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INULIN AND LENTIL FLOUR AS FAT REPLACERS IN MEAT-VEGETABLE PÂTÉ – A MIXTURE DESIGN APPROACH

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ABSTRACT

In the present work, the possibilities of using inulin gel and/or lentil flour as fat replacers in recipes for canned meat products were investigated. Different inulin gel and/or lentil flour concentrations were applied in canned poultry meat pâté samples. The influence of functional replacers on the emulsion stability, texture, water content, and organoleptic parameters were studied. Significant differences were found as a result of the replacement or reduction of fat, the type and quantity of functional ingredients (inulin gel and/or lentil flour), ($P < 0.05$). A direct relationship was observed between the hardness and quantity of lentil flour and inulin gel. The hardest ($P < 0.05$) samples were obtained with the highest lentil flour concentration as fat content substitute. To sum up, the substitution of half of the fat with inulin and lentil flour in the formulations of the sterilized meat pâtés can be used to improve the texture and emulsification, nutrition value and health benefits of the product.

1.Introduction

Meat pâtés in hermetically sealed cans are ubiquitous meat products that are consumed by a wide consumer segment. However, they have a fat content of around or above 30%, which has a negative impact on their health quality (Decker and Park Y., 2010; Felisberto *et al.*, 2015; Hygreeva *et al.*, 2014; Lorenzo *et al.*, 2014; Oostindjer *et al.*, 2014). This is the reason for the growing interest in the development of healthier products with an optimized concentration of functional ingredients. Nowadays, there is a widespread tendency towards the production of foods with reduced fat content, not just meat-based foods but also products in the entire food industry (Olmedilla-Alonso *et al.*, 2013). However, fat content reduction in meat emulsions such as pâtés is often accompanied by loss of fat and water content during the thermal process (Alvarez *et al.*, 2007). On the other hand, the formulation of a stable meat "emulsion" has been an area of interest for scientific research for many years (Acton and Dick, 1984; Gordon and Barbut, 1991; Lee and Carroll, 1981; Smith, 1988), and is considered a factor of paramount importance for obtaining a product with optimal technological and quality parameters (Bertram *et al.*, 2000; Bertram *et al.*, 2002; Bertram *et al.*, 2003; Bertram *et al.*, 2004; Di Luca *et al.*, 2011). Dietary fiber has the

ability to form a stable gel structure, retain fat and water (Fernández-Ginés *et al.*, 2005), and improve texture and yield (Tokusoglu and Ünal, 2003). At the same time, however, researchers report that fat replacement with vegetable fiber may lead to a reduction in organoleptic quality because the product consistency can become less dry, hard, friable or soft (Berry and Leddy 1984; Keeton, 1994). Inulin has been successfully used in different food products for fat substitution, as energy content reducer, and improver of the structure, viscosity, emulsion and water holding parameters of food products (Boeckner *et al.*, 2001). It is also considered a functional ingredient that carries a number of health benefits. In turn, legumes, lentils in particular, are regarded as a good source of protein, slow-release carbohydrates, dietary fiber, minerals and vitamins (Iqbal *et al.*, 2006; Antipova and Mishenko, 2016).

The purpose of this study was to investigate the possibility of fat content substitution with inulin and/or lentil flour in order to assess their influence on the emulsion stability, texture, sensory evaluation and water content of sterilized meat pâté using a simplex-centroid plan for the three-component mixture.

2. Materials and methods

2.1.Experimental statement

The following modified recipe was used for the production of the meat vegetable pâté: turkey meat (300 $g.kg^{-1}$), poultry liver (100 $g.kg^{-1}$), egg mélange (150 $g.kg^{-1}$), leaf fat (250 $g.kg^{-1}$), corn starch (20 $g.kg^{-1}$), water (150 $g.kg^{-1}$), sodium nitrite (0.05 $g.kg^{-1}$), black pepper (3 $g.kg^{-1}$), nutmeg (0.5 $g.kg^{-1}$), coriander (1.5 $g.kg^{-1}$), and polyphosphates (2 $g.kg^{-1}$). Ten types of samples were prepared with different concentrations of leaf fat, inulin and lentil flour according to Table 1. The formulation numbers correspond to Fig. 1.

Table 1. Experimental design of three components in meat vegetable pâté formulations

Sample number	Ingredient proportion		
	Fat (X_1)	Inulin (X_2)	Lentil flour (X_3)
1	1.0	0.0	0.0
2	0.0	1.0	0.0
3	0.0	0.0	1.0
4	0.5	0.5	0.0
5	0.5	0.0	0.5
6	0.0	0.5	0.5
7	0.33	0.33	0.33
8	0.667	0.167	0.167
9	0.167	0.667	0.167
10	0.167	0.167	0.667

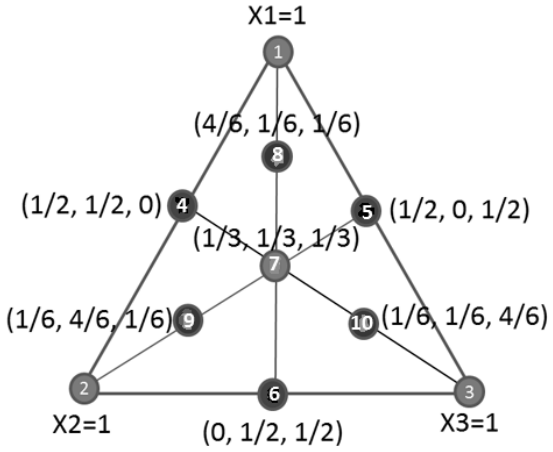

Figure 1. Ten point simplex-centroid design for the interaction of pork bacon (X_1), inulin (X_2), and lentil flour (X_3) in meat vegetable pâté formulations

The turkey meat, leaf fat (bacon) and liver were supplied by meat processing companies. Commercially purchased lentil was milled to flour and used. Inulin (Orafti®HPX) was provided by the ARTEMIS Ltd. company. The inulin was used in the form of a gel obtained by hydration in a 1:4 (w/v) ratio (Latoch *et. al.*, 2016). For the gel preparation, the inulin water suspension was heated to 85 °C until complete dissolution and then cooled to 50 °C. The two

functional ingredients were added during processing in the cutter.

Experimental pâtés were prepared from defrosted and sliced poultry meat by grinding in a cutter (Fimar CL/5), with the addition of poultry liver. During the cutting, egg mélange, auxiliary additives and inulin and/or lentil flour were added. The cutting was continued until a fine and homogenous meat mixture was obtained. During the cutting, water was added up to 15% of the meat weight. The prepared filling mass was heated to 70 °C, and then filled into 160 gram cans. The cans were closed with a sealing machine (Lanico Maschinenbau, Otto Niemsch KG, Braunschweig) and sterilized at 121ºC for 45 min in an autoclave purchased from the Hydroplastform Ltd. company, Haskovo, Bulgaria.

2.2.Analytical methods

Texture Profile Analysis (TPA) of the finished product (Bourne, 1978) was performed using a TA-XT.Plus texture analyzer (Stable Micro Systems, Surrey, GB), cylinder in tube configuration. The cylinder was 30.37 mm in diameter and 51.75 mm in height. It was filled with about 30 g of sample up to a height of about 40 mm. The samples were compressed twice at a rate of 2 mm s^{-1} up to 20 mm deformation. Relaxation time between two compressions was set to 5 s. Hardness (Ha), adhesiveness (Ad), cohesiveness (Co) and friability (Fr) were calculated for further analysis (Bourne, 1978; Bourne, 2002; Chorbadzhiev *et al.*, 2017).

The moisture content (MC) of the tested canned pâté samples was determined by drying at 104±1 °C to a constant weight using a KERN MLS-A labor scale (Kern & Sohn GmbH, Germany).

For determination of the emulsion stability, the method described by Ockerman (1985) and Zorba et al. (1993) was used.

The sensory evaluation of the samples was performed using a five-digit hedonic scale, where 5 corresponded to the highest value and 1 to the lowest value of the assessment for the given indicator. The tasting panel included a total of 10 tasters. When the cans were opened, the samples were spread on bread slices and evaluated for the following qualities: appearance, color, texture, taste, aftertaste, flavor, spreadability, and overall assessment of the perception of the products.

A three-component, simplex centroid mixture design was used. The mixture components consisted of pork bacon (X_1), inulin (X_2) and lentil flour (X_3). The component proportions were expressed as fractions of the mixture with a sum (X_1+ X_2 + X_3) of one (Table 1). The ten points were three single components, three two-ingredient mixtures and four three-ingredient mixtures (Fig. 1).

Scheffe's canonical special cubic equation for three components was fitted to data collected at each experimental point using backward stepwise multiple regression analysis as described by Cornell (2002). The canonical special cubic equation Eq. 1 was postulated:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

where Y is a predictive dependent variable (emulsion stability, moisture, texture, and sensory evaluation); and β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, and β_{123} are the corresponding parameter estimates for each linear and cross-product term produced for the prediction models for pork bacon, inulin, and lentil flour, respectively. An analysis of variance was performed on the data and response surfaces were generated for each response using predictive models.

The terms in the canonical mixture polynomial have simple interpretations which can be found in specialized texts (Myers and Montgomery, 2002). The usual way of summarizing mixture proportions is via triangular (ternary) graphs.

3.Results and discussions

The experimental data obtained in the texture analysis (Table 2) demonstrated that hardness showed significant differences between the individual samples. According to some authors, hardness in meat products is associated with fat content. Ventanas *et al.*, (2010) and Alvarez and Barbut (2013) have found that the decrease in fats in meat products

leads to a decrease in hardness as well as in other texture parameters. In our samples, the reduction of fat and its substitution with inulin gel resulted in a decrease in hardness. This difference was proportional to the amount of inulin used. The addition of inulin gel led to values lower than those recorded in the lentil flour samples when added in the same amounts and at the same fat reduction rate of the formulation. The samples with fat, inulin gel, and lentil flour showed a gradual increase in hardness, which was most notable in the samples in which lentil flour prevailed.

The higher values found for this indicator in lentil additions were associated with the presence of large and unequally distributed clusters of flour within the thermally denatured protein matrix. Apart from the increase in the protein content due to the use of a larger amount of lentil flour, the reduced fat (Table 1) and the increased water content (Table 2), as well as the probability of formation of a greater number of intercellular bonds can be seen as the reasons for the increase in hardness. On the basis of these results and the unanimous agreement of the tasting panel members on the "too dense" consistency in these pâté samples (Table 3), it could be suggested that a direct relationship might exist between the reported values for the hardness index and the amount of lentil flour used.

Taking into account the significant differences in the adhesiveness (stickiness) of the product determined between the individual samples, the influence of the two functional additives on the characteristics of the meat-vegetable pâté should be noted. The established decrease in the adhesiveness of the test samples where the fat had been completely or partially replaced with inulin gel was a direct consequence of the amount used and the moisture content of the samples.

Cohesiveness (homogeneity) is a dimensionless parameter with values ranging from 0 to 1. The ability of cohesive products to adhere to themselves (Bourne, 2002) should be directly related to the ways in which such products form their structure capable of resisting the application of external stresses, i.e. compression or stretching. Considering the chemical composition of the additives used, it can be argued that the variations in cohesiveness in the individual samples were directly related to the higher protein content in the samples with lentil flour and the direct involvement of the lentil proteins in the building of new bonds both with meat and with non-meat proteins. High levels of stickiness are uncommon for meat products and may have a negative impact on sensory parameters. However, the very low values are also indicative of a non-typical meat product structure (Table 3).

Friability as a parameter has a complex character that gives a generalized idea of the structural and mechanical properties of the tested product, affecting its behavior in consumption. The reasons for the higher friability values in the samples with the addition of lentil flour and fat in close concentrations were the same, leading to the previously established solidity and cohesion (homogeneity) data for these samples. Lower scores for inulin supplements compared to lentils added were an indicator of pâté texture improvement by a gradual increase in the value of the indicator while avoiding the adverse effect of the strong modification of some other texture parameters.

Table 2. Texture profile analysis (TPA), moisture content and emulsion stability of the meat-vegetable pâtés

Sample №	Texture profile analysis (TPA)				MC g/100g	ES %
	Ha, N	Ad, Nmm	Co, -	Fr, N		
1	61.67^{b}	-64.63^{b}	0.20^{a}	12.69^{b}	54.81^{ab}	97.92^{c}
2	24.71^{a}	-12.06^{c}	0.25^{abc}	6.14^{a}	67.66^{f}	90.73^{a}
3	127.75^{d}	-130.15^{a}	0.27^{cd}	34.91^{d}	67.66^{f}	100.00^{d}
4	26.98^{a}	-21.61^{c}	0.25^{bcd}	6.71^{a}	58.62^{bc}	96.37^{b}
5	170.68^{f}	-137.23^{a}	0.28^{cd}	47.30^{e}	54.03^{a}	99.96^{d}
6	69.33^{b}	-64.79^{b}	0.25^{bcd}	17.62^{bc}	62.75^{de}	99.91^{d}
7	63.10^{b}	-72.66^{b}	0.25^{abcd}	15.79^{bc}	59.1^{cd}	99.83^{d}
8	87.90^{c}	-81.22^{b}	0.22^{ab}	19.07^{c}	53.53^{a}	99.88^{d}
9	23.94^{a}	-18.10^{c}	0.23^{abc}	5.56^{a}	$64.97e^{f}$	96.48^{b}
10	143.75^{e}	-129.08^{a}	0.29^{d}	42.24^{e}	60.33^{cd}	99.97^{d}

The values for the respective sample are the arithmetic mean of 5 measurements for the given indicator
$^{a-f}$ *-values within the same column bearing a common superscript did not differ statistically ($P < 0.05$)*

The comparison of the data on the water content of meat-vegetable pâtés (Table 2) showed that the addition of the two functional additives, inulin and lentil flour, resulted in a product with higher moisture content. This became more pronounced when the fat content was lowered and fat was replaced with higher amounts of inulin or lentil flour. The reason for this was undoubtedly the high water holding capacity and hygroscopic properties of the additives, which favored the ability of the product to retain the added water. A number of other authors have also reported these properties (Felisberto *et al.*, 2015; Kaur, *et al.*, 2007).

To assess the influence of the functional additives (inulin and lentil flour) used on the gel forming and emulsifying capacity of the filling mass and the finished sterilized pâtés, tests were conducted to determine the resultant emulsion stability (Table 2). A statistically significant difference was found in this indicator between sample 1, which contained 100% fat, and the other samples. The results showed that the addition of lentil flour significantly improved the emulsion stability of the filling mass and the resulting products in all the samples, with values exceeding 99.00%. The highest concentration of lentil flour resulted in the most stable meat emulsion: 100.00% in sample 3; however, according to the numerical expression of this optimization, it was statistically the same with all samples containing lentil flour ($P > 0.05$). In contrast, the lowest value for the emulsion stability was recorded in the sample formulations where the fat was replaced only by inulin gel (sample 2). Felisberto et al. (2015) indicated the formation of a more brittle protein gel with the involvement of inulin and indicated the need for another ingredient to reduce this destabilizing effect and improve the meat emulsion stability. The advantage of the addition of lentil flour as a protein supplement in terms of meat emulsion stability is associated with its ability to participate in the formation of a stronger protein structure that favors the capturing of water molecules and fat globules (Table 2) (Gordon et *al.*, 1992) as compared to the protein matrix formed only by inulin gel or

inulin gel and fat. In sample 2, the absence of fat and its replacement with inulin led to water loss, resulting in visible phase separation after heat treatment. Similarly, the results of Cáceres *et al.*, (2004) explained the decreased stability of the emulsion with the limited ability of the meat proteins to retain water in the compact structure of the inulin gel. In addition (Carballo *et al.*, 1996), gels formed with polysaccharides lead to the formation of a more compact and stronger thermally induced protein matrix, reducing its ability to retain water and thereby increasing the loss of liquid after thermal processing.

Table 3. Sensory analyses of the meat-vegetable cans

Sample №	Parameter							
	Appearance	Color	Spread-ability	Aroma	Consistency	Taste	Aftertaste	General acceptance
1	4.05^{g}	4.35^{f}	4.35^{e}	4.00^{d}	4.15^{e}	4.55^{e}	4.55^{f}	4.60 ± 0.66^{d}
2	2.70^{cde}	3.40^{cdf}	3.75^{de}	3.35^{cd}	2.30^{ab}	3.60^{bcd}	3.70^{def}	3.45 ± 1.21^{bc}
3	1.25^{a}	1.15^{a}	1.55^{a}	2.05^{a}	1.70^{a}	2.20^{a}	2.25^{a}	1.85 ± 0.94^{a}
4	3.50^{fg}	3.75^{df}	4.25^{e}	3.75^{cd}	3.35^{cde}	3.85^{cd}	4.00^{ef}	3.85 ± 1.16^{cd}
5	2.25^{bc}	2.45^{bc}	2.25^{ab}	2.85^{abc}	3.10^{bcd}	2.70^{ab}	2.45^{ab}	2.55 ± 1.17^{ab}
6	3.25^{ef}	3.20^{cd}	2.70^{bc}	3.05^{bc}	3.75^{cde}	2.75^{ab}	2.60^{abc}	3.15 ± 0.91^{bc}
7	3.25^{ef}	2.90^{cd}	3.60^{de}	3.45^{bcd}	3.90^{de}	3.60^{bcd}	3.45^{bcde}	3.65 ± 0.67^{c}
8	3.25^{ef}	3.35^{cd}	3.10^{cd}	3.65^{cd}	3.85^{cde}	3.10^{abc}	3.35^{bcde}	3.40 ± 0.99^{bc}
9	3.25^{ef}	2.95^{cd}	4.30^{e}	3.40^{bcd}	3.20^{bcd}	4.15^{e}	3.65^{cdef}	3.65 ± 1.20^{c}
10	3.25^{ef}	1.80^{ab}	2.20^{ab}	2.55^{ab}	2.95^{bc}	2.90^{abc}	2.75^{abcd}	2.70 ± 1.03^{ab}

The values for the respective sample are the arithmetic mean of 5 measurements for the given indicator
$^{a\text{-}f}$ *-values within same column bearing a common superscript did not differ statistically ($P < 0.05$)*

The results of the sensory analysis for the individual indicators as well as for the general acceptance as shown in Table 3 made it possible to assert that the type and quantity of the functional additives added had a significant effect on the organoleptic characteristics of the finished canned meat samples.

A lower score for the indicator was recorded in the samples with a higher concentration of inulin that was associated with the presence of surface-migrated fat and segregated water and corresponded to the observed lower emulsifiability of these pâtés. Sample 2 demonstrated the largest amount of fluid released on the surface, and samples 1, 5 and 8, fat removal. The highest grade was given to the visual appearance of sample 1, which was statistically distinct from all other samples ($P < 0.05$), and the closest to it was the estimate for sample 4, in which 50% of the fat had been replaced with inulin gel. A similar trend was also found with the organoleptic scores for the color indicator. The highest grade for this indicator was excellent for sample 1, followed by sample 4. The lowest scores were given to samples 3, 5, and 10, in which the color was assessed as uncharacteristic for this product range. Unfavorable color changes were found in the samples with the largest amount of lentil flour. The deterioration of the color characteristics was expressed as darkening of the color of the product and a less pronounced red color. The darkening was probably due to non-enzymatic reactions occurring during the subsequent sterilization regime. The data obtained

corresponded to those from the pictures of the captured samples (not shown).

In the samples prepared with the addition of lentil flour, consistency deficiencies were also observed, with the highest deviations found in sample 3 (100% fat replacement with lentil flour). The atypical consistency was described as "too stiff and crumbly". Here too, there was a coincidence of the established trends with those recorded in the previous analyses related to the structural and mechanical properties and, more precisely, to the established hardness, homogeneity, and friability of the product.

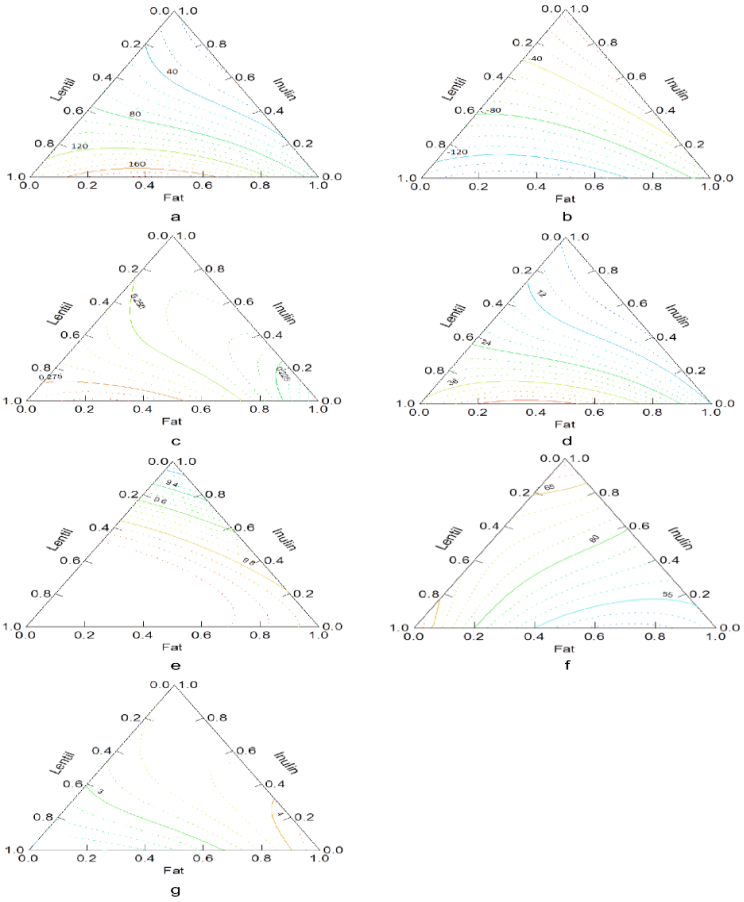

Figure 2. Two-dimensional triangular coordinate system showing the hardness (a), adhesiveness (b), homogeneity (c), friability (d), emulsion stability (e), moisture (f), and sensory evaluation (g).

Samples 1, 4, 6, 8, and 9 were characterized by good homogeneity, i.e. no particles with dimensions differing from the total mass, which is an essential condition in the production of pâtés, were observed. Samples 3 and 10, where the amount of lentil flour used was predominant, and sample 2, in which fat was completely replaced by inulin gel, exhibited non-homogeneous consistency with the presence of larger particles. Regarding spreadability, the highest value was observed in sample 1 and was statistically indistinguishable ($P > 0.05$) from samples 4 and 9. These close values were most likely due to the amount of inulin incorporated into the respective samples and its effect on the filling mass. The data on this indicator successfully corresponded to the data obtained from the TPA analysis, more specifically, for the hardness and friability indicators.

The analysis of the results obtained by the tasting panel on the taste and aroma indicators revealed differences in the pâtés studied. The testimonies were as close as possible to the expectations and perceptions of sample 1, followed by samples 4, 8, 9, and 2. The reduction in the fat amount and its replacement with inulin gel resulted in products having good taste and aroma. Similar results have also been reported for the production of boiled sausages with added inulin (Šojić *et al.*, 2011). In low-fat samples and samples with lentil flour substitute, there was a decrease in taste and aroma intensity, which made it non-specific for the product, i.e. sterilized pâté.

