total Phenolic Content (mg GAE/g)	3.68
Total Carbonyl (%)	3.44
Radical Scavanging Activity (%)	91.35
Polycyclic aromatic hydrocarbons (PAHs) (ppm)	
Naphtalen	286.40
Acenaphtane	106.35
Phenantrene	11.70
Phyrene	30.00
Benzo-α-Antrazene	67.10
Benzo-α-Phyrene	47.55

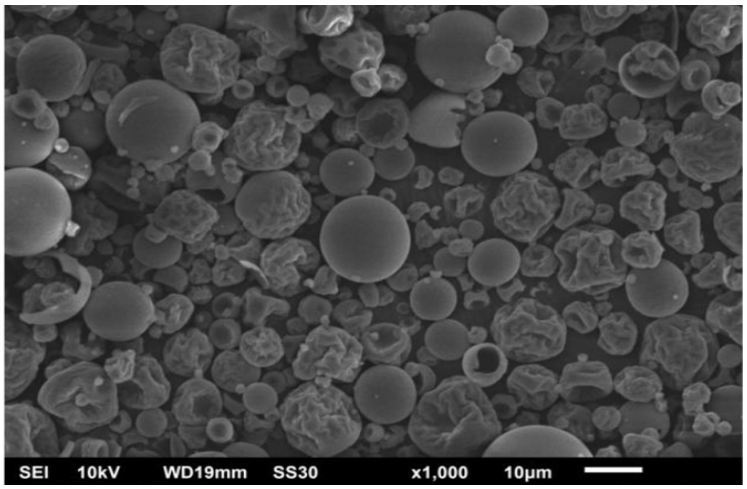


Figure 1. Microstructure of Liquid Smoke Nanocapsules

The composition of the coating material also affected the content of total phenols. The use of the coating material for one portion of alginate composition could trap phenolic content of liquid smoke during the spray drying process. This research was accordance with Novianty *et al.,* (2015) that the encapsulation process of liquid smoke with alginate 1% was able to trap the phenol content with the release of phenol for 20 minutes.

Total carbonyl content of liquid smoke nanocapsules was also affected by carbonyl content of liquid smoke. The carbonyl content of corncob liquid smoke was greater than coconut shell liquid smoke. Because of the corncob liquid smoke contains cellulose degradation products that were more than the liquid smoke coconut shell (Hardianto and Yunianta, 2015). In addition, the alginate composition as a coating material can protect the carbonyl during the spray dryer. Alginate can form a gel (Novianty *et al.,*2015). Alginate was polysaccharide that contain of homoploymeric mannoronic (M) and guluronic (G) block. The gel characteristic of alginate was affect by M/G ratio (Fertah *et al.,* 2017). This character was used to protect the phenolic content and carbonyl component during nanoencapsulation process. Nanocapsules oxidative capability of liquid smoke was measured by Radical Scavanging Activity. The RSA of liquid smoke nanocapsules was 91.35%. It was indicated that the coating materials was able to inhibit the oxidation of liquid smoke associated with total phenolic content and total carbonyl, where the component acts as an antioxidant and antimicrobial in food (Leha, 2010).

According to the table 1, it was known that liquid smoke nanoencapsulation contain PAH especially benzo-α-phyrene. Benzo-α-phyrene was known to be carcinogenic and mutagenic to human. Based Swastawati (2008), coconut shell liquid smoke had benzo-α-phyrene contents of 11.351 ppm, while corn cob liquid smoke was not detected (Swastawati *et al.,* 2007). According to the table 1, it showed that the coating material can trap nanocapsules PAH compounds.

Based on morphological observation of liquid smoke nanocapsules (Figure 1), it could be detected that the liquid smoke nanocapsules produced a perfect numerous circle. Novianty *et.al.,*(2015) showed that the concentration of 1% alginate microcapsules produced liquid smoke morphology with an unbroken sphere. This showed that alginate as a coating material was capable of protecting the liquid smoke during nanoencapsulation process.