The comparison of the total scores obtained from the experiment showed that the highest scores were given to samples 1 and 4 ($P < 0.05$).

Using the simplex method and the modeling and optimization procedures related to it and after processing of the results, equations for texture (hardness, adhesiveness, homogeneity and friability), emulsion stability, moisture, and sensory evaluation were obtained as follows:

$$Y_1(hardness) = 62.56X_1 + 20.74X_2 + 134.03X_3 + 70.99X_1X_2 + 318.22X_1X_3 + 23.00X_2X_3 + 665.80X_1X_2X_3 \ (R^2 = 0.97) \quad (2a)$$

$$Y_2(adhesiveness) = -65.83X_1 - 7.41X_2 - 134.52X_3 + 73.85X_1X_2 - 170.50X_1X_3 + 25.83X_2X_3 + 46.13X_1X_2X_3 \ (R^2 = 0.98) \quad (2b)$$

$$Y_3(homogeneity) = 0.20X_1 + 0.24X_2 + 0.28X_3 + 0.09X_1X_2 + 0.16X_1X_3 + 0.02X_2X_3 + 0.54X_1X_2X_3 \ (R^2 = 0.78) \quad (2c)$$

$$Y_4(friability) = 12.03X_1 + 5.08X_2 + 37.55X_3 - 14.27X_1X_2 + 97.93X_1X_3 - 8.44X_2X_3 - 217.25X_1X_2X_3 \ (R^2 = 0.95) \quad (2d)$$

$$Y_5(emulsion\ stability) = 98.16X_1 + 90.70X_2 + 99.76X_3 + 8.62X_1X_2 + 4.00X_1X_3 + 17.64X_2X_3 + 5.05X_1X_2X_3 \ (R^2 = 0.98) \quad (2e)$$

$$Y_6(moisture) = 54.32X_1 + 68.33X_2 + 67.48X_3 - 10.10X_1X_2 - 30.16X_1X_3 - 18.66X_2X_3 + 61.42X_1X_2X_3 \ (R^2 = 0.97) \quad (2f)$$

$$Y_7(sensory\ evaluation) = 4.50X_1 + 3.47X_2 + 1.88X_3 - 0.85X_1X_2 - 2.84X_1X_3 + 2.09X_2X_3 + 40.69X_1X_2X_3 \ (R^2 = 0.97) \quad (2g)$$

The resulting equations describe with high accuracy the change in the contents of the dependent variable ($R2 > 0.9$), except for the equation for homogeneity, where it was 0.78. The two-dimensional contour plots are shown in Fig. 2.

4. Conclusions

On the basis of the analysis, a conclusion can be drawn that the experimentally obtained data were objective evidence of the effect of the functional additives used on the texture, emulsion stability, water content and sensory evaluation of the sterilized meat-vegetable pâtés. The main reason for the differences established between the tested samples was the type and amount of the functional additives incorporated (inulin gel and lentil flour). In the inulin gel containing samples, hardness and homogeneity were less pronounced and depended on the amount of inulin used. More undesirable texture characteristics were found in the samples with higher amounts of lentil flour added and reduced fat content, which corresponded very well to the results of the sensory analysis. The low scores given by the tasting panel to these samples were a direct consequence of the deviations in texture and consistency that were not typical of the product range. However, the incorporation of lentil flour into the filling mass resulted in a better emulsion stability, which meant that meat-based vegetable pâtés having the necessary characteristics for this product range could be manufactured.

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INVESTIGATION OF EXPERIMENT DATA AND SENSITIVITY COEFFICIENT DATA WITH ARTIFICIAL NEURAL NETWORK IN THE OHMIC HEATING PROCESS FOR SOUR ORANGE JUICE

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ABSTRACT

In this investigation, an ohmic heating system was constructed and applied to the heating process at three voltage gradient inputs (8.33, 10.83, 13.33 V/cm) and three percent weight loss sample (10, 20 and 30%) compared to total weight was selected. During the thermal process, the power consumption, electrical conductivity and coefficient performance system were calculated. All experiments were performed in three replications. An artificial neural network was used to predict experimental data. In this study multi-layer perceptron were selected and radial basic function artificial neural network by 1 hidden layers and 4, 8 and 12 neurons hidden layers, and with two activation function (hyperbolic tangent and sigmoid). The highest R values were for power consumption (0.998), electrical conductivity (0.996) and Coefficient performance systems (0.999) in a MLP network with 8 neuron in hidden layer and sigmoid activation. Also the fastest network with lowest EPOCH was in a network of 12 neuron. According to the results obtained for R, MSE and learning cycle, it can be said that the neural network has ability to predict power consumption, electrical conductivity and coefficient performance systems to an acceptable level for ohmic processing.

1. Introduction

Artificial neural network (ANN) seems very appropriate for the investigation and simulation of the data. ANN is, in fact, a collection of mathematical methods mostly including artificial intelligence and it attempts somehow to imitate human brain. During the past two decades, the neural network has exhibited a very high potential in a great many of the science and engineering areas for its exceptional performance, internal organization and self-learning, overcoming the challenges and high solidity rate. Recently, there has come about an increase in the interests in utilizing neural networks as a modeling tool in agriculture and food industry technologies. Neural networks have been successfully employed in several foodstuff processing technologies such as drying, post-harvest technologies, rheology of the foodstuff, microbial predictions, fermentation and thermal processing (Lu et al., 2010). Artificial neural networks are also considered as most effective tools for processing a large volume of information that was once a

big challenge in various respects. The development trend of the neural networks is suggestive of the importance of using them for information processing because they have been proved highly successful in data analysis and they have been capable of undergoing development in various grounds. Moreover, the use of neural networks is promising in food production and foodstuff quality processing and evaluation methods wherein old methods of data processing might not provide us with accurate information or be substantially costly. Two important abilities of neural networks, to wit prediction and classification scales, have drawn a large deal of attention. According to the internal competencies of the artificial neural networks, they can be successfully applied in agriculture sector (Hosu, Cristea, & Cimpoiu, 2014). The artificial neural network is a topic discussed in artificial intelligence and it is an information processor trained using a percentage of input and output data and the system's performance method is stored in its memory(Mazloumzadeh, Alavi, & Nouri, 2008). Artificial neural networks are trained based on calculations on numerical data or examples. One feature of the neural networks is their ability in extracting the relationships between the inputs and outputs of a process with no need to complex environmental conditions. They are capable of connecting a multidimensional space to another space even if the information is imperfect and erroneous. These characteristics have made them appropriate for the problems related to the estimation and prediction in agriculture and industry and the neural network displays a good efficiency when the relations are nonlinear (Beale & Jackson, 1998; Menhaj, 2000). Moreover, the artificial neural network (ANN) modeling is widely used in many fields. This method is of high efficiency in solving the complex and non-linear equations in dryers (Özdemir, Aktaş, Şevik, & Khanlari, 2017). They also researcher used neural network in thermal processes:

Mattar and et al (2004) on modeling thermal conductivity, specific heat, and density of milk with neural network reported that artificial neural networks presented a better prediction capability of specific heat, thermal conductivity, and density of milk than polynomial modeling(Mattar et al., 2004).

Chegini et al. (2007) used predictive process and orange juice from artificial neural network, the results of which showed that the properly trained ANN model was able to produce simultaneously seven outputs, unlike traditional models where one mathematical model was required for each output. Radial Basis Function neural networks were not able well to learn the relationship between the input and output parameters. ANN parameters had a significant effect on learning ability of the ANN models(Chegini, Khazaei, Ghobadian, & Goudarzi, 2008).

The objective of this research is the power consumption, electrical conductivity and Coefficient performance systems analyses of ohmic processing with three ohmic voltage gradient in order to reduce the weight loss sour orange with new processes. For this purpose, the ANN (multilayer perceptron and radial basic function) was applied to verify the accuracy of the numbers obtained. Additionally, the sensitivity coefficient test was applied to relate the power consumption, electrical conductivity and coefficient performance systems factors to voltage gradient and weight loss percentage.

2. Materials and methods

2.1 Preparation of the sample

The oranges were purchased from a garden located in the city of Gorgan, Golestan province. The prepared oranges were washed and split into two halves in the middle and immediately after the purchase in the same condition for all samples (ambient temperature and applied uniform pressure), the manual removal was carried out.

2.2. Method of testing

For this processing was considered one tank and the sample were poured into the ohmic tank and between the two electrodes, and their initial temperature was recorded after stability. After recording the temperature, the voltage was applied to the set and the samples were heated. Three heating gradients of 8.33, 10.83 and 13.33V/cm were selected for the heating process and, using this voltage gradient, 10% (from 90 g to 81 g), 20% (from 90 g to 72 g) and 30% (from 90 g to 63 g), the percentage of the total weight of the samples of sour orange discharged inside the cell is steamed during the heating process. All samples were weighed 90 g and the temperature of all specimens was 26 °C to initiate the heating process. In Figure 1, a schematic representation of the heating process and system components is shown.

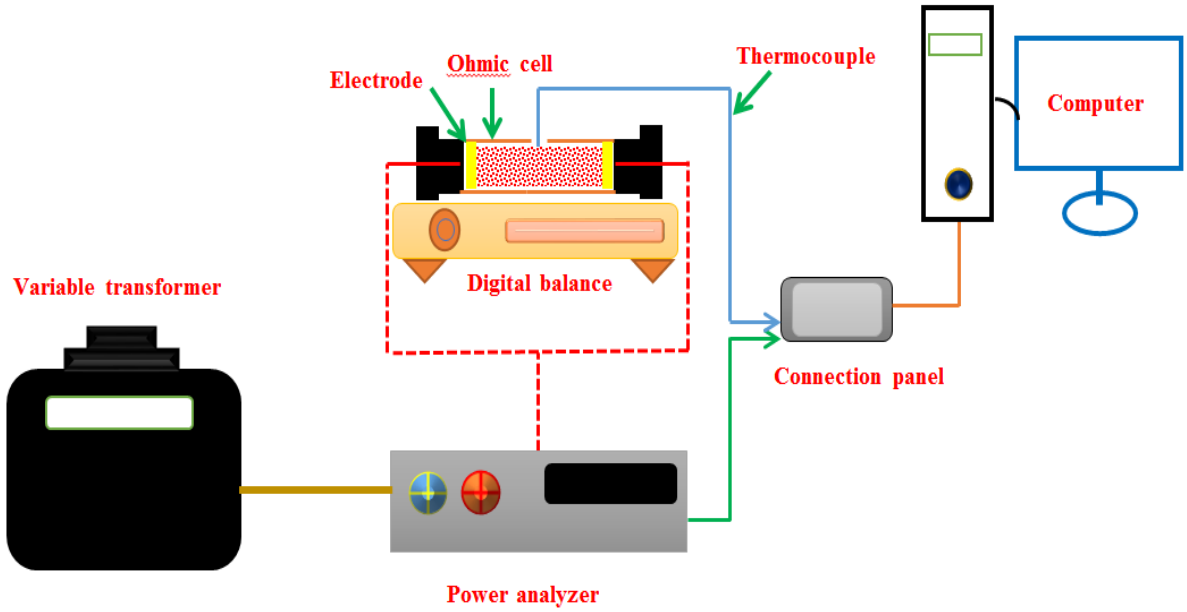

Figure 1. Schematic of equipment used for the ohmic heating process

The experiments were carried out in a home-based heating system. The system specifications used are shown in Table 1. All experiments were carried out at the department of bio systems mechanical engineering, Gorgan University of Agricultural Sciences and Natural Resources

Table 1. The system specifications

Length	6 cm	Distance electrode	6 cm
Width	6 cm	Power controller	(3 kW, 0–300 V, 50 Hz, MST – 3, Toyo, Japan
Height	3 cm	Balance accuracy	0.01 g
Thickness	0.3 cm	Electrode Thickness	0. cm
Electrode	Steel		

2.3. The equations of the heating process of ohmic

Electrical conductivity was calculated using the resistivity of the samples within the cell geometry used in equation (1)(Castro, Teixeira, Salengke, Sastry, & Vicente, 2004)（ Cappato et al., 2017）

$$\sigma = R\frac{L}{A} = \frac{LI}{AV} \quad (1)$$

In this formula, σ = the electrical conductivity of the sample L: the distance between the two electrodes (m) from each other, A: the cross-sectional area of the plates (m^2), V: the input voltage (V), I: the input current (A)

During the heating, the contact surface between the samples and the electrode decreases due to the vapor output, the contact surface can be calculated using the equation below.（ Darvishi, Hosainpour, Nargesi, & Fadavi, 2015： ）

$$A = \frac{M_t}{\rho_t L} \quad (2)$$

$$\rho_t = 1340 - 3.26M_t^2 \quad (3)$$

M_t Humidity content at any moment

Power consumption was also calculated using formula 4（Kanjanapongkul, 2017):

$$P=VI=I^2R \quad (4)$$

In this equation, P is the power consumption (W)

The energy given to the system in accordance with the relationship provided by icier and Hammers in 2005 is as follows (Srivastav & Roy, 2014)

$$E_{given} = E_{taken} + E_{loss} \quad (5)$$

$$\sum(VIt) = mc_p(T_f - T_i) + E_{loss} \quad (6)$$

The energy of the system is equal to the sum of the energy needed to increase the temperature of the cell, the energy dissipated to the environment through the displacement and the electrical energy converted to heat. In the above equations, the volatility value was determined and the amperes and time values were calculated by the software. The initial temperature and final temperature of the orange water were measured by a thermometer and the mass of water in the orange water was calculated by the balance. The system performance coefficient is given by the energy ratio taken by the system to the energy and calculated from the following equation（Darvishi, Khostaghaza, & Najafi, 2013．）

$$SPC = \frac{E_{taken}}{E_{given}}$$

$$SPC = \frac{mc_p(T_f - T_i)}{\sum(VIt)} \quad (7)$$

In this formula, the energy $_{given}$ to system (j), T_f is the final temperature (C), Etaken energy taken from the system (j), T_i input temperature, E_{loss}, the energy lost in the system (j), t (s), SPC is the coefficient of performance system, m is the mass of the sample (kg).

2.4.Artificial Neural Network Modeling

In this research, the artificial multilayer perceptron (MLP) and radial basic function (RBF) neural network were used for modeling the Investigating sour orange components during voltage and percent decrease mass different to predict electrical conductivity by one hidden layer and 4, 8, and 12 neurons using the Neuro-Solution 5 software. Hyperbolic tangent and sigmoid activation functions (Equation 3,4), which are the most common type of activation functions, were used in the in hidden input and output layer. In this paper, the Levenberg-Marquardt algorithm was used to learn the network(Taheri-Garavand, Karimi, Karimi, Lotfi, & Khoobbakht, 2018). Additionally, 70% of the data were used for training, 10% of them were used for network evaluation (Validating Data), and 20% of the data were used for testing the network (Testing data) (Table 3). The voltage, decreasing mass value, current input and ohmic time as network inputs and power consumption, electrical conductivity and Coefficient performance systems were the considered network outputs. Five repetitions were considered to achieve the minimum error rate and maximum network stability as a mean of 5000 Epoch for the network. Error was estimated using algorithm with back propagation error. Statistical parameters including, Root Mean Square Error (RMSE), R^2, and Mean Absolute Error (MAE) were calculated for inputs and relationships were calculated using the formulas shown in Table 2.

Table 2. Neural Network Relationships

Formula	Formula Number	Reference
$\text{Tanh} = \frac{e^{x}-e^{-x}}{e^{x}+e^{-x}}$	(8)	(Soleimanzadeh, Hemati, Yolmeh, & Salehi, 2015)
$\text{Sig}=\frac{1}{1+e^{-x}}$	(9)	(F. Salehi, Gohari Ardabili, Nemati, & Latifi Darab, 2017)
$R^2 = 1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{(P_i-O)^2}$	(10)	(Azadbakht, Torshizi, & Ziaratban, 2016)
$r = \sqrt{1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{(P_i-O)^2}}$	(11)	(Fakhreddin Salehi & Razavi, 2012)
$\text{RMSE} = \sqrt{\sum_{i=1}^{n}\frac{(P_i-O_i)^2}{n}}$	(12)	(B. Khoshnevisan, Sh. Rafiee, M. Omid, 2013)
$\text{MAE} = \frac{\sum_{i=1}^{n}\lvert P_i-O_i\rvert}{n}$	(13)	(Azadbakht, Aghili, Ziaratban, & Vehedi Torshizi, 2017)

Table 3. Optimization values for artificial neural network parameters

Number of hidden layers	Learning rule	Type of activation function	The number of hidden layer neurons	Testing data %	Validating data %	Training data %
1	Levenberg Marquardt	Hyperbolic tangent and sigmoid	4	20%	10%	70%
1	Levenberg Marquardt	Hyperbolic tangent and sigmoid	8	20%	10%	70%
1	Levenberg Marquardt	Hyperbolic tangent and sigmoid	12	20%	10%	70%

3. Results and discussions

For to predict power consumption, electrical conductivity and coefficient performance systems, MLP and RBF neural network model were used. As lower error value was obtained by using the hyperbolic tangent and sigmoid activation function, this type of function was selected as the activation function in the hidden layer and the output. Based on the test method, 70% of the data were used for training and the network could learn the relationships between inputs and outputs well and 20 % of the data were used to test the network and 10 % of the data were used to Cross Validation network. The value of Mean squared error, Normalized Mean squared error, Mean absolute error, Correlation coefficient are shown Table 4.The results showed that best neural network for 4 neurons in the hidden layer was in tangent hyperbolic activation function and MLP network for power consumption (R = 0.991-MSE=91.419), and for Coefficient performance systems (R= 0.9832 - 0.0003) and best value for electrical conductivity (R = 0.9164 – MSE=0.0072) was in RBF network with tangent hyperbolic and sigmoid. Also for neural network with 8 neurons in hidden layer, Sigmoid activation function and MLP network have best amount for power consumption (R = 0.99832-MSE=3.5E+1), electrical conductivity (R = 0.9963 – MSE=3.1E-4) and Coefficient performance systems (R= 0.99963 -7.8E-5). In neural network with 12 neuron in hidden layer were best amount R=0.9996, 0.9782, 0.999 for power consumption, electrical conductivity and coefficient performance systems in tangent hyperbolic, respectively and best amount for MSE were 18.800, 0.00089, 0.00001 respectability in MLP network and tangent hyperbolic tangent activation function. In total MLP network with 8 neuron in hidden layer and sigmoid activation function have best amount R and MSE for power consumption, electrical conductivity and coefficient performance systems. The results showed in table 4. According to MSE and R value, network 8 neuron in hidden layer was best network for predication power consumption, electrical conductivity and Coefficient performance systems value, because this network has lowest MSE and highest R. Table 5 shows the best network between input data and the data simulated by the network for each of the neurons in the hidden layer. Smaller epochs suggest that the number of neurons in the layer successfully learned by the neural network compared to other neurons. As shown in table 5, the fastest learning speed network for predicting data with sigmoid activation function and tangent hyperbolic were in network by 12 neuron in hidden layer and RBF network by 795 and 115 EPOCH and RUN 1 for training, respectability. Also according to result in table 5 all network created by RBF has Lowest EPOCH than MLP network. But according to result in table 4, lowest MSE and R was in MLP network, sigmoid and tangent

hyperbolic activation in 8 and 12 neuron in hidden layer respectively, so the best EPOCH and RUN are 1093-1 for 8 neuron in hidden layer and 795-1for 12 neuron in hidden layer. In total speed training for tangent activation function is highest than sigmoid activation function. Also result for cross validation showed in table 4 for data experiment. The results of the sensitivity analysis for power consumption, electrical conductivity and Coefficient performance systems are shown in Figure 2, 3, 4. Based on this figures, the highest sensitivity for training data were obtained for the Voltage gradin and weight loss percentage in the hidden layers with 8 neurons and sigmoid activation in MLP network and highest sensitivity process time and input current for electrical conductivity and Coefficient performance systems were in hidden layer 8 , 12 and hyperbolic tangent , sigmoid activitaion function and RBF , MLP, respectivity. overall, the voltage gradient sensitivity was higher than the other three inputs, meaning the voltage had a greater effect on power consumption, electrical conductivity and Coefficient performance systems. Also, the sensitivity coefficient of the process time and the input current are exactly the same for power consumption, electrical conductivity and Coefficient performance systems.

Table 5. Some of the best MLP and RBF neural network topologies to predict test value

Sigmoid							
12		**8**		**4**			
MLP	**RBF**	**MLP**	**RBF**	**MLP**	**RBF**		
795	695	4377	1093	4407	1934	Training	**EPOCH**
7	5	215	73	11	10	Cross Validation	
1	1	1	1	5	1	Training	**RUN**
4	1	1	3	5	5	Cross Validation	
Tangent hyperbolic							
12		**8**		**4**			
MLP	**RBF**	**MLP**	**RBF**	**MLP**	**RBF**		
157	115	4999	457	5000	500	Training	**EPOCH**
32	21	25	6	15	7	Cross Validation	
1	1	1	1	4	1	Training	**RUN**
3	4	3	2	4	3	Cross Validation	

Table 4. Error values in predicting experimental data using optimal artificial neural network

Power consumption													
R			**MAE**			**NMSE**			**MSE**				
12	**8**	**4**	**12**	**8**	**4**	**12**	**8**	**4**	**12**	**8**	**4**		
0.9534	0.99832	0.9815	14.97	4.7526	16.1328	0.1019	8.2E-03	0.0978	481.45	3.5E+01	473.2174	S	MLP
0.91236	0.9690	0.9636	29.65	10.37	17.24	0.863	0.0639	0.0845	580.65	309.14	413.43		RBF
0.99683	0.99288	0.991	2.519	4.84	6.636	0.00649	0.02	0.019	18.800	78.30	91.419	T	MLP
0.9822	0.9827	0.9655	14.01	7.3861	16.2073	0.0812	0.0370	0.0855	279.28	107.7434	388.5924		RBF
Electrical conductivity													
0.8215	0.99633	0.9157	0.0762	0.0117	0.0696	0.3808	1.1E-02	0.1937	0.0116	3.1E-04	0.0077	S	MLP
0.7936	0.9465	0.8258	0.156	0.0579	0.0838	0.456	0.1222	0.3323	0.0793	0.0050	0.0130		RBF
0.97824	0.94909	0.883	0.01512	0.04	0.076	0.04368	0.10	0.227	0.00089	0.0036	0.009	T	MLP
0.5661	0.9088	0.9164	0.2582	0.0589	0.0670	4.7372	0.2098	0.1911	0.0864	0.0059	0.0072		RBF
Coefficient performance systems													
0.97813	0.999750	0.9799	0.01632	0.0059	0.0144	0.04714	7.7E-03	0.0403	0.00044	7.8E-05	0.0004	S	MLP
0.9336	0.9936	0.9832	0.0296	0.0093	0.0143	0.089	0.0133	0.0357	0.00245	0.0001	0.0003		RBF
0.99963	0.98228	0.973	0.00187	0.01	0.020	0.00077	0.04	0.060	0.00001	0.0002	0.001	T	MLP
0.9916	0.9851	0.9375	0.0228	0.0122	0.0265	0.0822	0.0314	0.1229	0.0008	0.0003	0.0010		RBF

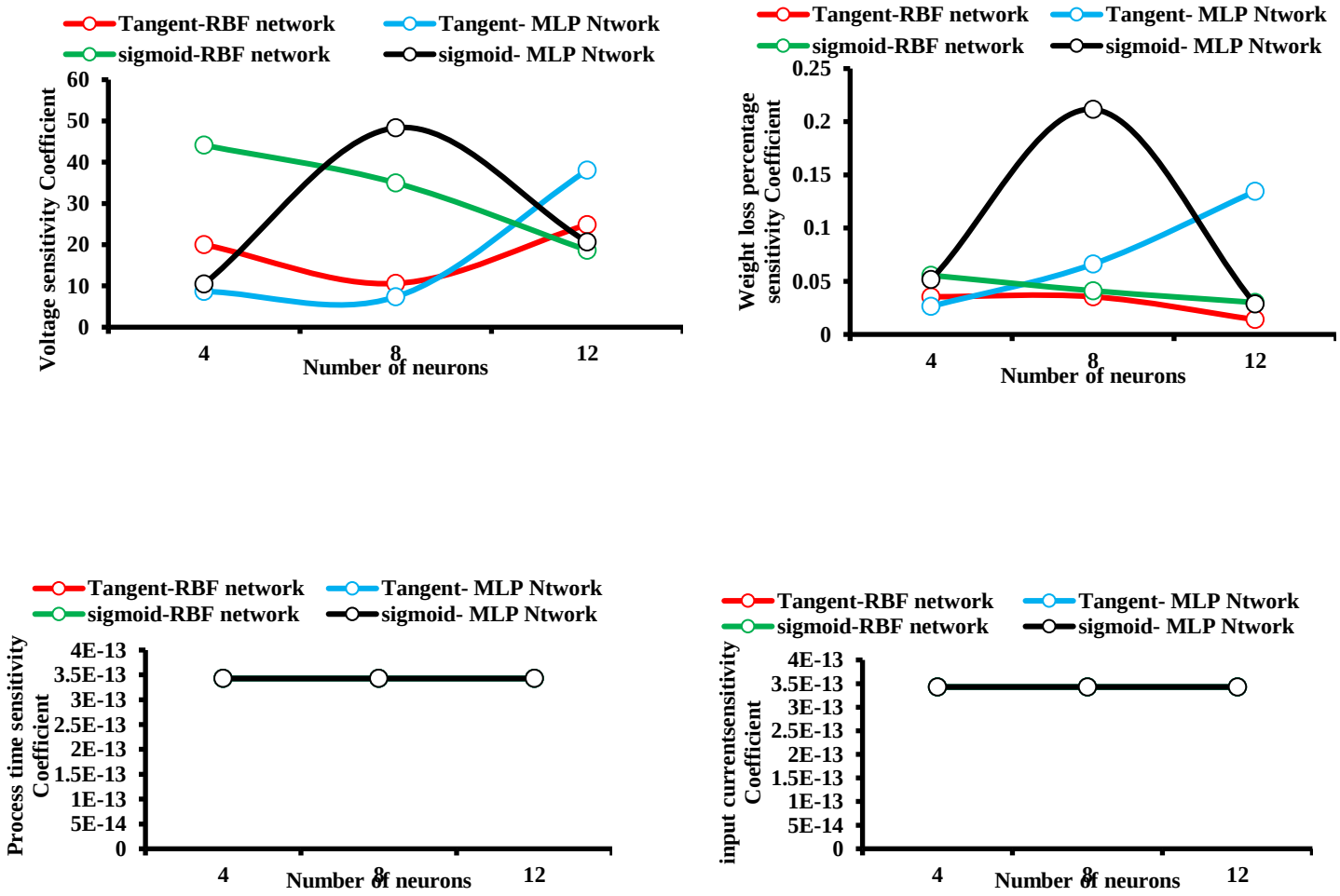

Figure 2. Sensitivity coefficient power consumption

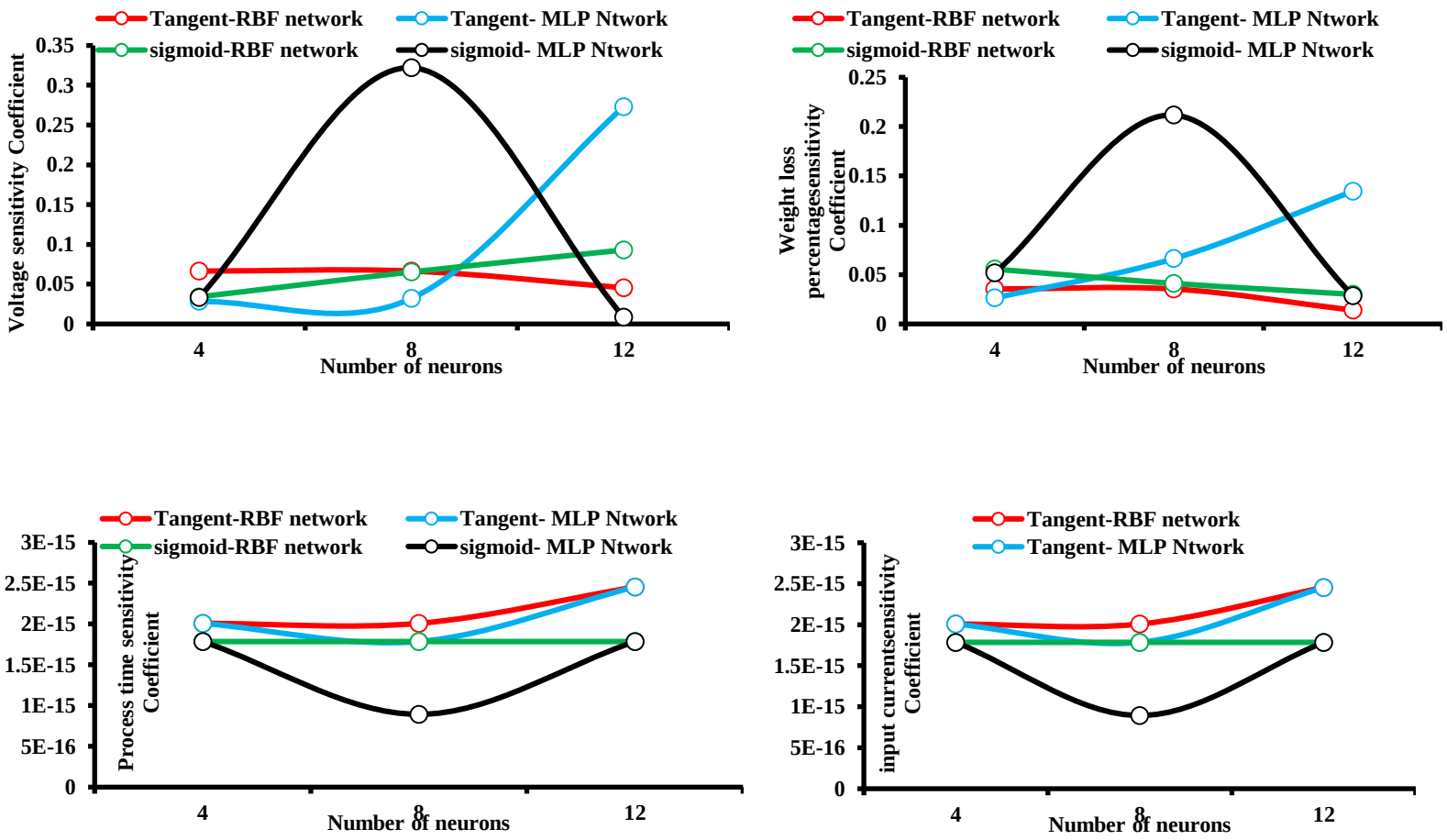

Figure 3. Sensitivity coefficient conductive electrical

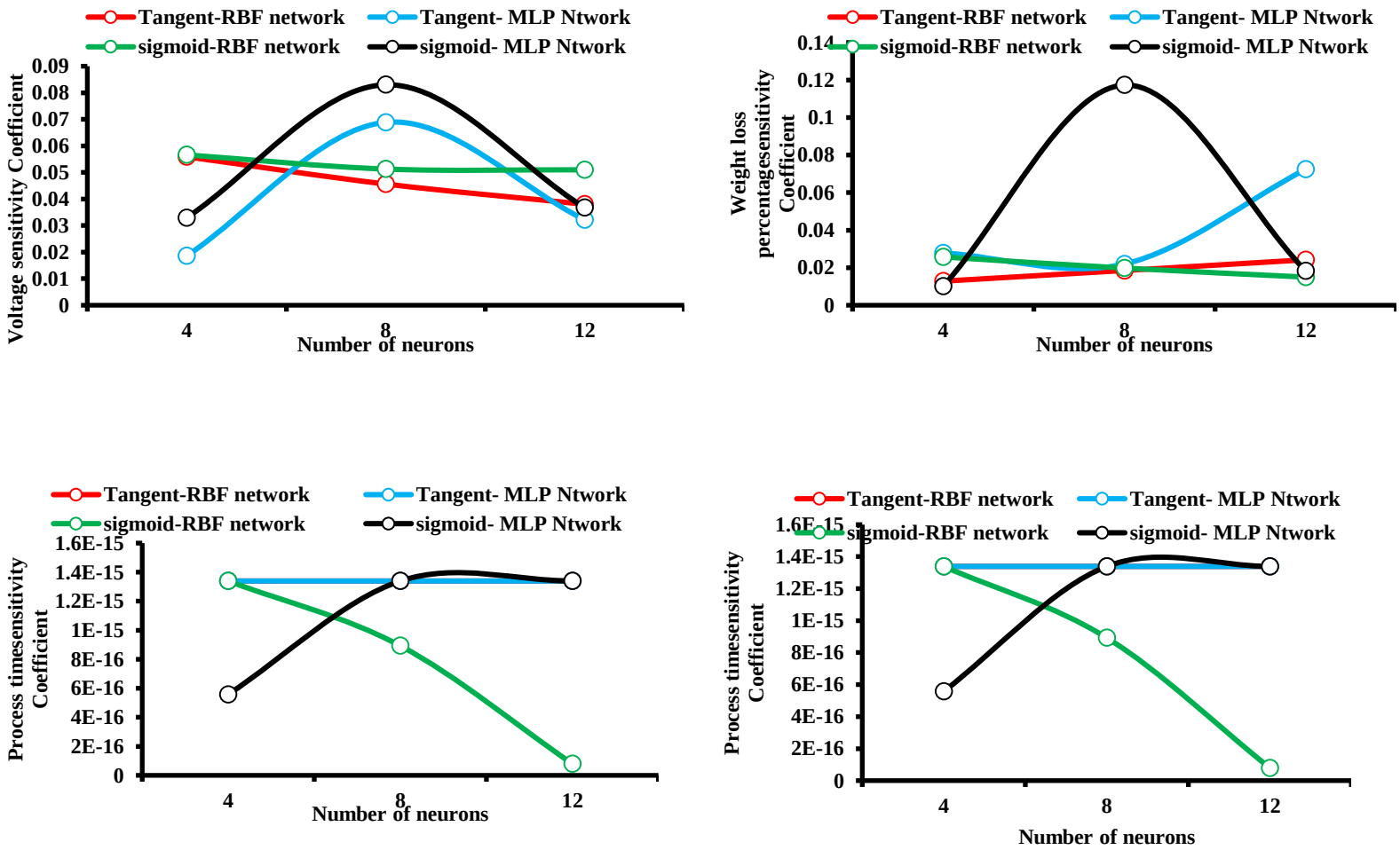

Figure 4. Sensitivity coefficient, coefficient performance systems

4.Conclusion

For power consumption, electrical conductivity and performance system, the best R value in the MLP network with 8 neurons in the hidden layer was the sigmoid activation function, But for power consumption and system efficiency, Sigmoid activation functions and tangent have been able to show R values in RBF and MLP networks, These values were good for all three numbers of input neurons for the network. But for electrical conductivity, the network with 12 neurons, and especially the RBF network, has not shown satisfactory results.

For power consumption, electrical conductivity and performance system were the lowest MSE in a network of 8 neurons, The MSE values for both the hyperbolic and sigmoid tangency activation function were lower for both the MLP and RBF networks than for the two networks with 4 and 12 neurons, which suggests a better formation of the network with 8 neurons.

According to the results of the network learning speed, as the number of neurons in the hidden layer has increased, the speed of network learning has increased to simulate data, and the fastest network with lowest EPOCH was in a network of 12 neuron. Also, the hyperbolic tangent activation function has a faster speed in network training than sigmoid activation function.

The sensitivity coefficient for the Voltage gradient relative to the other parameters of the network input has a greater effect on the power consumption, the electrical conductivity coefficient, and the coefficient performance of the system.

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TEA WITH HERBAL ADDITIONS: THEIR ANTIOXIDANT ACTIVITY AND ITS DEPENDENCE ON HIGH PRESSURE PRE-TREATMENT BEFORE EXTRACTION

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ABSTRACT
The antioxidant effects of developed tea drinks on the lipid peroxidation in rats' blood during swimming stress were investigated by malondialdehyde and conjugated dienes levels' measurement. Components of tea drinks from common Ural plants were selected based on their antioxidant action, contributing to prevention of oxidative stress, as well as high consumer properties. Developed tea drinks have high organoleptic characteristics, high content of flavonoids and high antioxidant activity. It was established that the use of developed tea drinks in the diet of rats can reduce the stress impact on the rat organism on biochemical and morphological levels. Processing of developed blends with high pressure (200 MPa, 60 s) significantly intensifies the subsequent yield of biologically active substances during extraction and the antioxidant activity of the extract. Good prospects of using common Ural plants for the development of tea drinks formulations with strong antioxidant effect are shown in our study.

1. Introduction

Tea and tea drinks are the most widely consumed beverages in the world and they are also an important agricultural product in many countries. A number of researchers have found that tea possesses a wide range of beneficial effects including depression of hypertension, reduction of cholesterol, anti-oxidation and anti-cancer activities (Kris-Etherton, Keen, 2002; Feng et al., 2016; Ganguly, 2017). These effects are commonly attributed to such chemical ingredients in tea leaves, as polyphenols, polysaccharides, alkaloids and amino acids, etc. (Nie & Xie, 2011; Wang et al., 2012).

Earlier it was generally believed that only green tea prepared by dehydration of *Camellia sinensis* leaves, which contain monomeric polyphenols, possesses antioxidant properties (Graham, 1992). Recent investigations, however, showed that black tea obtained by fermentation of tea leaves and containing only a small amount of catechins and theaflavins and thearubigins--whose biological activities are less studied, also has antioxidant activity (Frei & Higdon, 2003). As a result, black tea has been proved to protect against cancer, heart and other diseases (Alferink et al., 2017; Feng et al., 2016; Singh et al., 2017; Sur & Panda, 2017). There are some data about the similar effect of different herbal teas (Tan et al., 2016; Shannon et al., 2017). However, the biological activity of black and herbal tea as a source of antioxidants required further investigation.

Therefore, scientists have searched for potent antioxidants, especially among natural products. One such potentially health-promoting beverage is herbal tea drinks or tea with addition of natural plants. It was found that some herbal

teas, which are a hot water infusions of dried or fresh plant parts: roots, leaves and fruits had excellent antioxidant capacities as compared with black tea (Dalar & Konczak, 2013; Deetae et al., 2012; Jin et al., 2016). Thus, it was found that is a kind of important resource worth of exploitation and utilization. Further studies showed that antioxidant properties and organoleptic parameters significantly varied among herbal teas and that total phenolic content in herbal teas was highly positively correlated with their antioxidant capacities (Qasim et al., 2017; Carabajal et al., 2017). So, these beverages could be important sources of antioxidant and antimutagenic compounds. The developed beverages from different herbs of different countries could be important dietary sources of antioxidant and antimutagenic compounds for prevention of chronic diseases (Carabajal et al., 2017; Kapepula et al., 2017).

On the other hand, it was found that most of herbal teas had lower antioxidant property values than green tea (Chan & Wong 2015; Pardau et al., 2017). It is known that extraction of bioactive compounds from plants strongly depends on the solvents used (Bhebhe et al., 2016) and extraction methods such as mechanical grinding, ultrasonic-assisted extraction (Wei & Yang 2015; Vinatoru et al., 2017), microwave-assisted extraction (Vinatoru et al., 2017), and also sample pretreatments using acid and alkali on the microstructure of plant sample (See et al., 2016). One of such promising extraction method which can intensify transition of biologically active substances in the extract from row material is the High Pressure Processing (HPP) (Balasubramaniam et al., 2015; Wang et al., 2016). Applying high-pressure processing can inactivate pathogenic and spoilage microorganisms, as well as modify structures with little or no effects on the nutritional and sensory quality of tea (Wang et al., 2016). Application of high-pressure reduces the thermal exposure of the food during processing; therefore protect a variety of bioactive compounds and can increase the extraction rate of these compounds from tea and herbs (Jun et al, 2011).

So, the aim of this study was to investigate antioxidant compounds extraction using conventional and HPP methods from different tea drinks made from Ural plants and the influence of these extracts on antioxidant activity in vivo.

2. Materials and methods

2.1. Plant materials

A number of plants were selected for study based on availability and popularity in the areas where research was being conducted (Yekaterinburg, Russia). This selection process was carried out on the basis of previous articles (Pastushkova et al., 2015) with the assumption that teas that are widely available and popular will represent an accurate cross-section of plants being consumed by the local population.

We used following dried plant raw materials for the study: stinging nettle (*Urtica dióica*), peppermint (*Mentha piperita L.*), Origanum ordinary (*Oríganum vulgáre*), Salvia officinalis (*Salvia officinalis*), Yarrow ordinary (*Achilléa millefólium*), Hypericum perforatum (*Hypéricum perforátum*), Thyme (*Thymus serpyllum L*), cowberry leaf (*Vitis idaeae* folia), black currant leaf (*Nigrum ribes* folia) and cherry leaf (*Cerasus* folia). Plant materials were harvested in 2015-2016 in ecologically clean areas: Nizhneserginsky and Shalinsky areas. Plant leaves from these locations of Ural region were washed and dried at ambient temperature for 7 days.

The end of drying process was determined by the following features: leaves, leaf stalks and grass stems should be without excessive fragility, the mass fraction of moisture should not exceed 14%. Next, the raw material was brought to a state that corresponds to the standard: parts that have lost their natural color, crushed parts, accidentally trapped impurities were removed

2.2. Extraction and pressure treatment

The technology used in our work for producing of tea drinks with addition of Ural

plants consists in the joint processing of plant materials, which is based on black tea «Greenfield. Classic Breakfast» and plant components, which were placed in a blending drum and mixed at a rotation frequency of 4-5 rpm within 5-6 minutes and their packaging in hermetic vacuum package (Patent RU2462873C1, 2012). Obtained doses for one-time brewing and packaged compositions of tea drinks with the addition of Ural plant materials were treated with a high pressure of 100-200 MPa for 60-90 seconds. Tea drinks were placed in a high-pressure chamber in vacuum packaging and then the working chamber was filled with liquid (drinking water) to capacity and sealed. Then the required pressure was applied to high-pressure chamber.

2.3. Sample preparation

Each tea bag packaging of samples was brewed in a pot made of ceramic materials in 200 ml of mineral water with 85-95 ºC temperature for 5 minute without closed (Sharpe et al., 2016), and was used for experiments within 20 minutes after brewing.

2.4. Organoleptic measurements

During organoleptic characteristics of tea drinks such indicators as tea drinks taste, aroma, aftertaste and infusion were measured according to Russia standard GOST 32572-2013.

All experiments were conducted at one study site (Ural State University of Economics, Yekaterinburg) with seven judges. Ethical approval was obtained from the University ethical panel. Judges used three-time replicate sets of samples, with each set being completed within one session with rest periods between sessions. All judges were well-experienced. For all experiments, judges were asked to rate each tea drinks sample on a 0 to 5 scale.

2.5. Animals

Adult Wistar rats of either sex (8 weeks of age, weighing 190±10 g, mean±SEM) were used in experiments. Rats were housed under controlled environmental conditions (12-h light/dark cycle and temperature 20 °C) in cages. Food and water were available *ad libitum*. The project was reviewed and approved by the university animal care review committee. All procedures were in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the Order of the Ministry of Health of the Russian Federation “On the approval of rules through laboratory practice" No. 267 on June 19, 2003”.

2.6 Experimental design. Swimming protocol

Rats were divided into three groups of 10 animals each as follows:

(a) The control group was treated intragastrically with 10 ml of physiological saline every day for 3 weeks (n = 10),

(b) The stress group was treated intragastrically with 10 ml of physiological saline every day for 3 weeks prior to stressing through the probe (n = 10), exercised for last 5 days and killed immediately after last exercise,

(c) The stress+tea group was treated intragastrically with 10 ml of black tea or tea drinks in a dose of 10 ml per animal every day for 4 weeks (n = 10), exercised for last 5 days and killed immediately after last exercise.

Stress was modeled by chronic forced swimming stress according to (Michailidis et al., 2007). The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37X37X30 cm), filled with water to a height of 25cm. The duration of exercise was about 45 minutes a day for 5 days at a water temperature of 27–28°C.

2.7. Blood and tissue sampling

All animals were deeply anesthetized with chloral hydrate and euthanized by decapitation at the end of experiment. Samples of thymus and right adrenal gland were collected immediately after decapitation. Adrenal glands were also dissected out at this time.

Blood samples from the control and experimental rats were collected into heparinized tubes after decapitation (Nayanatara et al., 2005).

2.8. Lipid peroxidation measurements

Lipid peroxidation was assayed by spectrophotometric measurement of conjugated dienes at 234 nm (Recknagel and Glende, 1984) and by malondialdehyde (MDA) measurement as malondialdehyde-thiobarbituric acid adducts (Londero & Greco, 1996). MDA in serum was separated by connecting with thiobarbituric acid and serum proteins were precipitated by TCA centrifugation. Then, TBA complex was measured at a wavelength of 534 nanometers.

2.9. Antioxidant activity measurements

For preliminary measurement of potential antioxidant activity (potential AOA) of herbal extracts a potentiometric method was used. The value of antioxidant activity was measured as the redox potential difference of the $K_3[Fe(CN_6)]/K_4[Fe(CN_6)]$ mediator system using an antioxidant activity measuring device (IVA Co. Ltd., Yekaterinburg, Russia) (Brainina et al., 2007).

2.10. Flavonoids measurements

The total flavonoid content in the tea was determined by a method described by Matyuschenko & Stepanova (2003). The flavonoid content was calculated from a calibration curve using rutin as reference standard. Flavonoid content was expressed as g of ruthin equivalents/100g of tea leaves (g RE/100g). Measurements were conducted in triplicates.

2.11. Statistical analysis

Data are reported as mean values with standard deviation. Statistica 8.0 software was used for data analysis. *P* values less than 0.05 were considered statistically significant.