3.2. Peroxide Value (PV) Analysis

The combination of liquid smoke nanoencapsulation was applied to the catfish fillets and stored at room temperature and cold temperature. The antioxidant and antimicrobial

effects were observed during storage. The number of peroxide value on a catfish fillet was presented in Figure 2. Based on the results obtained, the peroxide value of catfish fillets increased on days 0 to day 4. After that, the peroxide value decreased until 10 days at all storage temperatures. Peroxide value was the number that indicated the degree of damaged oil or fat by oxidation. The oil reacted with oxygen and form peroxides, especially when it contains unsaturated fatty acids (Panagan, *et al.,*2011). Catfish fillets had a fat content of 0.12 to 1.42% (Rario, 2015). Catfish fat contains omega-3 (Panagan, *et al.,*2011) as an unsaturated fatty acid, that potentially forms peroxides due to oxidation.

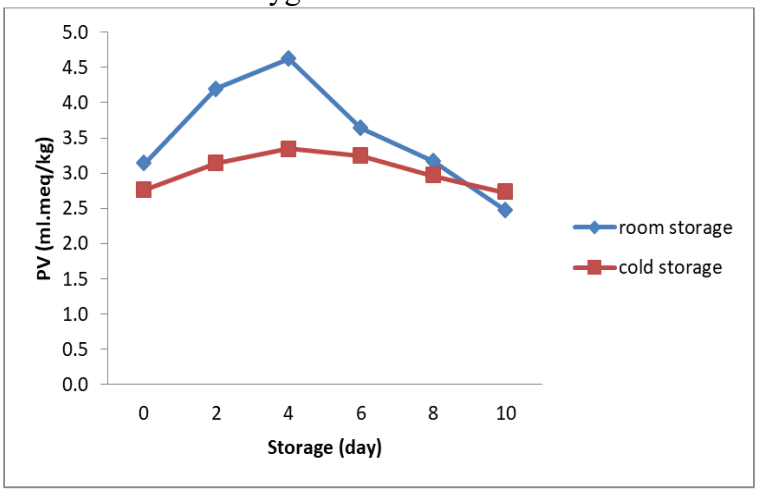


Figure 2. The Peroxide Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

The combination of liquid smoke nanocapsules had a total phenolic content of 3.68% and the RSA of 91.35%, that can inhibit the oxidation process of catfish fillet. The result showed that the storage of catfish fillet for 4 days had a good peroxide value at room temperature and cold temperature, there were 4.70 meq/kg and 3.35 meq/kg, respectively. The peroxide value was decreased for 10 days of storage at room temperature and cold temperature, there were 2.47 meq/kg and 2.73 meq/kg, respectively. The different results were shown by Adebowale *et al.,* (2012) that the catfish storage at room temperature for 21 days obtained peroxide value for 5.12 meq/kg. A maximum limit for foodstuffs peroxide value was 5 meq/kg. This result showed that the catfish fillet after 10 days of storage was feasible for consumption.

3.3. Thiobarbituric Acid (TBA) Analysis

The TBA value of catfish fillet during storage was presented in Figure 3. TBA measured the amount of malonadehid which is the final product of fat oxidation (Piccolo *et al.,* 2014). Based of figure 3, it could be seen that the TBA value of catfish fillet increased until 4 days of storage, for storage of room temperature from 3.53 mg malonaldehid/kg to 4.73 mg malonaldehid/kg. Meanwhile the TBA value of catfish fillet at cold temperature storage were 3.29 mg malonaldehid/kg to 4.05 mg malonaldehid/kg. The TBA value decreased until 10 days of storage, there were 3.21 mg malonaldehyde/kg at room temperature and 2.80 mg malonaldehid/kg at cold temperature. Swastawati *et al.,* (2012) applied the coconut shell liquid smoke on a stingray, showed the TBA value decreased after 6 days of storage. The maximum number of malonaldehyde was 5 mg/kg (Gunsen *et al.,* 2011). This result showed that catfish fillets were still feasible for consumption either on the storage at room temperature or cold temperature until 10 days of storage. The combination of liquid smoke nanocapsules applied to the catfish fillet was able to inhibit the oxidation of fat. The

decreasing of TBA value indicated that the secondary oxidation products formation which not detected with TBA value (Piccolo *et al.,* 2014).