3. Results and discussions

3.1. Development of tea drinks based on common Ural plants

Based on the literature data about the pharmacological properties of Ural herbs and preliminary results of organoleptic compatibility studies (Pastushkova et al., 2015), we selected the most promising plants for use in herbal teas. Due to industrial production requirements, among the most significant selection criteria we used plant availability and best organoleptic characteristics of the plant (color and transparency of infusion, aroma and the influence on herbal tea taste).

Among selected herbs, the content of flavonoids and ascorbic acid, as well as their potential antioxidant activity (potential AOA) according to Brainina et al., (2007) were studied (Table 1). Black tea («Greenfield. Classic Breakfast»), which usually used as a main part of tea drinks, was used as a control.

Table 1. The content of biologically active substances in dry plant materials of common Ural plants (mean±SD**)**

Plant raw material	**Ascorbic acid, (mg/100 g)**	**Total flavonoids, (g RU/100 g)**	**Potential AOA, (equiv/l)**
Urtica dioica	3,40±0,04	0,20±0,03	8,53±0,41
Méntha piperíta	12,13±0,37	0,31±0,03	4,87±0,15
Oríganum vulgáre	5,65±0,04	0,41±0,02	4,91±0,10
Salvia officinalis	5,34±0,01	0,12±0,01	6,73±0,35
Achillea millefolium	1,70±0,02	0,34±0,03	2,67±0,15
Hypericum perforatum	6,58±0,03	0,16±0,02	3,84±0,15
Thymus vulgaris	1,89±0,04	0,29±0,01	5,38±0,20
Vaccinium vitis-idaea (folia)	1,41±0,03	0,11±0,02	2,47±0,15
Ríbes nígrum (folia)	3,20±0,01	0,50±0,03	2,16±0,10
Prunus cerasus (folia)	2,10±0,05	0,10±0,03	2,13±0,10
Camellia sinensis (black tea)	1.11+0.03	0.11+0.01	4.90+0.14

According to the data obtained, we can assert that at all plants studied ascorbic acid content was higher than in black tea (*Camellia sinensis)*. Especially high it was in *Méntha piperíta*, Hypericum perforatum, *Oríganum vulgáre*, *Salvia officinalis* and *Urtica dioica*. Total flavonoids content was highest in *Ríbes nígrum* (folia), *Oríganum vulgáre*, *Achillea millefolium*, *Méntha piperíta* and *Thymus vulgaris* and in all of them it was higher, then in *Camellia sinensis*. In *Urtica dioica*, *Salvia officinalis* and *Thymus vulgaris* potential antioxidant activity higher, than in black tea (*Camellia sinensis)* was detected.

Therefore, we can suppose, that addition of this medicinal raw material can significantly increase the potential antioxidant activity of tea drinks due to the high content of antioxidants of different nature. Thereby, based on these data and on the organoleptic compatibility of tea drinks components, a number of herbal tea compositions were developed (Table 2).

Table 2. The composition of tea drinks with addition of plant materials

No.	**Composition**	**Content of components,%**
No. 1	*Prunus cerasus* (folia); *Ríbes nígrum* (folia); *Camellia sinensis* (black tea)	2,8 : 10,6 : 86,6
No. 2	*Urtica dioica*; *Hypericum perforatum*; *Salvia officinalis*; *Camellia sinensis* (black tea)	2,8 : 1,2 : 0,5 : 95,5
No. 3	*Urtica dioica*; *Hypericum perforatum*; *Vaccinium vitis-idaea* (folia); *Camellia sinensis* (black tea)	4,8 : 5,2 : 2,5 : 87,5
No. 4	*Oríganum vulgáre*; *Achillea millefolium*; *Thymus vulgaris*; *Camellia sinensis* (black tea)	2,5 : 10,5 : 1,6 : 85,4
No. 5	*Urtica dioica*; *Méntha piperíta*; *Ríbes nígrum* (folia); *Camellia sinensis* (black tea)	5,9 : 5,3 : 5,7 : 83,1
No. 6	*Oríganum vulgáre*; *Achillea millefolium*; *Salvia officinalis*; *Vaccinium vitis-idaea* (folia); *Camellia sinensis* (black tea)	4,8 : 13,9 : 2,8 : 2,9 : 75,6

These tea drinks has increased ascorbic acid content (up to 75% to black tea at No 5) and increased total flavonoids content (up to 40% to black tea at No 6). So, we developed a number of tea drinks from common Ural plants. The high content of natural antioxidants in herbs used allows us to achieve an increase of antioxidant activity using minimum dosage of added plant materials: the proportion of added in various combinations to black tea plant materials is not more than 15% by dry weight. It allows us to save consumer and organoleptic properties of tea drinks developed.

3.2. Influence of tea drinks on lipid peroxidation during swimming stress in rats

It is well known that green tea consumption had no effect on basic physiological parameters of rats (Alessio et al., 2002). It also had no effect on the basic rate of lipid peroxidation in different organs in non-stressed rats (Alessio et al., 2002). On the other hand, it has antioxidant activity *in vitro* and *in vivo* during swimming stress (Alessio et al., 2002) and this effect also known to other herbal drinks (Kothiyal & Ratan, 2011). So, we study the influence of consumption of 6 developed tea drinks on rate of lipid peroxidation in rats during stress.

The study of influence of developed tea drinks consumption on rate of lipid peroxidation in rats' blood showed that in animals consuming water and subjected to swimming stress ("stress" group) level of MDA content in blood significantly increased after acute exercise as compared to control (Figure 1A), wherein MDA levels in animals consuming black tea before stress did not significantly differ from those in stressed variant. These results differed from

those obtained with animals that consumed tea drinks № 1- №6 (1 - 6 groups) in which swimming stress did not caused such significantly change in levels of MDA content from non-stressed variant (Fig. 1A), sowing protection against oxidative stress.

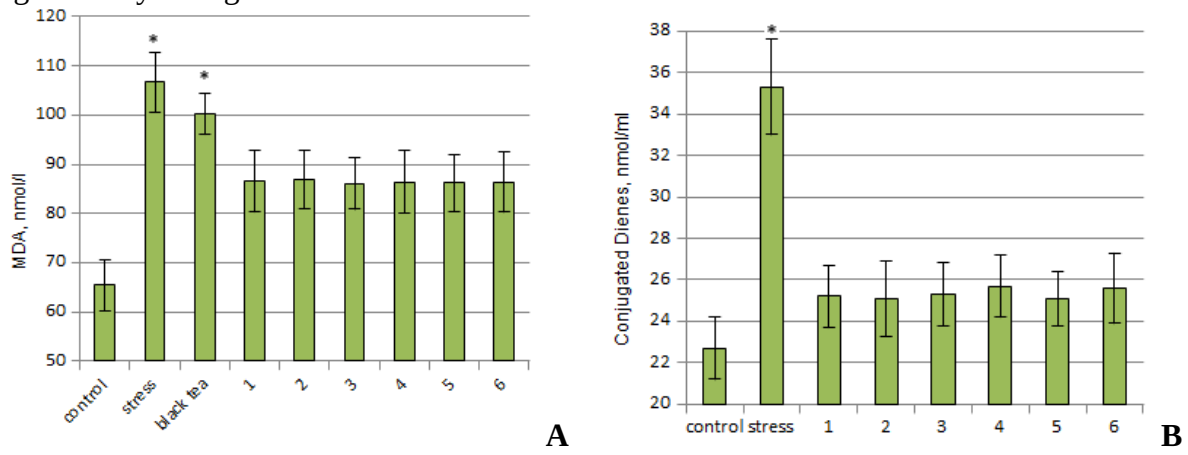

Figure 1. The influence of different tea compositions on MDA (A) and conjugated dienes content (B) in rats' blood during swimming stress.
* P < 0,05 compared to control non-stressed group

It is interesting to note that there was not any statistically significant difference between the influences of all tea drinks developed on the level of MDA content in blood during swimming stress (Figure 1A).

Conjugated dienes content in rat's blood showed even brighter picture (Figure 1B). If difference in MDA levels between "control" non-stressed group and groups which consumed tea drinks № 1- №6 during stress was statistically significant, the difference in conjugated dienes content between these groups was not statistically significant at all.

So, based on these data and the data about the organoleptic quality of 6 tea drinks developed, tea drink № 4 which has the best organoleptic indicators was chosen for the further experiments.

Evaluating the data obtained in general, we can say that the data about the influence of tea drinks consumption on rate of lipid peroxidation in rats' blood are in accordance with the data of about the influence of green tea on decrease of malondialdehyde level after green tea intake (Yokozawa et al., 1999, 2002) and the data of about the antioxidant effect of different herbal teas (Tan et al., 2016; Shannon et al., 2017).

3.3. Influence of tea drinks on morphological changes in rats during swimming stress

It is known that swimming stress cause changes in the biochemistry and even structure of many internal organs in rats (Avital et al., 2001), as particular in thymus mass (Živkovića et al., 2005) and in adrenal glands (Nayanatara et al., 2005). On the other hand, it is known that tea did not influence on structure and weight of internal organs of intact Ross broiler (Sarker et al., 2010). Therefore we studied the influence of tea drink № 4 consuming on stress-induced changes in these rats' organs. As a result of swimming stress, a significant decrease in the thymus mass in "stress" group was noted as compared to control (Figure 2A), in "stress+tea" group significantly less decrease of thymus mass was detected which showed good stress-protective properties of the tea drink tested. In addition, in animals of "stress" group the weight of the right adrenal gland significantly increased (Figure 2B). This hypertrophy of the adrenal cortex was observed due to the expansion of the beam zone as compared with control group. In the group of animals, which were given a tea drink ("stress+tea" group), the pathological

changes in the adrenal glands after stress were less pronounced. In addition, it is noted that stress caused multiple hemorrhages on the gastric mucosa of the stressed group of animals, which were not given a tea drink ("stress group") which were not detected in "stress+tea" group.

The data about protective action of herbal tea drink studied on thymus mass and adrenal gland showed that herbal tea drink consumption can show not only biochemical (Tan et al., 2016; Shannon et al., 2017) and physiological (Lindenmuth & Lindenmuth 2000) impact, but morphological impact on organism too.

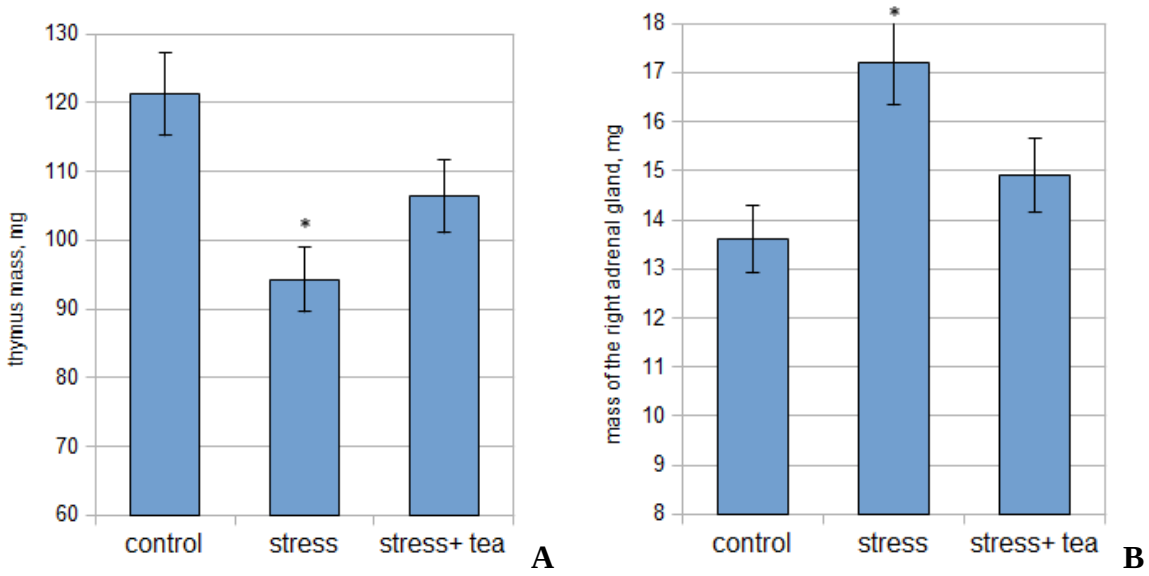

Figure 2. The influence of tea drink № 4 on mass of the right adrenal gland (A) and thymus mass (B) content in rats during swimming stress.
* P < 0,05 compared to control non-stressed group

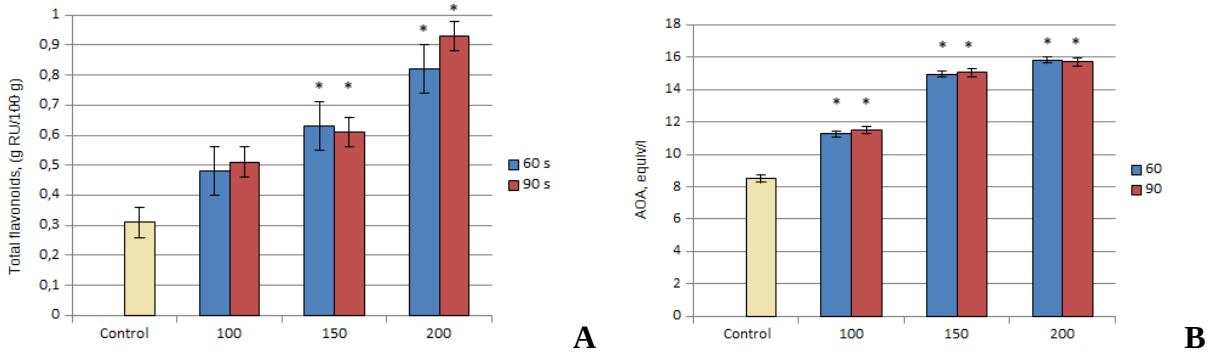

Figure 3. The influence of pressure (100, 150, 200 MPa) and treatment duration (60 and 90 secunds) on total flavonoids content (A) and potential AOA (B) in extracts of tea drink No. 4
* P < 0,05 compared to non-treated control Similar changes these processing caused in AOA. The highest AOA (15.82 mol eq. / l) was observed when a plant raw material was processed at pressure 200 MPa for 60 s. Further increase of treatment time did not cause any AOA increase in extract.

3.4. Influence of High Pressure Processing on the antioxidant features of tea drink

The next part of our research was to study the influence of High Pressure Processing of dried raw plant material on the antioxidant features of tea drink № 4 - total flavonoids content and antioxidant activity.

From the data of Figure 3 one can see, that the processing of tea drinks with high pressure significantly increase extraction of biologically active substances. In the samples of developed tea drinks, the content of Total flavonoids under pressure treatment 100 MPa for 60 s was about 40% higher, than in control, under pressure treatment 150 MPa for 60 s was about 100% higher, than in control and under pressure treatment 200 MPa for 60 s was about 130% higher, than in control sample (Figure 3A). The increase in the time of high pressure impact on plant materials does not lead to a significant increase in the intensity of extraction of Total flavonoids to infusion. On average, the difference in the amount of Total flavonoids, extracted into the drink during 60 or 90 seconds, was not statistically significant.

Therefore, one can say that the treatment of plant materials with a pressure of 200 MPa for 1 minute allows maximize the yield of biologically active substances and antioxidant activity in comparison with traditional technology.

The study of the organoleptic factors of tea drink with the addition of plant materials № 4 showed that, irrespective of the technological regimes, they correspond to the quality indicators. Taste and aroma were pronounced, well-coordinated, with pleasant, harmonious aftertaste and infusion was transparent.

The data about the influence of High Pressure Processing on the antioxidant features of tea drink showed, that this treatment can not only inactivate pathogenic and spoilage microorganisms or can intensify extraction of biologically active substances from fresh material (Balasubramaniam et al., 2015; Wang et al., 2016) bat can be used as pre-treatment of dried plant material with the aim of intensifying of biologically active substances extraction from it.

4.Conclusions

So, formulations and processing technology of tea drinks using some common Ural plants dried material are developed. Developed tea drinks have high organoleptic characteristics, high content of flavonoids and high antioxidant activity. It was established that the use of developed tea drinks in the diet of rats can reduce the stress impact on the rat organism on biochemical and morphological levels. Processing of developed blends with high pressure (200 MPa, 60 s) significantly intensifies the subsequent yield of biologically active substances during extraction and the antioxidant activity of the extract.

The study of the organoleptic characteristics of tea drinks with the addition of plant materials showed that, irrespective of the technological regimes, they correspond to the regulated indicators. Taste and aroma were pronounced, harmonious, aftertaste was pleasant, harmonious and infusion was transparent.

Therefore, good prospects of using common Ural plants for the development of tea drinks formulations with strong antioxidant effect are shown in our study. Moreover, because of the fact, that some of the common Ural plants studied are common for whole Europe, these formulations can be interesting not only in the investigated region.

5. References

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PHYSICOCHEMICAL CHARACTERISTICS, FATTY ACID COMPOSITION, AND FUNCTIONAL PROPERTIES OF THE TRADITIONAL SALTED DRIED MEAT OF *CAMELUS DROMEDARIUS* FROM ALGERIAN EASTERN SAHARA: "EL KADID"

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ABSTRACT

This is the first report describing physicochemical characteristics, fatty acid composition, and functional properties of traditional salted dried meat *El Kadid* produced from *Camelus dromedarius*. The samples were prepared according to the traditional Saharan method. The pH values ranged from 5.01±0.01 to 5.97±0.06; water activity ranged from 0.684±0.003 to 0.689±0.002; ash content ranged from 2.25±0.08 to 2.85±0.02%; moisture level was < 1%, and dry matter content exceeded 99%. The acid index, peroxide index, and acidity were in the range of 13.76 ± 0.14 to 20.66 ± 0.12 mg $KOHg^{-1}$, 0.45 ± 0.03 to1.0 $meqkg^{-1}$, and 0.16 ± 0.01 to 1.80 ± 0.02%, respectively. Protein, fat, and salt content were 19.73–22.52%, 3.17–7.14%, and 23.37–57.86% respectively. 19 fatty acids were identified, The oleic acid C18:1 was the predominant monounsaturated fatty acid (1.80%–59.98%) and palmitic acid C16:0 was the major SFA (25.98%–48.31%).regarding functional properties, Water Absorption Capacity and Oil Absorption Capacity values varied between 2.42 ± 0.03 -5.30 ± 0.05 and10.34 ± 0.05 to 13.34 ± 0.05 mLg^{-1}respectively.

1.Introduction

The dromedary camel (*Camelus dromedarius*) is an animal whose services to the human kind can easily be underestimated even though its adaptability to the harsh climate of the desert is widely known. This (the dromedary or one-hump camel) is the most populous camel species in the world. The dromedary camel has unique physiological characteristics, including a high tolerance to temperature changes, solar radiation, water scarcity, rough topography, and poor vegetation (Suliman et al., 2016). Camel meat is an excellent source of many nutrients, especially amino acids, B vitamins, iron, and zinc (Geay et al., 2001). The proximate composition of camel meat is similar to meats from other species, witham inverse relationship between the moisture and protein and fat content. Besides from its importance in determining the nutritional value of the meat, the proximate composition is also an indicator of meat functionality (Kadim et al., 2006) as protein and fat content determine its manufacturing quality. Because of its potential high dietetic quality, camel meat might be particularly beneficial for human consumers

(Kadim et al., 2008). Fat is a vital nutrient with many functions in the human body (e.g., energy provider, carrier of fat-soluble vitamins, component of cell membranes, basic substance of hormones and second messengers). It is also important for sensory characteristics of food (e.g., flavor and texture), and therefore plays an essential role in meat quality. However, the dietary fat intake is also associated with health problems (Schmid,2011). Not only the ingested amounts but also the nature of the fatty acids (saturated vs. unsaturated fatty acids, n-6/n-3ratio, etc.) is pertinent to the health of human beings (Dilzer and Park, 2012).Different types of meat products are on offer, varying in their methods of preparation and preservation. *El Kadid* is a sun-dried product commonly found in North Africa and the Middle East. It has been consumed in Arabia since pre-Islamic times (Bekhit and Farouk,2013a). Large amounts of meat products are customarily prepared for the Eid-ul-Adha festival in Algeria; the excess has to be preserved for later consumption. Raw meat is prone to microbial contamination during its production and handling, which might result in serious illnesses. Therefore, it is crucial to preserve it promptly and in an appropriate manner to prevent spoilage. In Algeria, the most common name for dried meat is *El Kadid*; it's often prepared by the Northerners using sheep and cow meat and by Southerners, using camel meat. Drying is one of the oldest methods of meat preservation. The method reduces the water content to such an extent that the bacteria cannot grow on such products. There are some reports on the quality of meat obtained by drying, curing, or freezing in the developed countries. Unfortunately, there is no data on the quality of dried salted camel meat, *El Kadid*, produced in Algeria. The current study was conducted to evaluate the effect of traditional methods (sun drying and salting) on the nutritional and functional quality of this local camel meat product.

2. Materials and methods

2.1.Salted dried meat Samples

Nine fresh samples of camel meat were collected from different animals in Oued Souf region of Algeria and parts of thigh were used. El Kadid was prepared using a traditional method. After slaughtering, the muscles of thigh were washed under running water and μthen cut into strips of thickness and size (about 1 cm wide and 10-20 cm long). In a bowl, afterwards, other ingredients such as salt (6.0 g salt/100g of meat), pepper and olive oil were added to the strips of meat and then left overnight to macerate at 4 °C. Then we proceed with the drying phase, in which the strips of meat were left to dry on ropes under the sun between 9 am and 15 am every day, under a temperature of 30°C and humidity percentage of 67% for a period of about 10 days and periodically turned, in order to obtain a uniform drying of the product. The drying phase ends when the meat reaches a brown color and the right fibrous appearance. Finally, the strips of meat were cut into 2-3 cm long pieces and placed inside a sealed jar, stored at room temperature.

2.2. Physicochemical characteristics

2.2.1. pH

pH was determined using a pH-meter (HANNA HI 2210-Romania). The samples (5 g of dried salted meat each) were homogenized in 50 mL of distilled water. Suspension was filtered and the titration was performed using 0.1N hydrochloric acid solution (Carlo ERBA, Val de Reuil, France), with five drops of phenolphthalein (Riedel-de Haen, Germany) as an indicator (AOAC, 1995)**.**

2.2.2. Water activity

The value of the water activity (a_w) was determined using the Aqualab CX3-TE instrument (Labo-Scientifica, Parma, Italy), by placing appropriate quantities of sample into the spacecraft appropriate.

2.2.3. Salt content

Salt content was analyzed following the method published in Application Bulletin No. 130/2. Ten g of dried salted meat was

homogenized in 190 mL of distilled water. Two mL of 2M potassium chromate solution (Fluka, Almaty, Kazakhstan) was added to 50 mL of the mixture. The titration was performed using silver nitrate solution (0.1M) (Biochem Chemopharma, Montreal, Canada).