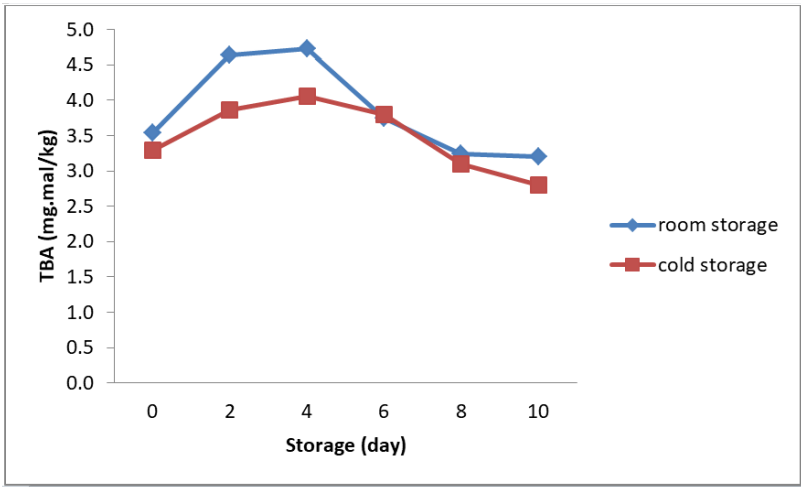


Figure 3. The TBA Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

3.4. Total Volatile Base Nitrogen (TVBN)

TVBN analysis measured the declining of fish quality. TVBN measured the protein degradation which is formed dimethylamine, trimethylamine, and ammonia Saloko *et al.,* (2014) that caused by bacterial activity (AOCS, 1990). The TVBN value of catfish fillet during storage was presented in Figure 4. The result showed that TVBN value increased during storage at 10 days. The TVBN value of catfish fillet increased in room temperature and cold temperature of storage, that was 15.08 mgN/100g to 22.58 mgN/100g for room temperature and 10.95 mgN/100g to 21.51 mgN/100g for cold temperature. This indicated that the longer of storage time, the growth of bacteria in catfish fillet was also increased.

The maximum limit of TVBN value for fish was about 30-35 mgN/100g. This showed that until the 10th day of storage, TVBN value is still below standard, consequently the catfish fillet was fit for consumption. These results related to the total phenolic content of liquid smoke nanocapsules that the phenol content of liquid smoke was able as antimicrobial agents (Saloko *et al.,* 2014).

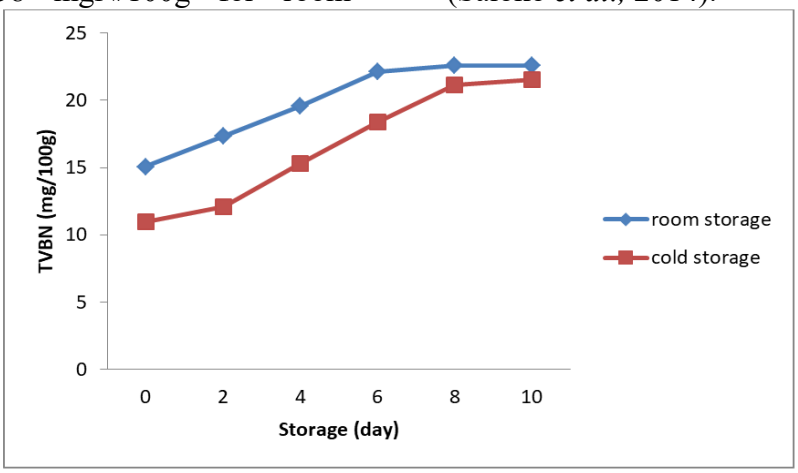


Figure 4. The TVBN Value of Catfish Fillet at Room Temperature and Cold Temperature