2.2.4. Moisture, dry matter, and ash content

Moisture, dry matter, and ash content were measured following the AOAC (2000) methods. Two-g *El Kadid* samples were dried at 105± 1 °C. Sample weight was monitored until its stabilization. Dry matter content was obtained after drying in the oven at 105°C ± 1°C. Then, the samples were transferred to a Thermolyne F48010-33 Muffle Furnace (Cole-Parmer,VernonHills,USA) and heated at 550°C to complete the combustion of carbon. Once the combustion residue became white, the sample was weighed to obtain the ash matter content.

2.2.5. Peroxide index

For the determination of peroxide index, one g of *El Kadid* sample was dissolved in acetic acid (Sigma-Aldrich)-chloroform (Biochem) solution (3:2 v/v). Then, the solution was added to 1 mL of saturated potassium iodide (Riedel-de Haen).The mixture was titrated with sodium thiosulfate solution (0.01N) (Enthone-OMI, Spain) in the presence of starch as an indicator. For the determination of acid index, two-g *El Kadid*sample was dissolved in diethylether-ethanol mixture (1:1 v/v) (Prolabo, Riedel-deHaen). The solution was titrated with 0.05M potassium hydroxide (Riedel-deHaen) in methanol (Biochem) using phenolphthalein as an indicator.

2.2.6. Fat content

Fat content was determined according to AOAC (2000), using anEV-16 Soxhlet extractor (Gerhardt, Bonn, Germany).The sample remaining after moisture determination was transferred to the fat extraction thimble, which was loaded into the chamber of the extractor. One hundred and fifty mL of petroleum ether (Sigma-Aldrich, Taufkirchen Germany) was added to the distillation flask of the device. The samples were extracted for 8 h over a water bath at 80°C. At the end of the extraction period, the thimble was removed from the device and most of the petroleum ether in the flask was distilled off. The petroleum ether was evaporated on steam bath at low temperature and was then dried at 100°C for 1 hour, cooled and weighed.

2.2.7. Protein content

Protein content was determined according to the AOAC method (2000). A 2 g sample of *El Kadid* was placed in a 250 mL Kjeldahl flask (Gerhardt) with 25 mL of concentrated sulfuric acid (Biochem Chemopharma, Montreal, Canada). The contents of the Kjeldahl flask were heated in the digestion chamber until the solution became clear. Then, the solution was cooled and diluted to 100 mL with distilled water. An aliquot of 5mL was taken for distillation. Forty mL of 40% sodium hydroxide (Biochem Chemopharma, Montreal, Quebec) was added to the distillation flask. The resulting ammonia was distilled into boric acid solution (Fluka chemicals, USA) until the color of the solution changed from bluish purple to bluish green. The contents of the boric-acid flask were titrated using the standard hydrochloric acid solution (0.01N) until the blue color disappeared.

2.3. Fatty acid profile

Fatty acid profile was determined following the method described in Arrete du 5 mai 1986 of the French Ministry of Commerce. *El Kadid* samples (0.2 g) were extracted by adding 4 mL of heptane (Prolabo BDH, France), and 0.1 mL of potassium hydroxide solution in methanol (2N) was added to the extract. The solution was stirred vigorously using a vortex (Stuart, UK) for 10 s. The solution was put to rest during 10 min to separate the clear layer of fatty acids from the cloudy aqueous layer. The upper, fatty acid layer was collected. The fattyacid solution was injected into a GCMS-QP2010 gas chromatograph (Shimadzu, Kyoto, Japan). We employed anon-polar capillary column SE-30 with a diameter of 0.25µm, temperature 180°C (or a gradient from 170 to 200 °C), FID detector, heptane as a solvent, dimethylpolysiloxane as

SE-30stationary phase, and helium as the mobile phase.

2.4. Mineral and heavy metal analysis

Mineral analysis was carried out according to the method of Faleye and Fagbohun (2012). Each ash sample was placed in1 mL of pure hydrochloric acid, and 10 mL of distilled water was added. The solution was heated for 5 min until complete dissolution, and then distilled water was added to the volume of 100 mL.The levels of lead, chromium, zinc, iron, copper manganese, and cadmium were examined using AA-6200 atomic absorption spectrophotometer (Shimadzu).

2.5. Functional properties

Foaming and emulsion capacity values were determined following the previously described methods (Yasamatsu et al.,1972). Hygroscopicity was obtained using the method described by Bhatty (1988). Five g of *El Kadid* sample was exposed to the temperature of 31°C and humidity of 64%. Hygroscopicity was expressed as an increase in the weight of samples (percent) after 48 h of exposure. Oil and water absorption capacity (OAC and WAC) were determined according to Beuchat (1977). Samples (1 g) were mixed with 10 mL of distilled water or oil for 30 sec, left at room temperature for 30 min, and centrifuged at 5000 × g for 30 min (using HettichEBA 20 centrifuge). The volume of the supernatant was measured in a 10-mL graduated cylinder. The density of water was assumed 1 g mL^{-1} and of oil, 0.911 g mL-1.

2.6. Statistical analysis

In our study the data analysis was performed with SPSS software (version 20.0) [SPSS Inc., France]. The results obtained for three replications were subjected to analysis of variance (ANOVA: Analysis of Variance at a Factor). At $P < 0.05$, the difference between samples was considered significant.

3.Results and discussions

3.1. Physicochemical characteristics of *El Kadid*

The acidity, pH, water activity, peroxide, and acid indices are presented in Table 1. We found that pH values varied between 5.01± 0.01 and 5.97 ± 0.06($P<0.05$). Kadim et al. (2006) have reported that pH of fresh camel meat ranges between 5.7 and 6.0. The pH of *El Kadid* after salting and drying process is due to the alkalinization caused by the addition of salt. Water activity was in the range of 0.684 ±0.003 to0.689± 0.002 ($P>0.05$).Acid values of *El Kadid* samples ranged from 13.76± 0.14 to 20.66± 0.12 mg KOH g^{-1} of meat ($P>0.05$). These high acid values could be attributed to lipolysis by lipolytic microorganisms or to lipase activity naturally present in meat. The examination of the acid values has been used to assess the degree of fat alteration (Amon et al. 2009). The peroxide index values varied from 0.45± 0.03 to 1.00 meq kg^{-1} of meat ($P<0.05$). The polyunsaturated fatty acids are very sensitive to oxidation and create peroxides. Susceptibility of lipid peroxidation in food depends on the lipid composition, the presence of prooxidants and antioxidants, oxygen levels, temperature, light and processing methods. PUFA-rich foods are more susceptible for lipid oxidation. Likewise, presence of prooxidants such as redox active metals (Fe, Cu) and hemeproteins, exposure to high oxygen levels and high temperature may accelerate oxidation process. Lipid oxidation often brings problems in food processing and storage. Oxidation of PUFAs produces a complex mixture of volatile secondary oxidation products, and these cause particularly objectionable off-flavors, lipid oxidation may reduce the nutritional value by causing the destruction of essential fatty acids and the lipid-soluble vitamins A, D, E, and K as well as the decrease in caloric content. Free radicals and metabolites formed during oxidation may exert adverse effects on human health (Tao,2015). The low levels of this type of fatty acids in our samples justify the obtained peroxide index values.

Moisture, ash, dry matter, fat, protein, and salt content are shown in Table 2. The level of moisture in *El Kadid* samples varied between 0.28 ± 0.09 and 0.84 ± 0.02% (P <0.05). Ash content was in the range of 2.25 ±0.08 to2.85± 0.02% (P <0.05). The samples contained 99% of dry matter (P <0.05). The moisture content of fresh camel meat is approximately 73% (Kadim and Mahgoub, 2013). The *El Kadid* contains very little moisture (< 1%) due to the drying process (evaporation) and salting, reducing the water level by salting out. This traditional salting–drying method increases the concentration of protein and other nutrients. There was an inverse relationship between the dry matter and moisture content. The ash content in fresh camel meat is in the range of 0.75–1.38%; this is lower than in our case. Some reports suggested that the ash content varies depending on the types of the muscle and meat cut (Babiker and Yousif, 1990; Dawood and Alkanhal, 1995; Gheisari et al., 2009) and the age of the animal (Gheisari et al., 2009).Our results showed a change in ash content caused by salt and condiments used in the preparation of *El Kadid*.Our results showed the fat content between 3.17 and7.14% (P <0.05),while the fat content of fresh camel meat ranges from 1.4 to 10.6% (Bekhit and Farouk ,2013b).The observed decrease in fat content was due to the oxidation of fat during sun drying. Slight differences in the fat content have been reported for different cuts and muscle types, with a significant variation. Clearly, the age of the animal has a strong effect on the amount of stored fat; old animals contain more fat than the young individuals (Elgasim and Alkanhal, 1992; Dawood and Alkanhal 1995; Kadim et al., 2006; Gheisari et al., 2009). It is important to note that camel meat contains less fat than beef, lamb, or goat meat and has only slightly higher fat content than ostrich meat. This makes the camel meat a healthy option to be used in special diets (Bekhit and Farouk, 2013b). Protein content ranged from 19.73 to 22.52% in *El Kadid* samples (P <0.05). Fresh camel meat contains 18.2–23.7% of protein and genetic and dietary factors might have a slight effect (Dawood and Alkanhal, 1995; Kadim et al.,2006).Based on the obtained results, we can conclude that *El Kadid* is a good source of protein with a high biological value. Moreover, salt content varied between 23.37 ± 0.04 and 57.86 ± 0.05 % (P< 0.05).In food industry, extending the shelf life of food products is often achieved by salting. This leads to desorption of large amounts of water and a substantial increase in the osmotic pressure, which lowers the water activity and thus inhibits the growth of microorganisms (Berkel et al., 2004).

.**Table 1.** Acidity, pH, water activity, peroxide and acid index of *El kadid* samples.

Sample	Acidity %	pH	Peroxide index Meqg^{-1}	Water activity	Acid index mg KOHg^{-1}
DCM1	0.81±0.09^{c}	5.07±0.05^{a}	0.75±0.00^{b}	0.689 ± 0.002^{d}	16.84±0.42^{b}
DCM2	0.99±0.00^{c}	5.21±0.07^{a}	0.50±0.00^{b}	0.689 ± 0.002^{d}	18.53±0.42^{b}
DCM3	1.07±0.20^{c}	5.01±0.01^{a}	0.70±0.00^{b}	0.688± 0.001^{d}	18.51±0.22^{b}
DCM4	1.80±0.02^{c}	5.85±0.03^{a}	0.45±0.03^{b}	0.688± 0.002^{d}	19.07±0.30^{c}
DCM5	0.90±0.00^{c}	5.97±0.06^{a}	0.47±0.07^{b}	0.684± 0.003^{d}	20.66±0.12^{c}
DCM6	0.48±0.02^{c}	5.36±0.01^{a}	1.00±0.00^{b}	0.685± 0.004^{d}	16.17±0.28^{b}
DCM7	0.43±0.02^{c}	5.65±0.01^{a}	0.50±0.00^{b}	0.685± 0.001^{d}	13.76±0.14^{a}
DCM8	0.16±0.01^{c}	5.75±0.02^{a}	0.80±0.00^{b}	0.686± 0.003^{d}	18.42±0.29^{b}
DCM9	0.21±0.01^{c}	5.78±0.01^{a}	0.80±0.05^{b}	0.686± 0.002^{d}	17.43±0.42^{b}

Results are expressed as means ± standard deviation of three measurements.
$^{a-d}$Means followed by a different letter in the same column aresignificantly different P <0.05

Table 2. Dry, ash matter, moisture, fat, protein and salt content of *El kadid*samples (%).

Sample	Dry	Ash	Moisture	Protein	Fat	Salt
DCM1	99.00±0.00^{a}	2.25±0.08^{b}	0.84±0.02^{d}	19.73±0.00^{c}	7.14±0.00^{e}	50.25±0.05^{f}
DCM2	99.00±0.01^{a}	2.44±0.00^{b}	0.82±0.04^{d}	22.08±0.00^{c}	3.80±0.04^{e}	44.41±0.03^{f}
DCM3	99.45±0.05^{a}	2.34±0.00^{b}	0.54±0.03^{d}	21.00±0.01^{c}	4.52±0.03^{e}	39.72±0.05^{f}
DCM4	99.60±0.03^{a}	2.56±0.01^{b}	0.42±0.05^{d}	20.83±0.03^{c}	5.00±0.05^{e}	37.40±0.03^{f}
DCM5	99.50±0.01^{a}	2.85±0.02^{b}	0.45±0.02^{d}	20.16±0.02^{c}	6.34±0.02^{e}	23.37±0.04^{f}
DCM6	99.60±0.00^{a}	2.65±0.08^{b}	0.31±0.04^{d}	22.52±0.00^{c}	3.17±0.00^{e}	57.86±0.05^{f}
DCM7	99.49±0.03^{a}	2.50±0.00^{b}	0.28±0.09^{d}	21.56±0.00^{c}	4.41±0.00^{e}	45.58±0.07^{f}
DCM8	99.77±0.03^{a}	2.82±0.01^{b}	0.32±0.01^{d}	20.75±0.00^{c}	5.01±0.00^{e}	52.60±0.08^{f}
DCM9	99.65±0.05^{a}	2.32±0.05^{b}	0.40±0.01^{d}	19.94±0.00^{c}	6.91±0.00^{e}	50.14±0.09^{f}

Results are expressed as means ± standard deviation of three measurements.
$^{a-f}$Means followed by a different letter in the same column are significantly different P <0.05.

3.2. Mineral and heavy metals analysis

Table 3. shows the content of minerals and heavy metals in our samples. *El Kadid* contains zinc (0.3 ± 0.06; *P*<0.05), iron (0.99 ± 0.20; *P*>0.05), manganese (0.03 ± 0.13; *P*<0.05), and copper (0.03 ppm; *P*<0.05). The animal species, age, and the environment (Bekhit, and Farouk, 2013b) affect the mineral composition of meat. Zinc is an activator of several enzyme systems; it is important in cell division and differentiation mechanisms and in the metabolism of nucleic acids. It is also involved in the production, storage, and secretion of hormones (Mgbabu, 2011).Manganese is a trace element that is essential in the diet of all animals. It is found in all body tissues; it is necessary in many ubiquitous enzymatic reactions, including synthesis of amino acids, lipids, proteins, and carbohydrates. While this metal can be inhaled from the air, the diet is normally a far better source of manganese. Because of homeostatic systems regulating the absorption and excretion of manganese, its levels in the tissues are usually very stable, regardless of intake levels. However, it can accumulate in certain brain regions following excessive exposure, and manganese-induced neurotoxicity can ensue. Copper is a trace element found in all tissues; it is required for cellular respiration, peptide amidation, neurotransmitter biosynthesis, pigment formation, and maintaining the connective tissue strength. Copper is a cofactor for numerous enzymes and plays an important role in the development of central nervous system; low concentrations of copper may result in incomplete development, whereas excess copper maybe harmful. It can be involved in free radical production, via the Haber-Weiss reaction, resulting in mitochondrial damage, DNA breakage, and neuronal injury (Desai and Kaler, 2008). Iron is a component of several metalloproteins and plays a crucial role in vital biochemical activities, such as oxygen sensing and transport, electron transfer, and catalysis. Iron is thus indispensable to life. The biological functions of iron exploit its chemical properties, e.g., its capacity to form a variety of coordination complexes with organic ligands in a dynamic and flexible mode. One of its notable properties is its favorable redox potential; it can switch between the ferrous, Fe (II), and ferric, Fe(III), states (+772 mV at neutral pH) (Papanikolaou and Pantopoulos, 2005). The meat is an important source of iron; lack of iron causes the most common nutritional deficiency worldwide (Warriss,2000). However, the analysis also revealed the presence of heavy metals, lead at 0.11±0.02 (*P*<0.05), chromium, 0.16±0.05 (*P*>0.05), and cadmium, at 0.11 ppm (*P*>0.05) in the samples of *El Kadid*. The heavy metals might have been be absorbed directly by the animal.

Table 3. Minerals and heavy metals composition of *El kadid* samples (ppm)

Sample	DCM1	DCM2	DCM3	DCM4	DCM5	DCM6	DCM7	DCM8	DCM9
Zinc	0.46 ±0.11[a]	0.16 ±0.00[a]	ND	ND	ND	0.36± 0.06 [a]	0.18 ±0.00 [a]	0.21 ±0.05 [a]	0.46 ±0.14 [a]
Iron	1.37 ±0.33 [a]	1.10± 0.12 [a]	ND	ND	ND	0.69± 0.06[b]	0.72 ±0.12[b]	1.14 ±0.23 [a]	0.94 ±0.37 [a]
Manganese	0.02 ±0.01[c]	0.02 ±0.01[c]	ND	ND	ND	0.04 ±0.01 [c]	0.04±0.00 [c]	0.06 ±0.04 [c]	0.05± 0.03 [c]
Copper	0.04± 0.00[d]	0.03± 0.00 d	ND	ND	ND	0.03± 0.00 [d]	0.06± 0.00 [d]	0.04± 0.02 [d]	0.03± 0.01 [d]
Plumb	0.10± 0.01[e]	0.13±.0.03 [e]	ND	ND	ND	0.13 ±0.04 [e]	0.10± 0.02 [e]	0.10 ±0.00 [e]	0.10 ±0.03 [e]
Chromium	0.03± 0.00[f]	0.02 ±0.01 [f]	ND	ND	ND	0.23± 0.16[d]	0.07 ±0.04 [f]	0.29 ±0.09 [d]	0.32± 0.01 [d]
Cadmium	0.16± 0.00[f]	0.46± 0.00[f]	ND	ND	ND	0.02± 0.00[g]	0.02± 0.00 [g]	0.02 ±0.00 [g]	0.02± 0.01 [g]

Results are expressed as means ± standard deviation of three measurements.
[a-g]Means followed by a different letter in the same row are significantly different P <0.05.
ND: not determined

3.3. Fatty acid profil

The fatty acid composition of *El Kadid*has not been documented before. Most of the available data focuses on the composition of the hump (Kadim et al., 2002).The saturated fatty acid (SFA) ratio total fatty acids in the camel meat are within the range of total SFA reported for beef(43–52%) and lamb (46–54%) (Aro et al. 1998).In our samples, 19 fatty acids were identified (Table 4).Extensive characterization of the fatty acids of camel meat has been published by Rawdah et al. (1994), who identified 22 fatty acids. We showed important differences between fatty acid compositions in *El Kadid* samples. The traditional salted and dried camel meat contained a high percentage of SFA and palmitic acid C16:0 was the major SFA (25.98±0.05%–48.31±0.01%), followed by stearic acid C18:0 (11.57±0.03%–32.35±0.01%) and margaric acid C17:0 (0.90±0.01%–3.35±0.02%).The oleic acid C18:1 was the predominant monounsaturated fatty acid (1.80±0.03%–59.98±0.02%) followed by palmitoleic acid C16:1 (3.46±0.02%–7.65±0.01%). The fatty acid composition of meat and fat of *Camelus dromedarius* showed that the major saturated and monounsaturated fatty acids are C16:0 (25.98%; 30.29%; and 28.50%) and C18:1(18.93%; 32.01%; and 33.50%, respectively). Although the published reports agree on the percentage of total SFAs (51.5–53%), different percentages of monounsaturated fatty acids have been reported (29.9% and 41.4%) (Rawdah et al., 1994; and Kadim et al., 2011). The composition of fatty acids is affected by the diet, the age of the animal and the low volume of olive oil added to the preparation. Olive oils an example of a natural functional food ingredients. It contains a lot of antioxidants, it is dominated by monounsaturated fatty acids, it is characterized by low contain of saturated fatty acids, and contains essential fatty acids with a balanced ratio between ω-6 and ω-3 (oleic acid and linoleic acid). In all published studies, olive oil contains low percentage (8-14%) of saturated fatty acids (SFA). Unsaturated fatty acids are an important factor by which the olive oil is distinguished from other fats (65-83). The most common monounsaturated fatty acid in olive oil is oleic acid (18:1 n-9); it has a great biological nutritional value and is easily digestible. That's why olive oil is a representative of the oleic acid oil group (Šarolić et al., 2014). The highest percentage of unsaturated fatty acids and the lowest percentage of SFAs are found in the animals less than 1 year old, whereas the opposite trend is observed in animals in the 1–3year age group (Kadim et al., 2002). As shown in Table 4, other fatty acids were also detected, such as iso-margaric acid, lauric acid,

heneicosanoic acid, carboceric acid, pentadecanoic acid, myristic acid, 14-pentadecanoic acid, 14-hexadecanoic acid, 17-octadecanoic acid, 7-hexadecenoic acid, 2-hydroxy-hexadecanoic acid, vaccenic acid, 10-octadecenoic acid, and 11,14-eicosadienoic acid.

Table 4. Fatty acid profiles of *El kadid* samples (% of each peak on total peak area).

Fattyacids	DCM1	DCM2	DCM3	DCM4	DCM5	DCM6	DCM7	DCM8	DCM9
Palmiticacid	25.98± 0.05^{a}	27.47± 0.06^{a}	32.57± 0.02^{b}	-	37.62± 0.05^{b}	48.31± 0.01^{c}	43.01± 0.02^{d}	40.65± 0.01^{d}	43.80± 0.02^{d}
Margaricacid	-	-	0.90± 0.01^{a}	3.35± 0.02^{b}	0.69± 0.03^{a}	0.72± 0.01^{a}	1.90± 0.02^{c}	-	1.69± 0.04^{c}
Isomargaricacid	-	-	-	-	-	2.73± 0.03^{a}	0.76± 0.03^{b}	3.01± 0.08^{d}	1.77± 0.05^{c}
Stearicacid	11.57± 0.03a	-	22.51± 0.01^{d}	32.35± 0.01^{d}	11.75± 0.01^{a}	13.00± 0.03^{b}	13.68± 0.01^{c}	22.80± 0.06^{e}	19.50± 0.02^{f}
Lauricacid	-	-	-	-	0.75± 0.02^{a}	-	1.04± 0.04^{b}	-	1.30± 0.05^{c}
Heneicosanoicacid	-	-	1.74± 0.03^{a}	-	-	-	-	1.23± 0.06^{b}	1.64± 0.01^{a}
Carbocericacid	-	-	-	-	-	-	-	1.36± 0.04	-
Pentadecanoicacid	-	-	-	-	2.91± 0.06^{a}	2.05± 0.02^{b}	3.41± 0.01^{c}	-	0.95± 0.02^{d}
Myristicacid	-	-	-		1.78±	-		-	1.81±
14-Pentadecanoic acid	-	-	1.03± 0.01^{a}	1.50± 0.03^{b}	-	0.56± 0.05^{c}	0.67± 0.02^{c}	-	0.60± 0.05^{c}
14-Hexadecanoic acid	-	-	0.75± 0.01^{a}	2.40± 0.01^{b}	-	1.63± 0.03^{c}	-	-	-
17-Octadecanoic acid	-	-	-	-	-	1.90± 0.02	-	-	-
7-Hexadecenoic acid	-	-	-	-	-	-	0.73± 0.03^{a}	-	0.46± 0.04^{b}
2-Hydroxy-hexadecanoic acid	-	-	-	-	-	-	-	0.82± 0.01	-
Palmitoleicacid	-	-	4.25± 0.06^{a}	3.46± 0.02^{b}	7.65± 0.01^{c}	5.27± 0.01^{d}	7.56± 0.01^{e}	-	7.56± 0.02^{e}
Oleicacid	59.98± 0.02^{a}	41.02± 0.02^{b}	27.27± 0.05^{c}	37.79± 0.02^{d}	17.29± 0.01^{e}	23.51± 0.02^{f}	22.40± 0.02^{g}	25.79± 0.01^{h}	1.80±0. 03^{i}
Vaccenicacid	-	-	-	-	-	-	-	-	1.07± 0.02
10-Octadecenoic acid	-	-	-	-	-	-	-	2.73± 0.01	-
11,14-Eicosadienoic acid	-	-	-	-	-	-	-	-	0.44± 0.02

Results are expressed as means ± standard deviation of three measurements.
$^{a-i}$Means followed by a different letter in the same row are significantly different P <0.05.