3.5. Total Plate Count (TPC)

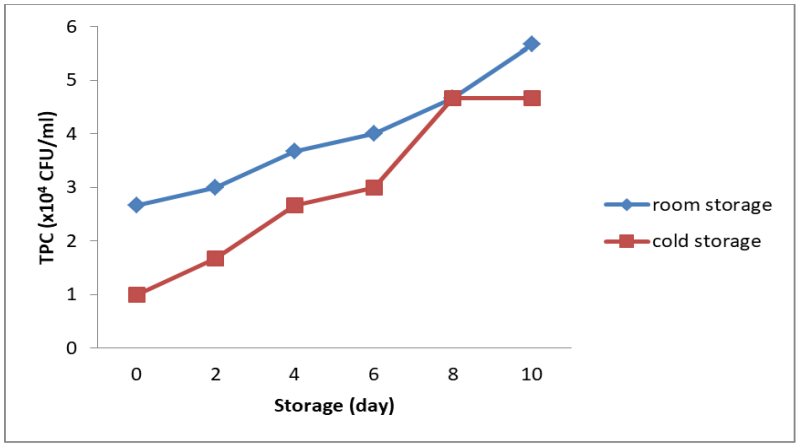


Figure 5. The TPC Value of Catfish Fillet at Room Temperature and Cold

Temperature TPC value of catfish fillet during storage was presented in Figure 5. The result showed that the number of microbial was increased during 10 days of storage. Both room temperature or cold temperature of storage, the number of microbial of catfish fillet were $2.67x10^4$ CFU/g to $5.67x10^4$ CFU/g at room temperature and $1x10^4$ CFU/g to $4.67x10^4$ CFU/g for cold temperature. Based on Indonesia National Standard, the TPC value of fish product was $5x10^5$ CFU/g (BSN, 2009). This result showed that until 10 days of storage, the catfish fillet was still feasible for consumption.

The combination of liquid smoke nanocapsules had total phenolic content that acted as an antimicrobial agent. Zuraida *et al.,* (2011) the coconut shell liquid smoke was able to inhibit microbial growth of fish balls on 20 days of storage with TPC value 1.8 log CFU/g. Ariestya *et al.,* (2016), also showed that the application of liquid smoke microcapsules on Tilapia meat could inhibit microbial growth with the TPC value 26 CFU/g at cold temperatures after 9 days of storage. The microbial growth inhibition because of the phenolic content of liquid smoke.

4.Conclusions

The liquid smoke nanocapsules application on catfish fillet was able to inhibit oxidation during storage, indicated by the PV and TBA value were under the limit standard until 10 days of storage. In addition, liquid smoke nanocapsules also able to inhibit microbial activity which was proved by the TVBN and TPC number was below the maximum limit.

The result showed that the liquid smoke nanocapsules was act as antioxidant and antibacterial agent.

5.References

Abbas, K.A, Mokhtar, A.S., Sapuan, S.M., Mawlood, M.K (2005). Quality deterioration of Malaysian patin fish during cold storage. *Journal of Food Agriculture & Environmental,* 3(2).

Adebowale, B.A., Ogunjobi, M.A.K., Olubamiwa, O., Olusola-Taiwo, M.O., Omidiran, V.A (2012) Quality improvement and value addition of processed fish (Clarias gariepinus) using phenolic compounds in coffee pulp smoke. *International Research Journal of Agricultural Science and Soil Science*, 2(13), 520-524.

Adilla, S.N, Utami, R., Nursiwi, A., Nurhartadi, E. (2017). The effect of nisin from lactococcus lactis subsp. lactis on refrigerated patin fillet quality. *IOP Conf. Series: Materials Science and Engineering*, 193, 1-7.

Ali, D.Y., Darmadji, P., Pranoto, Y. (2014). Optimation of coconut shell liquid smoke nanoencapsulation using response surface methodology and nanocapsules

characterization, *Jurnal Teknologi dan Industri Pangan*, 25(1), 23-30.

Alice, T.K, and Peter, E.S., Recovery of some volatile components from mango and guava, *Technical Progress Report* 128: Hawai, 1961.

Anggraini, S.P.A., and Nurhasiza, T. (2017). Performance optimization of liquid smoke device with agricultural waste material. *International Journal of Chemical Technology Research*, 10(13).

AOCS. 1990. Official methods and recommended practices of the American oil chemists' society (4th ed.). Urbana, IL: American Oil Chemists' Society.

Ariestya, D.I, Swastawati, F., Susanto, E. (2016). Antimicrobial activity of microencapsulation liquid smoke on tilapia (oreochromis niloticus (linnaeus, 1758)) meat for preservatives in cold storage (± 5°C). *Aquatic Procedia*, 7, 19-21.