3.4. Functional properties

The functional properties are shown in Table 5. Our results revealed that the Water Absorption Capacity (WAC) values varied between 2.42± 0.03 and 5.30 ± 0.05 mLg^{-1} (P< 0.05). This is probably due to the lower carbohydrate content and reduced space in the structure due to salting and sun drying. The

WAC of proteins is a criterion used to determine the quality of food texture, in particular for meat products. The production yield and sensory qualities depend on the WAC (Selmane,2010). Microscopic investigation of kilish (meat product) has shown that the size and shape of the starch granules as well as the distribution of the protein clusters has an important effect on the WAC (Muir et al., 2000). The OAC values ranged from 10.34 ± 0.05 to 13.34 ± 0.05 mLg^{-1} (P <0.05). These values could be explained by the reduced fat content, resulting from lipid oxidation during sun drying. Our results suggest that hygroscopic properties of the protein in *El Kadid* remained almost unchanged. This agrees with the results of Hayta et al. (2002), who have reported that the OAC of any food material depends on the degree of hygroscopicity of the system. Here, the hygroscopicity of the samples varied between 1.58 ± 0.01 and 3.43 ± 0.03 % (P<0.05). These results may be due to the prolonged exposure to the sun. The hygroscopicity is an important parameter in food formulation; it is affected by the polarity, texture, size, and microstructure of the protein particles (Kinsella, 1979). Moreover, the emulsion and foaming capacity values (P<0.05) of *El Kadid* samples ranged between 3.36 ±0.04%and5.34± 0.03% and between 6.20 ± 0.02% and8.95 ± 0.09%, respectively. These foaming capacity values are due to a reduced fat content and high protein level. The foaming capacity of a substance depends on the surface-active properties of the proteins involved. Kinsella (1979) has found that some food proteins can form foams; their capacity to foam and retain stable foams depends on the type of protein, degree of denaturation, pH, temperature, and processing method. Emulsion capacity values were due to interactions of the proteins with other components of the samples.

Table 5. Functional properties of *El kadid* samples.

Sample	Hygroscopicity %	WAC mLg^{-1}	OAC mLg^{-1}	FC %	EC %
DCM1	2.20±0.01^{b}	4.50±0.05^{a}	10.34±0.05^{c}	6.25±0.03^{d}	3.36±0.06^{e}
DCM2	1.86±0.01^{b}	4.50±0.05^{a}	13.25±0.02^{c}	8.62±0.01^{d}	5.33±0.03^{e}
DCM3	2.32±0.03^{b}	3.50±0.02^{a}	12.20±0.01^{c}	7.95±0.01^{d}	4.45±0.01^{e}
DCM4	3.34±0.04^{b}	4.35±0.01^{a}	12.03±0.02^{c}	7.62±0.05^{d}	4.35±0.03^{e}
DCM5	1.58±0.01^{b}	2.42±0.03^{a}	11.36±0.01^{c}	7.22±0.02^{d}	4.15±0.05^{e}
DCM6	2.32±0.02^{b}	4.30±0.01^{a}	13.34±0.05^{c}	8.95±0.09^{d}	5.34±0.03^{e}
DCM7	2.06±0.01^{b}	4.09±0.02^{a}	12.86±0.04^{c}	8.21±0.07^{d}	4.56±0.02^{e}
DCM8	3.43±0.03^{b}	5.30±0.05^{a}	11.68±0.01^{c}	7.32±0.02^{d}	4.23±0.03^{e}
DCM9	2.56±0.02^{b}	4.80±0.04^{a}	10.50±0.05^{c}	6.20±0.02^{d}	3.36±0.04^{e}

Results are expressed as means ± standard deviation of three measurements.
$^{a-e}$Means followed by a different letter in the same column are significantly different P <0.05.

4. Conclusions

From the obtained results, we can conclude that important differences were found in physicochemical, fatty acids composition and functional properties of traditional salted-dried meat *El Kadid* produced from camel meat (*Camelus dromedarius).* Thus, the present study is expected to encourage more research in quality of traditional Algerian salted-dried meat.

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EFFECT OF ADDITION OF WHEAT AND PIGEON-PEAN ON THE RHEOLOGICAL CHARACTERISTICS OF RICE FLOUR

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Wheat.

ABSTRACT

Evaluation of the pasting characteristics, dough mixing properties and dynamic rheology of composite flour comprising wheat, rice and pigeon-peas flours was carried out in order to determine suitability of the composite flour for some bake products production. The pasting characteristics, dough mixing properties and dynamic rheology were evaluated using amylograph, farinograph and rheometer respectively. The blend with wheat and rice flour only had zero breakdown viscosity at 50% wheat/50% rice flour and 30% wheat/70% rice flour while 100% wheat flour had the highest breakdown viscosity of 167 BU. However, wheat/rice flour blends had the highest setback viscosity, meaning it will enhance starch retrogradation most. Addition of pigeon-pea reduced the setback viscosity (347-376 BU) compared to 100% wheat flour (387 BU). The 50%:50% wheat-rice flour blend had the highest (802 BU) final viscosity followed by 100% wheat flour (746 BU) while 100% wheat flour had the best pasting temperature and peak viscosity. Inclusion of pigeon-pea flour to rice-wheat blends produced mixing quality close to 100% wheat flour in terms of dough constitency and water absorption. The stability of the blends with pigeon-pea was better than 100% wheat flour. Blend, 30% wheat/70% rice flour had poor mixing quality. Addition of pigeon-pea enhanced mixing quality and the dynamic rheology properties (G', G", shear stress vs shear rate) of the blends.

1.Introduction

Rice (Oritza sativa) is a staple food of consumed by over approximately half of the world's population (Ahmed et al., 2015). Rice flour is gluten-free, hence, a good source of flour for people who are gluten intolerant (Helms. 2005). Rice flour has been described as a viable alternative to wheat flour in the production of baked products (Martínez et al., 2014; de la Hera et al., 2013). Several researches have been carried out using rice in the production of extruded products (Awolu et al., 2015; Martínez et al., 2014), bread production (Nozawa et al., 2016; Araki et al., 2016; Therdthai et al., 2016), and cookies (Rai et al., 2014). The search for viable alternative to wheat flour and development of composite flour has several objectives such as the need to diversify wheat utilization, cost effectiveness, health purposes especially for people who are gluten intolerant (Awolu et al., 2017). A more formidable reason is the development of functional baked products which will be nutritionally enriched with protein, fibre and antioxidants (Awolu et al., 2017; Awolu et al., 2016).

The major problem with the development of composite flours without wheat has been the inability of the composite flour to have proper viscoelastic behaviour suitable for bread production in particular. So the many researches

were aimed at improving the rheological characteristics of the composite flours, or rice-base flours. Several approaches are being adopted to enhance the rheological properties of non-wheat composite flour, including but not limited to addition of hydrocolloids (Awolu, 2017), starch (Awolu and Oseyemi, 2016), fibre (Awolu et al., 2016), legumes. Addition of fibre and legumes have particularly gained interests because in addition to improving the rheological characteristics of the dough, it enhanced its nutritional properties by improving its protein, fibre and antioxidant contents (Marco and Rosell, 2008). In fact, the addition of legumes enhanced minerals contents, protein contents and antioxidant contents of the flour blends.

Pigeon-pea (Cajanuscajan) is an underutilized legume widely grown. It is has rich protein content up to about 26% (Oshodi et al., 1985) and also rich in fibre and mineral contents (Fasoyiro et al., 2010). It serves as a viable alternative to many rural dwellers especially in the tropics and subtropical regions where it is widely grown and affordable (Fasoyiro et al., 2010). Its utilization has been limited because of the presence of antinutrients and difficulty in cooking (Nene et al., 1990). However, effective processing had been successful at reducing such antinutrients to safe levels (Awolu et al., 2015)

Rheological properties are vital in the analysis of flow conditions in product processes as well as in the prediction of product stability (Wang et al., 2016; Li et al., 2014). It has been stablished to have direct relationship with textural attributes, which in turn affect the sensory characteristics and consumer acceptability of the products (Li et al., 2014). Small oscillatory frequency sweep using rheometer had been found to be extremely sensitive to the structure information of samples thereby making it useful in the evaluation of gelation behavior and kinetics (Liu et al., 2015).

The aim of this study is to explore various rheological measurement techniques, precisely rheometer and farinograph to evaluate the rheological properties of composite flour consisting wheat, rice and pigeon-pea flours. The effect of heating and shearing on the composite flour starch will also be explored by using viscoamylograph. The best blend in terms of rheological and pasting characteristics will be determined considering rice-wheat flour blend only, and also rice-wheat-pigeon pea flour blends; thereby establishing the effect of the addition of pigeon-pea on the blend rheology.

2. Materials and methods

2.1. Materials

Whole wheat flour (9.1% moisture content, 11.0% protein and 13.9% dietary fibre) by Avent Agro Pvt. Ltd, Narela, Delhi 110040, India, was sourced from Mysore, India. Rice grains (Superior) and Pigeon-pea were sourced from Mysore, India.

2.2. Preparation of rice flour

Rice flour was prepared using the method adopted by Awolu et al. (2015). The rice grains (1 kg) were sorted, washed, oven-dried and milled into flour using hammer mill (Model: CMC=CM- Q=753=97, M=s Cadmach Machinery Company) It was later passed through 0.037 mm test sieve (Jayant Test Sieves, Bombay, India).

2.3. Preparation of pigeon-pea flour

Pigeon-pea grains were soaked in hot water for 15 min, dehulled manually, and oven-dried at 65 oC for 24 h. It was milled using hammer mill as described above, sieved using 0.037 mm test sieve (Jayant Test Sieves, Bombay, India) (Fasoyiro et al., 2010, modified).

2.4. Blends formulation

The blend formulation for rice, wheat and pigeon-pea flours are presented in Table 1.

2.5. Pasting characteristics

The pasting characteristics were determined by using Micro-Visco-Amylograph (model 803201 by Brabender, Germany). About 10 g of the samples were mixed with appropriate water as obtained from the instrument after inputting the

moisture content. The sample was placed inside the amylograph and allowed to run for about 25 min.

2.6. Dough Rheological

2.6.1. Farinograph

The farinograph evaluation was carried out according to the standard AACC (2000) methods. The amount of water added was generated by inputting the moisture content of the composite flour into the farinograph (Model: E-380, Brabender OHG, Duisburg, Germany) system and allowed to run for about 30 min. Parameters measured include water absorption, dough development time, dough stability and mixing tolerance index.

2.6.2. Dynamic rheology

The dynamic rheology was carried out by using modified method of Demirkesen et al. (2010). The composite flour was carried out by using Modular Compact Rheometer (NCR52, Anton Paar). The position was set at 2.000 mm, temperature at 25.00 oC while the measuring system was PP76. The dough samples (without yeast) were placed between the parallel plates and the edges were carefully trimmed with a spatula. The flow experiments were carried out under steady-shear condition (Shear rate ranged from 1 to 50 1/s) while for the frequency sweep test, the strain rate was kept constant below 0.5%; the dough elastic modulus and complex viscosity were measured as a function of frequency (between 0.1 and 100 rad s−1)

3.Results and discussions

3.1 Pasting characteristics

The results of the pasting characteristics of the composite blends are shown in Fig. 1. Pasting characteristics represents the behaviour of the composite flour starch to heating and cooling. The 100% wheat flour had the best pasting temperature (65.5 oC) and peak viscosity (521 BU). The flour blend with pigeon-pea flour had lower pasting temperature than blends with only rice-wheat blends. Wheat-rice flour blend (50:50) performed better than blends with pigeon-pea in all pasting parameters measured except for the setback viscosity. The blend with 70% rice flour, however, had the least pasting quality. So, rice flour up to 50% could be recommended for flour blends requiring good pasting property. The challenge of setback viscosity of the wheat-rice flour blends could be solved by the incorporation of pigeon-pea flour. The samples with pigeon-pea had the least set back viscosities (347 and 376 BU for 20% and 10% pigeon pea incorporation respectively). The values were lower than what was obtained for 100% wheat flour (387 BU). The pasting temperature represents the temperature at which the viscosities first increase by at least 2 RVU over 20 s periods (Adegunwa et al., 2012). It is the minimum temperature at which starch granules in the flour swells (Awolu, 2017). The peak viscosity represents the pastes strength from gelatinization; it is the maximum viscosity attained during cooking (Adebowale et al., 2011). The holding strength measures of ability of granules to remain undisrupted during holding at high temperature (92 oC) and high mechanical shear stress (Adegunwa et al., 2012). A lower breakdown viscosity is required, as it signifies a higher stability.

An increase in viscosity as the temperature reduced from 92 oC to 50 oC is a reflection of the ability of the elements in pastes to associate as the paste temperature drops (Ocloo et al., 2010). This increase in viscosity is the setback viscosity. Setback viscosity corresponds to retrogradation

3.2. Farinograph

The results of the farinograph are shown in Fig. 2. Farinograph method has been described as the leading standard tool for rheological behaviour assessment of flour during bread dough development and mixing processes (Miś, et al., 2017). It measures dough resistance to the mixing blades during a prolonged and gentle mixing action at constant temperature. The dough development time (DDT) of 100% wheat flour (sample 1) dough was 3.16% while that of

sample 3 (50% wheat/30% rice/20% pigeon pea composite flour) was 9.14%. The other samples had DDT ranged from 19.10 to 19.57%. DDT is an indication of the dough mixing time. Sample 1 had the best DDT, followed by sample 3. Inclusion of pigeon pea (a legume) at 20% (sample 3) improved not only the DDT but the entire farinograph parameters of the samples. Other parameters that distinctively reflected the inclusion of pigeon pea were dough stability, mixing tolerance index (MTI), farinograph quality numbers, time to breakdown which were 2.48 min, 93 FU, 48 mm, 4.47 min and 7.45 min, 37 FU, 133 mm, 13.17 min for samples 1 and 3 respectively. There were no dough stability, mixing tolerance index (MTI), farinograph quality numbers and time to breakdown for samples 2, 4 and 5. While samples 2 and 5 consisted of only wheat and rice flour blends (between 30 and 50% wheat flour inclusion), sample 4 had 10% pigeon pea flour added to wheat and rice flours. These results showed that the promotion of rice flour as a viable alternative to wheat flour for bread production may not be viable afterall, except where the amount of wheat flour inclusion is above 50%, or addition of legumes and hydrocolloids to enhance the rheological characteristics of rice flour. Awolu and Oseyemi (2016) had shown that inclusion of wheat flours up to 72% to wheat-cocoyam-bambara groundnut composite flour produced farinograph properties that are very close to 100% wheat flour.

Low mixing tolerance index (MTI) and longer stability is preferred; sample 3 is better in terms of stability and MTI. Flours with low MTI have been reported to possess good tolerance to mixing, while flours with high MTI's were reported to be critical to mixing and overmixing in particular

The dough constitency for samples 1, 2, 3, 4 and 5 were 522, 552, 574, 594 and 406 FU respectively. The water absorption of sample 1 was 76%, samples 3 and 4 had 64.8 and 65.3% water absorption respectively. The water absorption of samples 2 and 5 were 61.3 and 57.0% respectively. Optimum water absorption for bread making had been reported to be between 50 and 64% (Mailhot and Patton, 1988), and it was found out that high water absorption is an indication of good baking performance (Zecevic et al., 2013). Awolu, et al. (2016) reported water absorption of 66.7% for composite flour consisting 72% wheat/19% cocoyam/9% bambara groundnut.

3.3. Dynamic rheology

The results showing the effects of shear stress and against shear rate (Fig, 3a to 7a), viscosity against strain (Fig, 3b to 7b) and modulus against frequency (Fig, 3c to 7c) are shown. All the samples showed shear-thinning (pseudoplastic) behaviour except for 100% rice flour dough (Fig. 8a). The same behaviour was also replicated in Fig, 3b to 7b. However, sample 1 had the highest initial viscosity (above 80,000 mPa.s) when considering samples 1 to 5, while sample 3 distantly followed (just above 10, 000 mPa.s). Sample 2 with 50% wheat flour and 50% rice flour had initial viscosity of about 9,000 mPa.s. The reason for the decrease in viscosity of shear-thinning fluid had been deduced to the break down in interaction between flour components under the action of shear (Deirmikesen, et al., 2010). Similar result was reported by Deirmikesen, et al. (2010) when the rheological properties of rice bread dough containing different gums with or without emulsifiers were determined. For pseudoplastic fluid, viscosity was high at low strains and dough structure seems to be intact; high strain, however, lead to the disorganization and destruction of the dough's structure leading to reduced viscosity (Weipert, 1990). In essence, Figures 3b to 7b indicated that samples 2 to 4 may not perform favourably with sample 1 in the terms of dough rheology. However, sample with 100% rice flour (Fig. 8b) had a very high viscosity which does not translate into better dough rheological characteristics.

The viscoelastic properties of samples (fig. 3c to 7c) showed that the samples displayed more viscous-like behaviour, with loss modulus greater than storage modulus. Such behaviour

had been reported for wheat flours (Autio et al., 2001) and gluten-free dough (Ronda et al., 2013; Lazaridou et al., 2007) as a result of dilution of constituents (Mancebo et al., 2015); reduction in dough water level had been found to reverse the trend by increasing the storage modulus (Mancebo et al., 2015), making the dough to be more elastic-like. Addition of fibre (pysillium) and thickening agents (like HPMC) had been discovered to reduce the water content, and hence, increase the storage modulus (Mancebo et al., 2015). However, bread dough had been reported to exhibit shear-thinning and thixotropic behaviour (Weipert, 1990; Muller, 1975).

Wheat flour (100%) as indicated by had the highest modulus while considering wheat-based composite flour (Fig. 3c to 7c), with G" greater than G'. With increase in strain, the G" was getting closer to G', a tendency to be more elastic-like. All samples containing wheat displayed this behaviour. In terms of modulus magnitude, sample 2 and 3 were similar and close, while samples 4 and 5 were also similar and close. Samples 2 and 3 also had similar complex viscosity (η) behaviour while samples 4 and 5 had similar complex viscosity behaviour. Rice (100%) dough had greater G" than G', which was an indication of elastic-like dough (Fig. 8). It could not, however, be said to possess good dough property. A wheat variety (Okapi) displayed behaviour similar to 100% rice dough with high G' and low G", while the difference between the two were high (hence, low loss tangent) had been reported to reflect rigid and stiff material with a lower dough processing value (Weipert, 1990).

Table 1. Blends Formulation for Rice, Wheat and Pigeon-Pea Flours

Sample	Wheat flour (g/100g)	Rice flour (g/100g)	Pigeon-pea flour (g/100g)
1	100.0	-	-
2	50.0	50.0	-
3	50.0	30.0	20.0
4	50.0	40.0	10.0
5	30.0	70.0	-

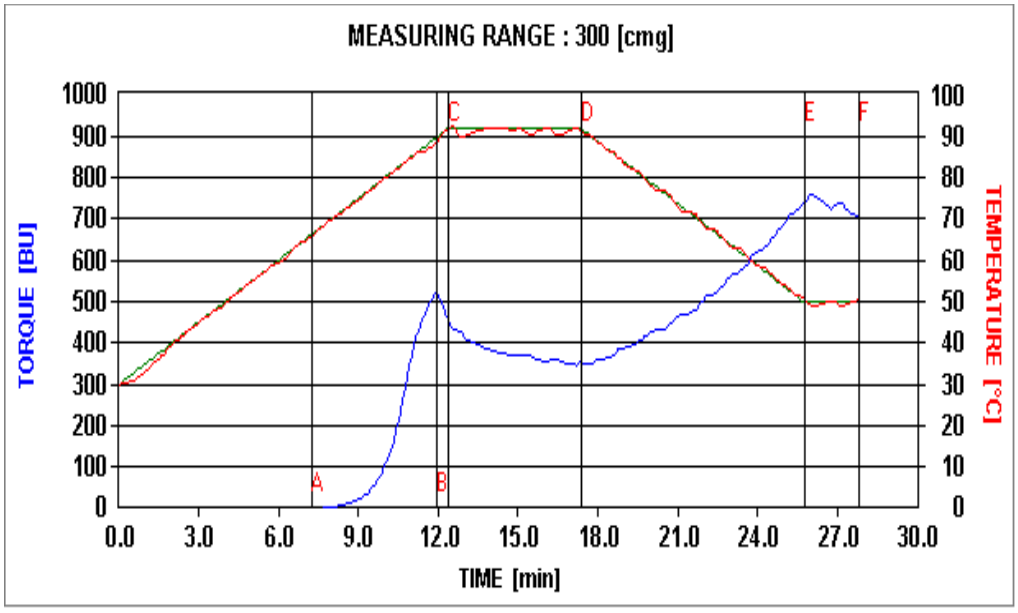

Figure 1a. Pasting profile of sample 1 (100% wheat flour)

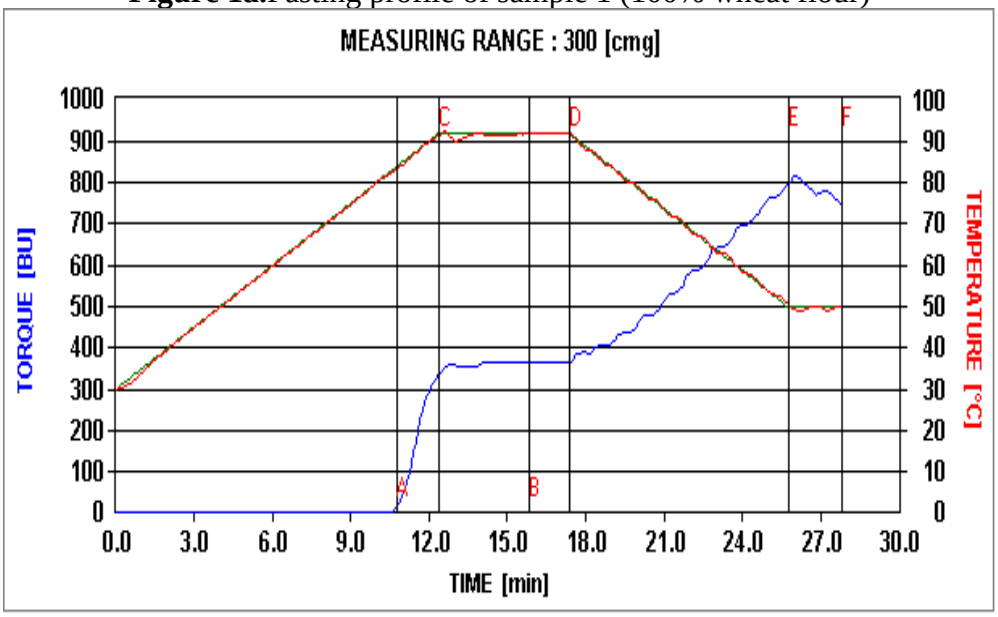

Figure 1b. Pasting profile of sample 2 (50% wheat/50% rice flour blend)