Badan Standarisasi Nasional. (2015). Microbiology Analysis Part 3: Determination of Total Plate Count (ALT) on Fisheries Product. Jakarta. Indonesia.

Badan Standarisasi Nasional. (2015). Chemical Analysis Part 8: Determination of Total Volatil Base Nitrogen (TVB-N) dan Trimetil Amin Nitrogen (TMA-N) on Fisheries Product. Jakarta. Indonesia.

Badan Standarisasi Nasional. (2009). Maximum Limit of Microbe Contamination in Food. Jakarta. Indonesia.

Castro, P., Millan, R., Penedo, J.C., Sanjuan, E., Santana, A., Caballero, M.J. (2012). Effect of storage condition on total volatile base nitrogen determinations in fish muscle extracts. *Journal of Aquatic Food Product Technology*, 21, 519-523.

Conte, R, Calarco, A., Napoletano, A., Valentino, A., Margaruci, S., Di Cristo, F., Di Salle, A., Peluso, G. (2016). Polyphenols nanoencapsulation for therapeutic applications. *Biomolecular Research & Therapeutics*, 5(2), 1-13. Etheridge, M.L., Campbell, S.A., Erdman, A.G. (2013). The big picture on nanomedicine : the state of investigational and approved nanomedicine products. *Nanomedicine : Nanotechnology, Biology, and Medicine*, 9(1), 1-14.

Ezhilarasi, P.N, Karthik, P., Chhanwal, N., Anandharamakrishnan, C. (2012). Nanoencapsulation techniques for food bioactive components : a review. *Food Bioprocess Technol*, 1-21.

Fertah, M, Belfkira, A., Dahmane, E. (2017). Extraction and characterization of sodium alginate from moroccan laminaria digitata brown seaweed, *Arabian Journal of Chemistry*, 10, 707-714.

Gunsen, U., Ozcan, A., Aydin, A., (2011). Determination of some quality criteria of cold storaged marinated anchovy under vacuum and modified atmosphere conditions. *Turkish Journal of Fisheries and Aquatic Sciences*, 11, 233-242.

Hardianto, L., and Yunianta. (2015). Effect of liquid smoke against chemical properties and appearance fish tuna (euthynnus affinis). *Journal of Food and Agro-Industry*, 3(4), 1356-1366.

Leha, M.A. (2010). Application of liquid smoke as biopreservatif in foodstuffs. *Prosiding Seminar Nasional Basic Science II*, 254-266.

Li, L.Y., Li, L.Q., Guo, C.H. (2010). Evaluation of in vitro antioxidant and antibacterial activities of laminaria japonica polysaccharides. *Journal of Medicinal Plants Research*, 4(21), 2194-2198.

Lombok, J.Z., Setiaji, B., Trisunaryanti, W. (2014). Effect of pyrolisis temperature and distillation on character of coconut shell liquid smoke. *Prosiding of International Conference of Research, Implementation, and Education of Mathematics and Sciences,* 87-96.

Masniyom, P (2011). Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. *Songklanakarin Journal of Science and Technology*, 33(2), 181-192.

Memon, N.N., Talpur, F.N., Sherazi, S.T.H., Banger, M.I. (2010). Impact of refrigerated storage on quality of oil from freshwater jarko (wallago attu) fish. *Pakistan Journal*

of Analytical & Environmental Chemistry, 11(2), 37-43.

Molla, M.R., Asaduzzaman, A.K.M., Mia, M.A.R., Zeb, M.A., Uddin, M.S. (2015). Extraction and characterization of oil and lecithin from boal (wallago attu) fish. *Journal of Food and Nutrition Research*, 3(10), 661-666.

Novianty, H, Darmadji, P., Pranoto, Y., Suharwadji. (2015). Utilization of alginate as an encapsulation model of coconut shell liquid smoke. *Marine Research in Indonesia*, 40(1), 1-8.

Nurilmala, M, Nurhayati, T., Syukur, A.G., Vitner, Y. (2015). Evaluation of nutritional and color on indonesian and imported patin fish (pangasius sp.,) fillets. *Advance Journal of Food Science and Technology*, 8(8), 576-582.