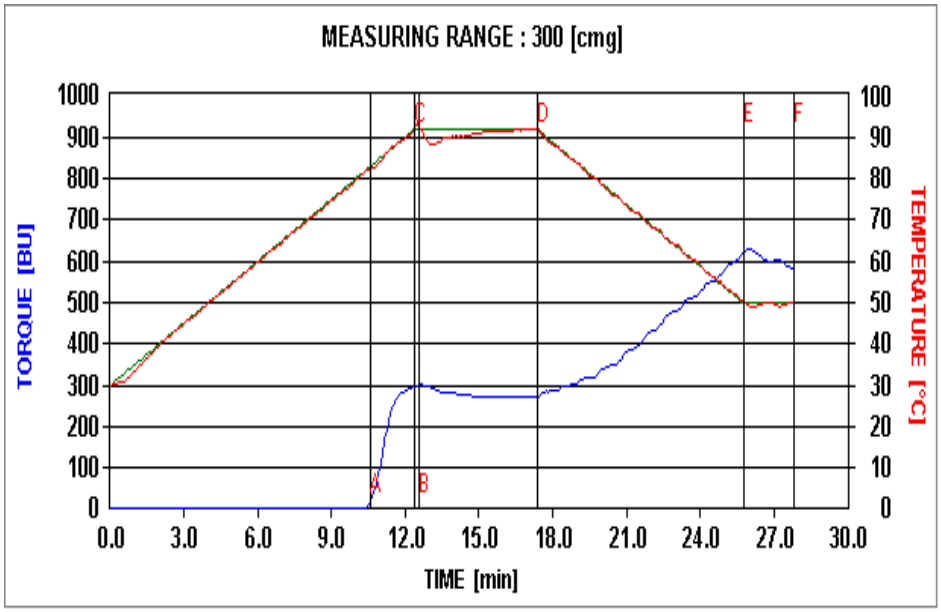

Figure 1c. Pasting profile of sample 3 (50% wheat/30% rice/20% pigeon-pea flour blend)

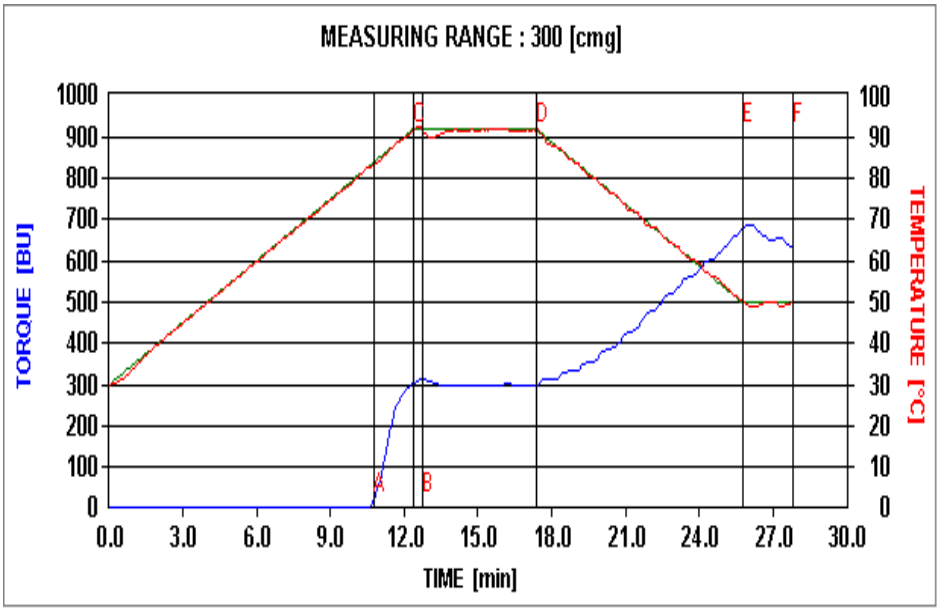

Figure 1d. Pasting profile of sample 4 (50% wheat/40% rice /10% pigeon-pea flour blend)

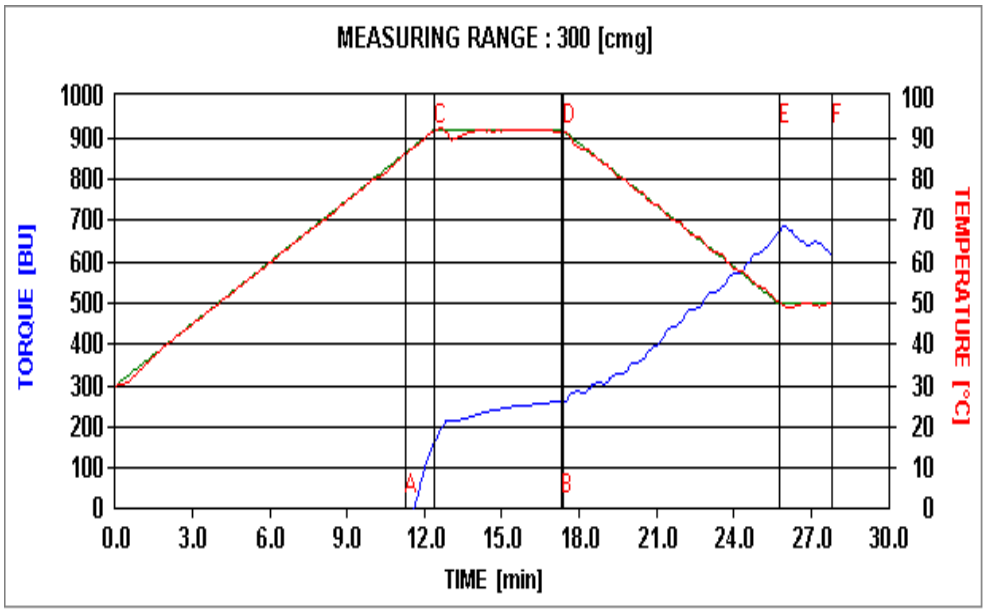

Figure 1e. Pasting profile of sample 5 (30% wheat/70% rice flour blend)

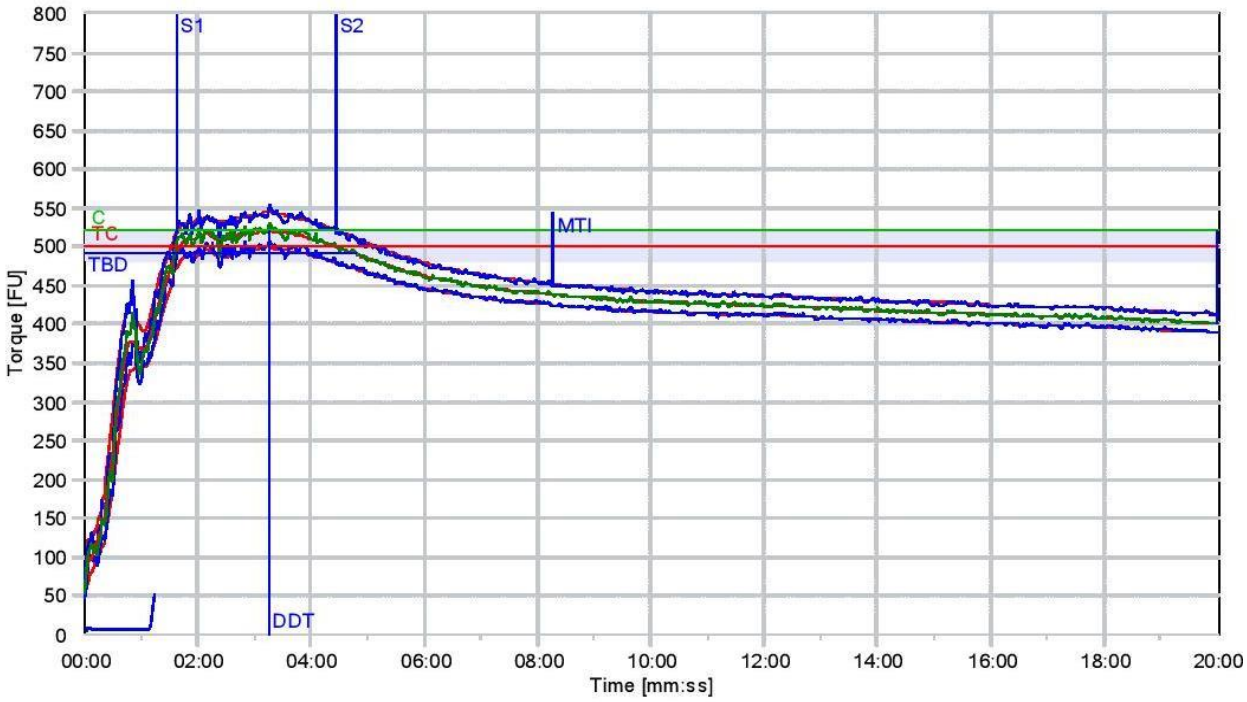

Figure 2a. Farinograph of sample 1 (100% wheat flour)

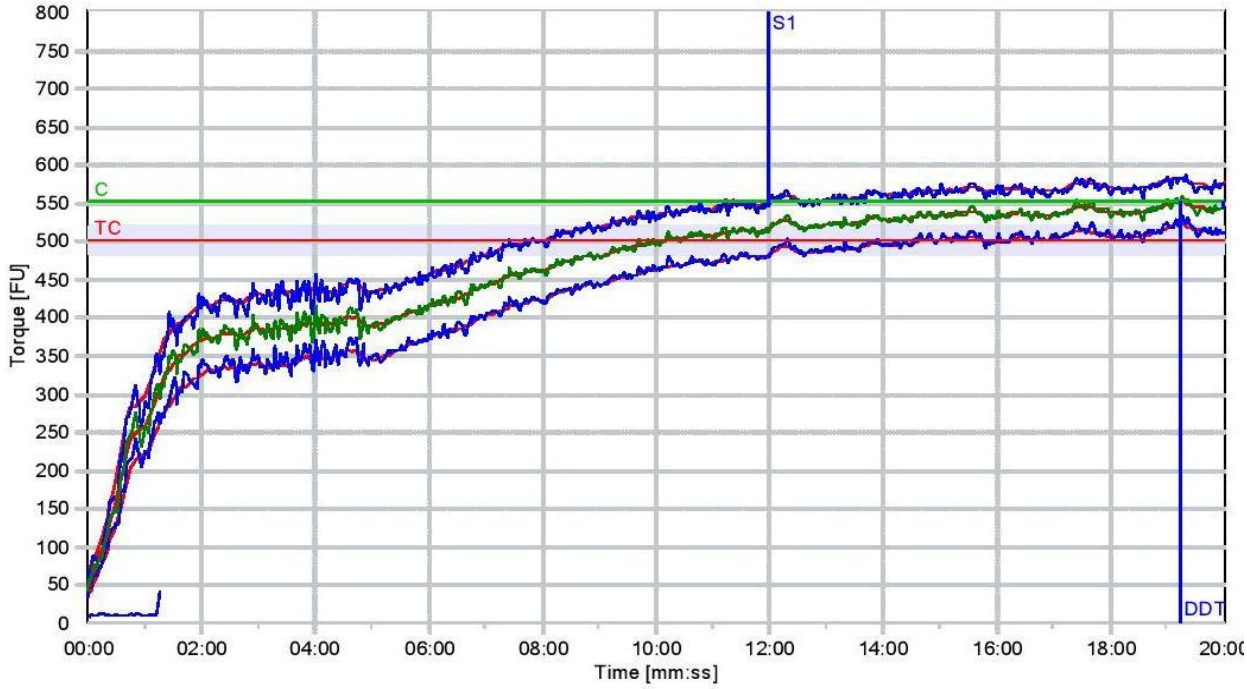

Figure 2b. Farinograph of sample 2 (50% wheat/50% rice flour blend)

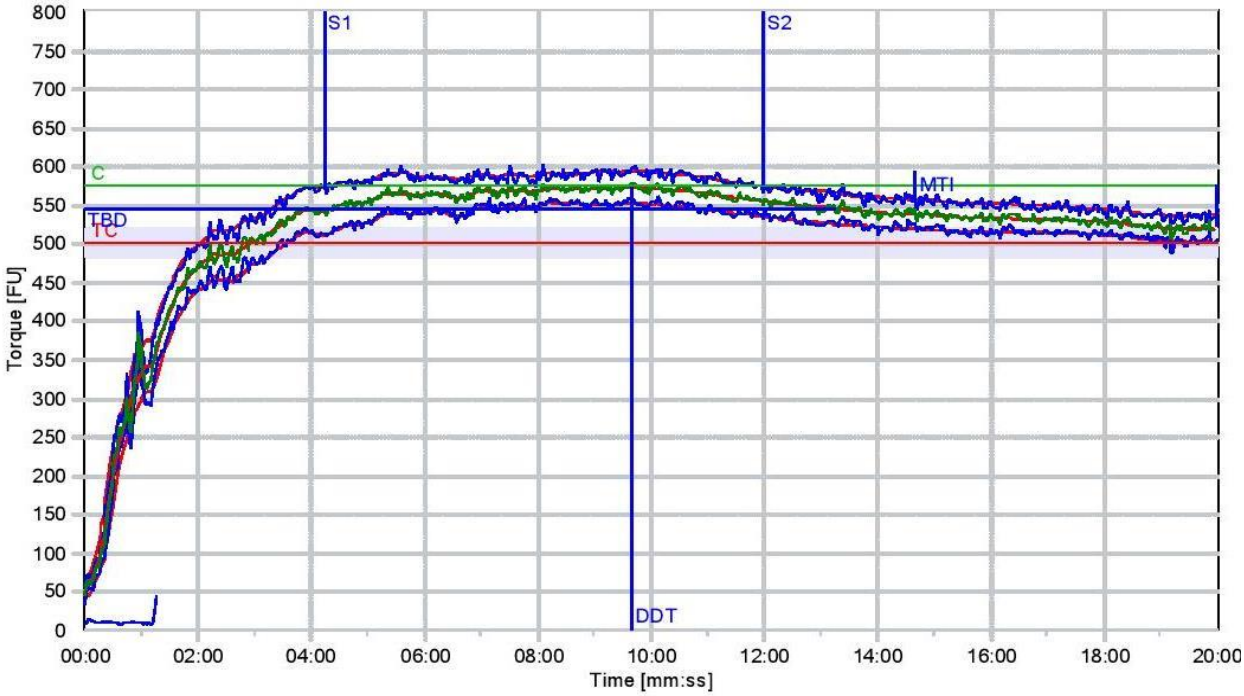

Figure 2c. Farinograph of sample 3 (50% wheat/30% rice /20% pigeon-pea flour blend)

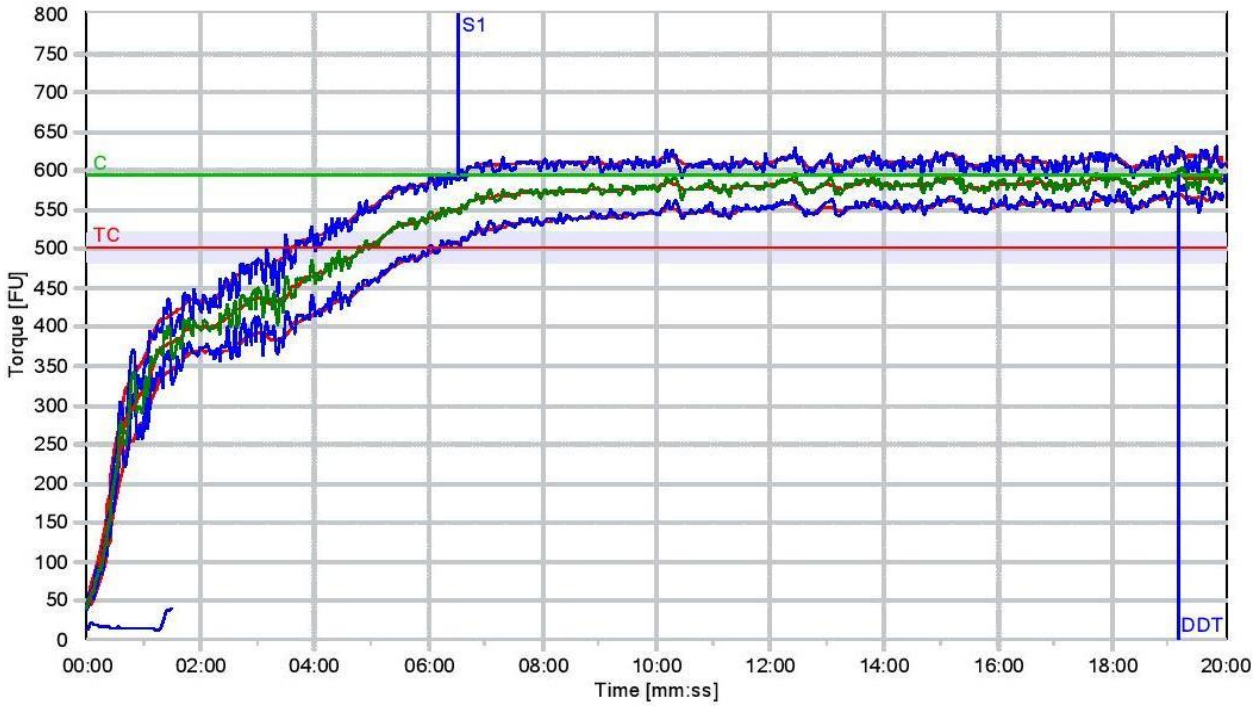

Figure 2d. Farinograph of sample 4 (50% wheat/40% rice /10% pigeon-pea flour blend)

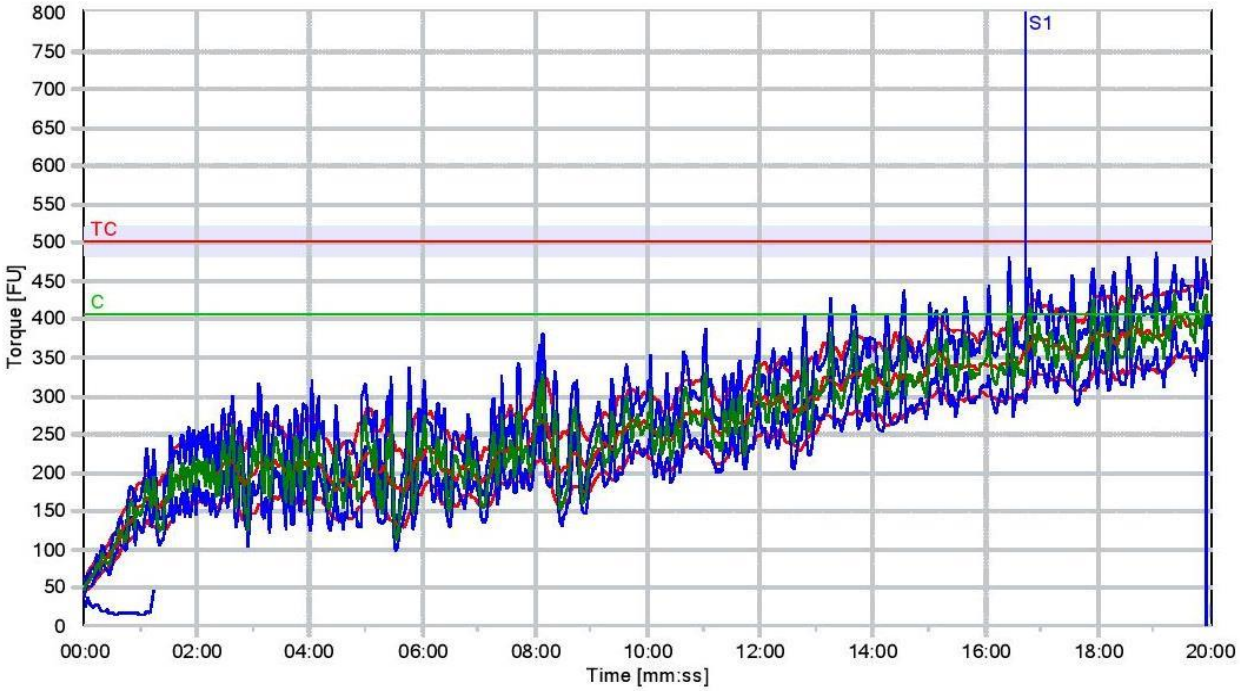

Figure 2e. Farinograph of sample 5 (30% wheat/70% rice)

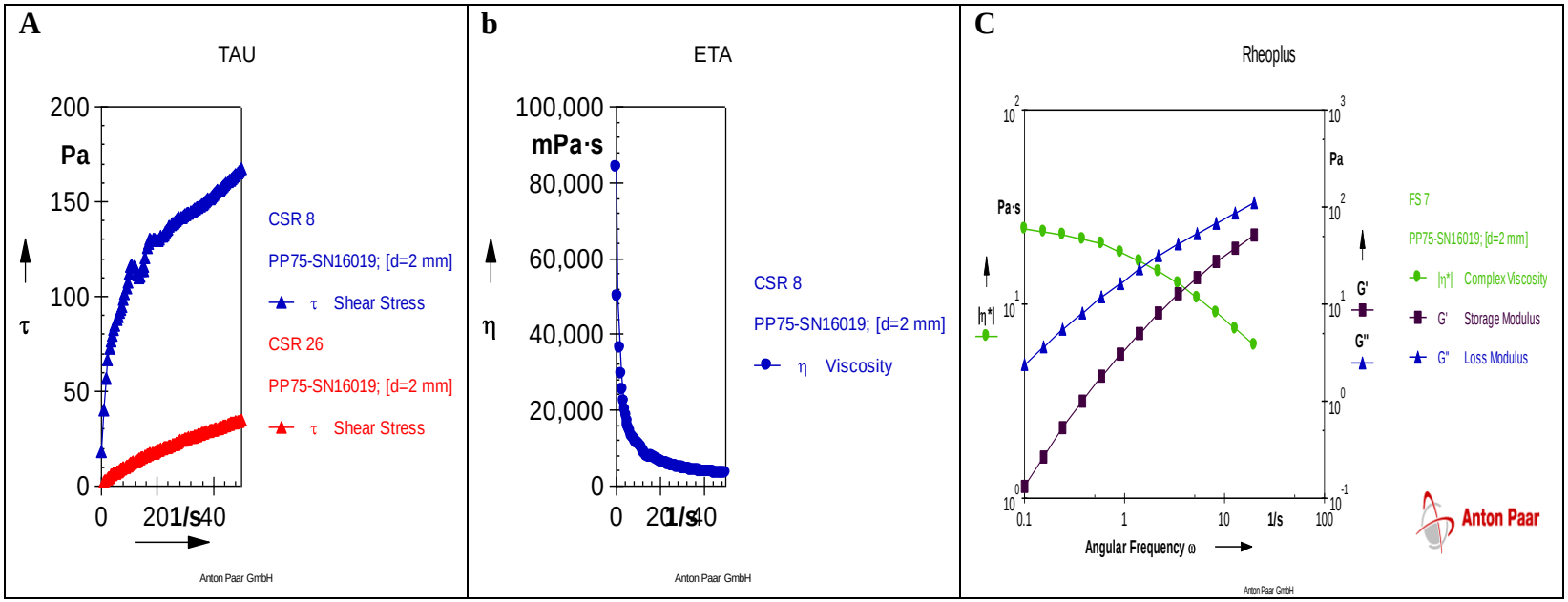

Figure 3. Dynamic rheology of sample 1 (100% wheat flour)