Panagan, A.T., Yohandini, H., Gultom, J.U. (2011). Qualitatif and quantitative analysis of omega-3 unsaturated fatty acid from patin fish (Pangasius pangasius) oil with gas chromatography method. *Jurnal Penelitian Sains*, 14(4) 38-42.

Piccolo, J., Daniel A. P., Klein, B., Ferreira, L.F., Ruviaro, A.R., Emanuelli, T., and Kubota, E.H. (2014). Oxidative stability of refrigerated fish pates containing loquat seed extract. *Ciencia Rural, Santa Maria*, 44(9), 1705-1710.

Rario. (2015). Fish fillet catfish processing technology from Central Kalimantan, Indonesia. *IOSR Journal of Environmental Science*, *Toxicology and Food Technology*, 9(5), 18-25.

Saloko, S, Darmaji, P., Bambang, S., Yudi, P. (2014). Antioxidative and antimicrobial activities of liquid smoke nanocapsules using chitosan and maltodextrin and its application on tuna fish preservation. *Food Bioscience*, 7, 71-79.

Saloko, S., Darmadji, P., Setiaji, B., Pranoto, Y. (2012). Structural analysis of spray-dried coconut shell liquid smoke powder. *Jurnal Teknologi dan Industri Pangan*, XXIII(2), 173-179.

Saloko, S., Darmaji, P., Pranoto, Y., Anal, K. (2013). Encapsulation of coconut liquid smoke in chitosan-maltodextrin based nanoparticles. *International Food Research Journal*, 20(3), 1269-1275.

Swastawati, F, Boesono, H., Susanto, E., Setyastuti, A.I. (2016). Changes of amino acids and quality in smoked milkfish [chanos chanos (forskal 1775)] processed by different redestilation methods of corncob liquid smoke. *Aquatic Procedia*, 7, 100-105.

Swastawati, F., Boesono, H. and Wijayanto, D. (2014). Antimicrobial activity of corncob liquid smoke and it's aplication to smoked milkfish (chanos chanos forsk) using electric and mechanical oven. *IPCBEE*, 67, 109-113.

Swastawati, F., Agustini T.W., Darmanto Y.S., Dewi, E.D. (2007). Liquid smoke performance of lamtoro wood and corn cob. *Journal Of Coastal Development*, 10(3), 189-196.

Swastawati, F., Susanto, E., Cahyono B. (2012). Sensory evaluation and chemical characteristics of smoked stingray (dasyatis blekeery) processed by using two different liquid smoke. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 2(3), 212-216.

Tian, X.Y, Cai, Q., Zhang, Y.M. (2012). Rapid classification of hairtail fish and pork freshness using an electronic nose based on the PCA method. *Sensors*, 12, 260-277. Valdez, H.S, Ezquerra-Brauer, J. M., Marquez-Rios, E., Torres, W.T. (2015). Effect of previous chilling storage on quality loss in frozen (scomberomorus sierra) muscle packed with a low-density polyethylene film containing butylated hydroxytoluene. *Food Science and Technology*, 35(1), 202-206.

Youssef, M.K.E., Abdel-Gawad, A.S., Darwish, B.M., Hashem, A.M.A. 2015. Assessment of the effect of liquid smokes on the chemical composition and quality attributes of fish balls during chilled storage. *Assiut Journal of Agricultural Sciences*, 46(4), 50-64.

Yuniningsih, S., and Anggraini, S.P.A. (2013). Characterization of liquid smoke from coconut shell to be applicated as safe food preservatives for human health. *Journal of Agriculture and Food Technology*, 3(2), 1-5.

Zuraida, I., Sukarno., Budijanto, S. (2011) Antibacterial activity of coconut shell liquid smoke (cs-ls) and its application on fish ball preservation. *International Food Research Journal*, 18, 405-410.

Acknowledgments

The authors special thank to Diponegoro University (DIPA PNBP of Diponegoro University – 2016) that was funding this research and financial support to attending The Japanese Society for Fisheries Science (JSFS) 85th Anniversary-Commemorative International Symposium, 22 – 24 September 2017 in Tokyo for presenting this research.