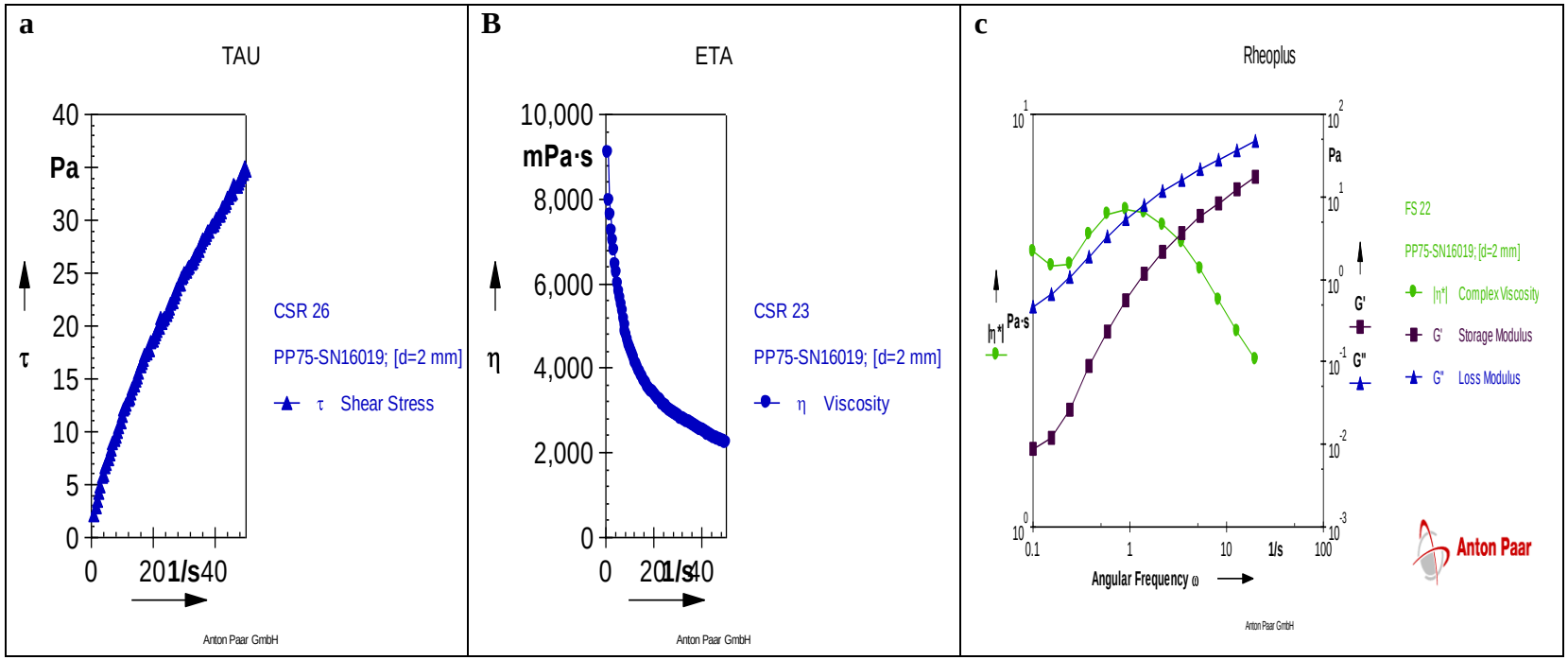

Figure 4. Dynamic rheology of sample 2 (50% wheat/50% rice flour blend)

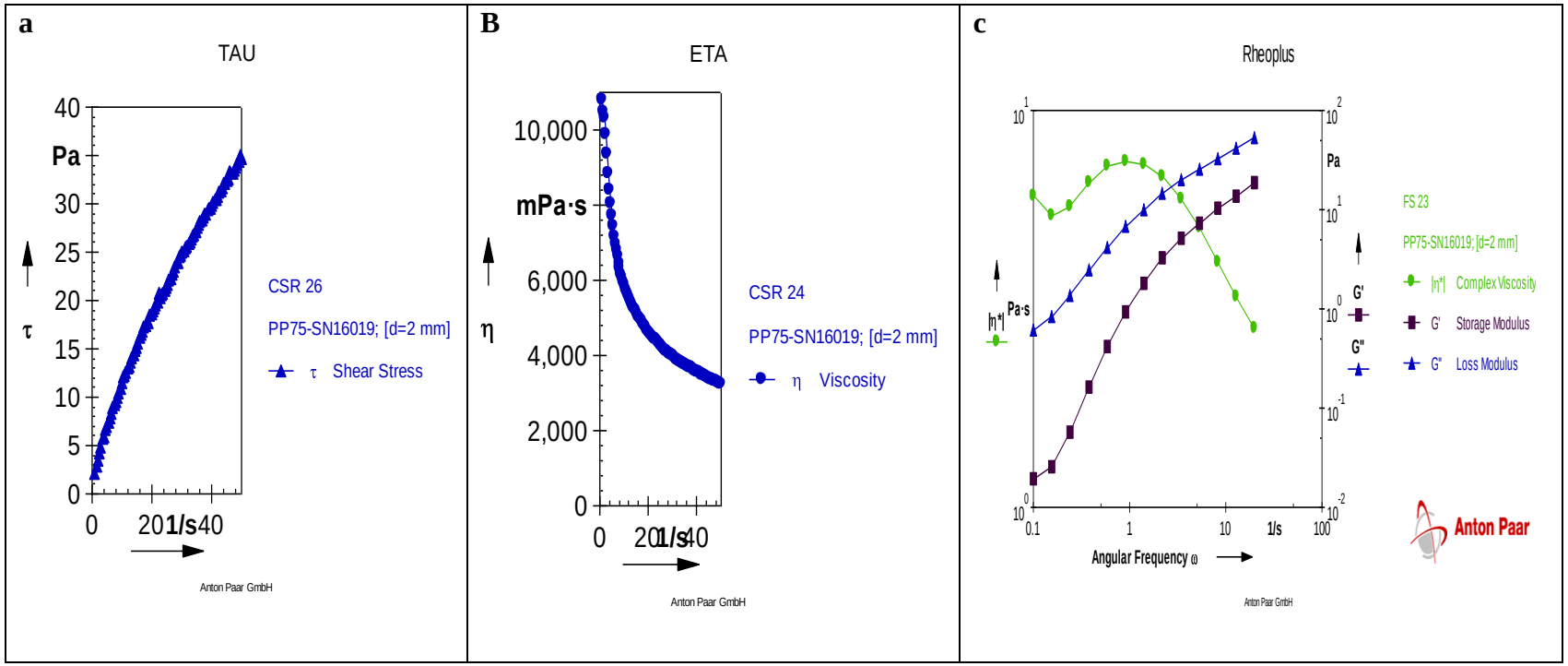

Figure 5. Dynamic rheology of sample 3 (50% wheat/30% rice /20% pigeon-pea flour blend)

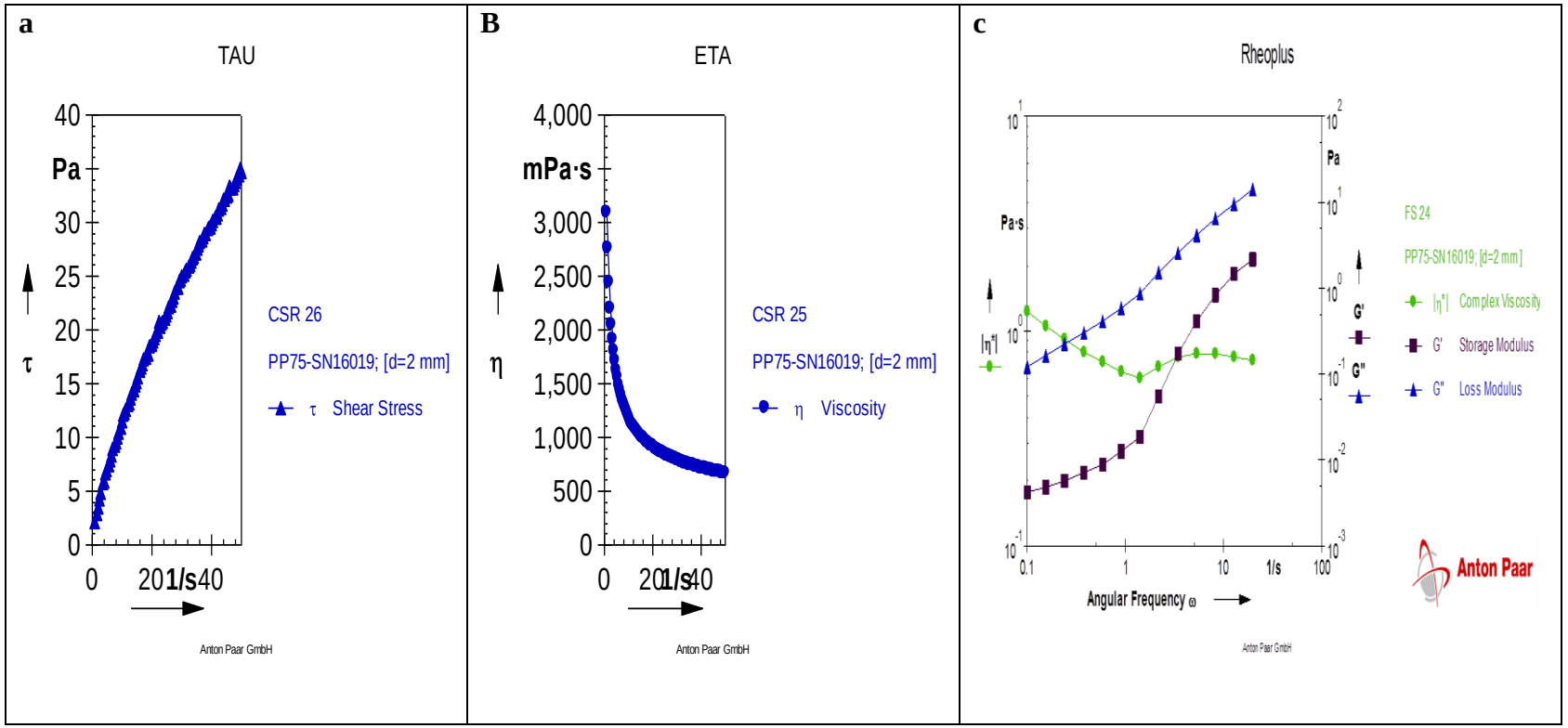

Figure 6. Dynamic rheology of sample 4 (50% wheat/40% rice /10% pigeon-pea flour blend)

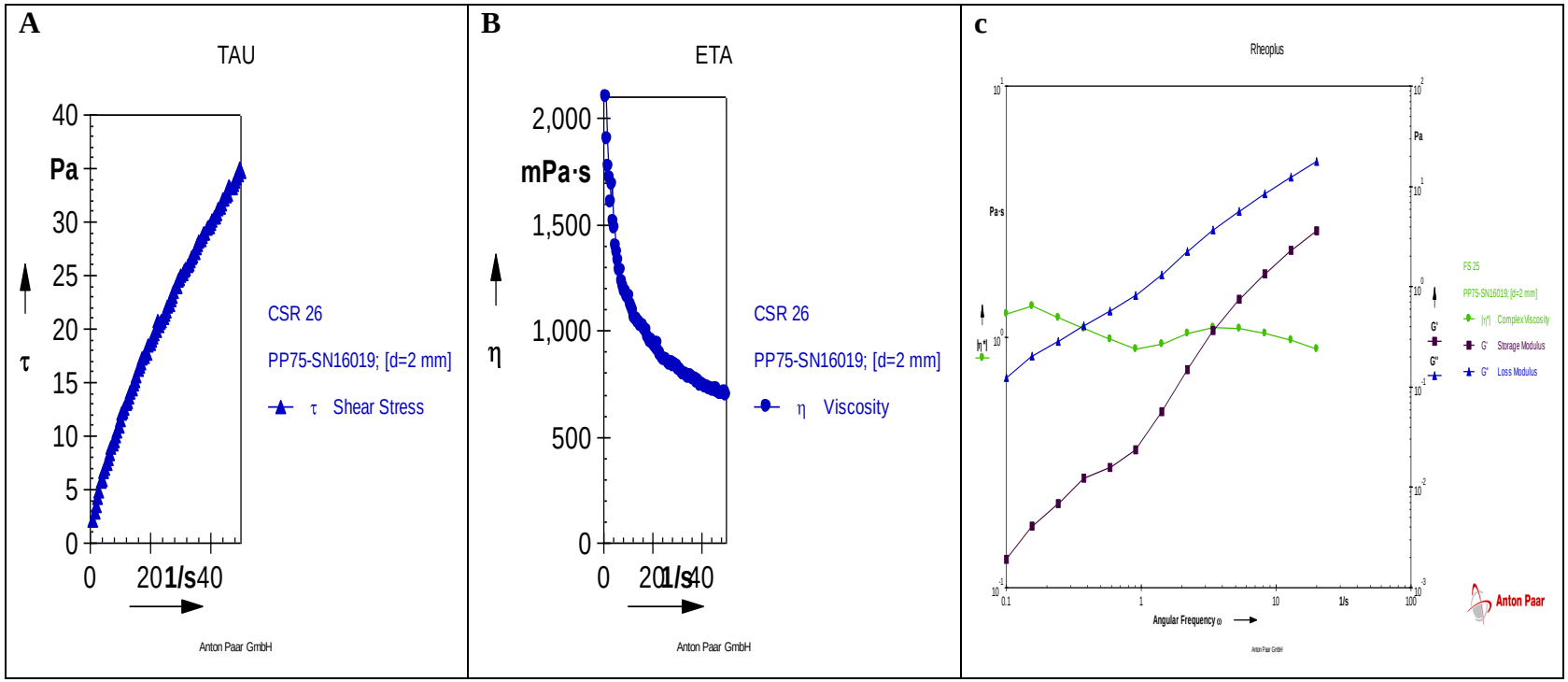

Figure 7. Dynamic rheology of sample 5 (30% wheat/70% rice flour blend)

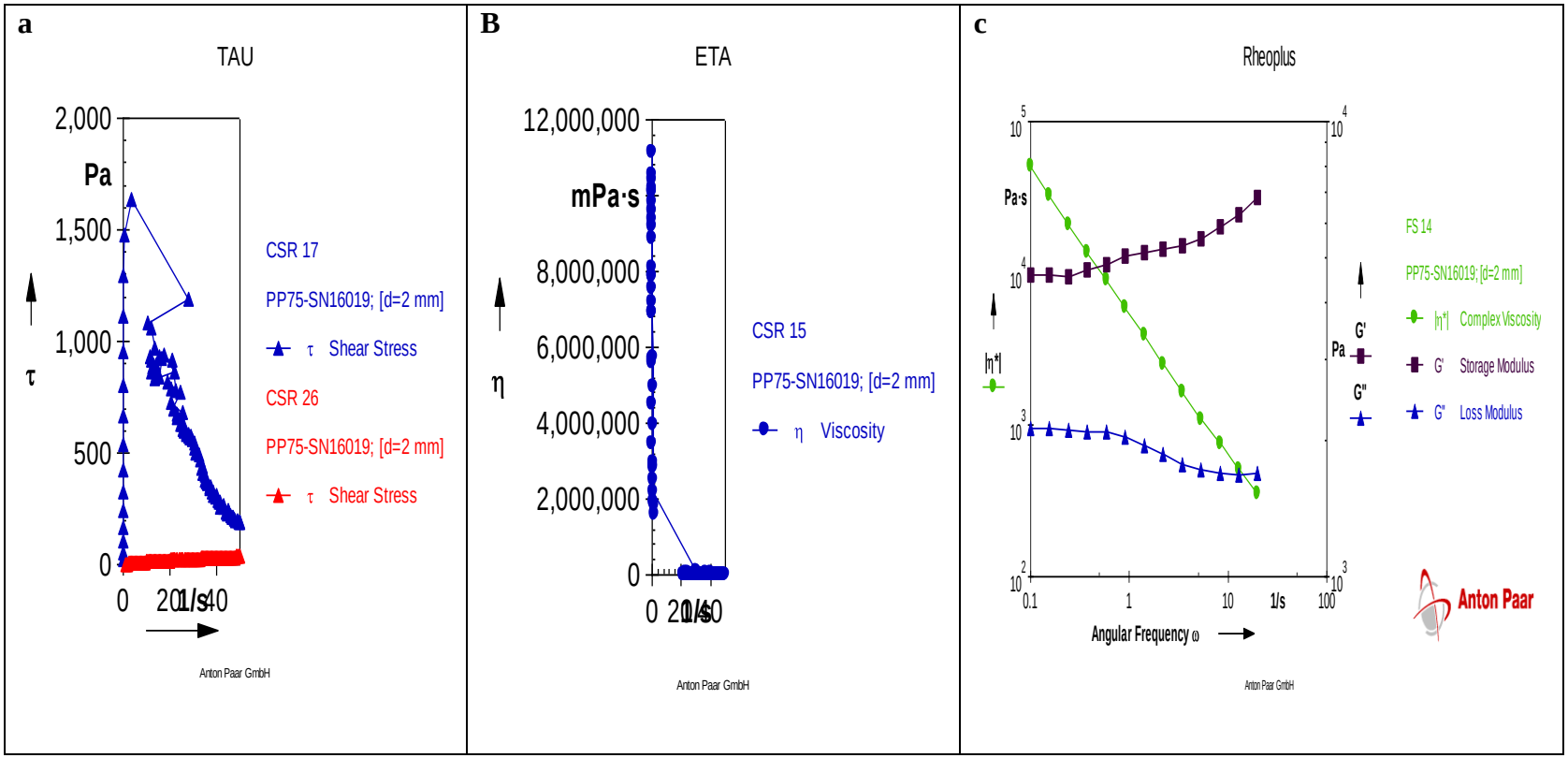

Figure 8. Dynamic rheology of sample 100% rice flour

4. Conclusions

The potential of the utilization of rice flour as a viable alternative to 100% wheat flour was reinforced in this study. However, it was established that 100% rice flour would be inappropriate rheologically. The incorporation of minimum of 20% pigeon-pea enhanced the farinograph and dynamic rheology properties of composite flour. The results showed that sample 3 as well as blends of wheat-rice with wheat flour component greater than 50% could be used in bread production. All other blends would be suitable for cookies and snacks production.

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COMPARISON OF TITULOMETRIC AND SPECTROPHOTOMETRIC APPROACHES TOWARDS THE DETERMINATION OF TOTAL SOLUBLE AND INSOLUBLE CARBOHYDRATES IN FOODSTUFF

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ABSTRACT

Total sugars and carbohydrates can be determined in foodstuff through several methods whose analytic principle may significantly differ. In view of this, the aim of this study was to evaluate the use of titrimetric and spectrophotometric methods to quantify soluble sugars and total carbohydrates in foods. Therefore, DNS, Somogyi-Nelson, Lane-Eynon, and Luff-Schoorl methods were used in the determination of total, reducing, and non-reducing sugars in three nectar-based products and soft drinks. Moreover, the analysis results of total carbohydrates were henceforth used to establish a comparison between the findings of wheat bran, cassava flour and canjica, which were assayed using Phenol-sulfuric and Anthrone methods. Results showcased that some samples presented different results according to the method therein used, what suggests the dependency of the findings to the analytical principle of the method. Thence, a deeper understanding of methodology might enhance results reproducibility regarding foodstuff analysis.

1.Introduction

Carbohydrates are chemical compounds whose structural diversity renders their segregation in several classes. Amongst these classes are simple sugars, which encompass monosaccharides and oligosaccharides, as well as those with a more complex structure, such as polysaccharides including starch, cellulose, hemicellulose, gums, and pectins. When total sugars in foodstuff are concerned, only metabolically active monosaccharides and oligosaccharides are considered, including glucose, fructose, and sucrose (BeMiller, 2019).

The demand for low-sugar foods has been steadily increasing in order to minimize the adverse health effects caused by high sugar intake (Başkan et al., 2016). The concentration of easily digestible carbohydrates in food is controlled in some instances by Brazilian Legislation. Regarding products under law monitoring, nectars pose as remarkable examples. These products are unfermented drinks obtained through dilution of fruit pulp in sweetened drinking water, and legislation recommends a minimum of 7% of total sugars for guava, mango and peach nectars (Brazil, 2003). Other noteworthy products are soft drinks, which are obtained by the dissolution of juice or vegetal extract in sparkling water. Albeit legislation specifies total sugars levels in nectars, soft drinks do not present minimum or maximum

amount of added sugars defined by any Brazilian law (Brazil, 2009).

The determination of sugars is common in carbohydrate research and the importance of quantifying total sugars and / or total carbohydrates is related to several instances of food quality control, namely: clear and informative labeling; nutritional information; fruit ripening indication; adequate fermentative control; jam-based products control; presence of adulterants, amongst others.

The concentration of carbohydrates or sugars is determinable in foodstuff either through titration, gravimetry, spectrophotometry or chromatography. In addition to quantifying, chromatographic methods also identify sugars which are present in the sample. Amongst the most common chromatographic techniques are layer chromatography, gas chromatography (GC), and high-performance liquid chromatography (HPLC). HPLC and GC can be coupled to nuclear magnetic resonance (NMR) or mass spectrometry (MS) in order to enable the identification of the chemical structure of the eluted chemical compounds (Koh et al., 2018).

Regarding titrimetric methods, Lane-Eynon and Luff-Schoorl are based on the reaction between reducing sugars and alkaline solution of copper sulphate, with a subsequent reduction of cupric copper to cuprous oxide. In the Luff-Schoorl method, Cu^{2+} ions which had not been reduced are determined iodometrically, while the reduced copper is determined in Lane-Eynon (Sá et al., 2018). Furthermore, total sugars are thence determined by converting non-reducing sugars into reducing sugars through acid or enzymatic hydrolysis (Marques et al., 2016; Tavares et al., 2010).

Concerning spectrophotometric approaches, Somogyi-Nelson method relies on the following procedures: reducing sugars are heated in alkaline medium to become enediols, which reduce Cu^{2+} to Cu^{+} ions. The formed cuprous oxide reduces the arsenic-molybdic reactive to a blue-colored molybdenum oxide (Shao & Lin, 2018). Furthermore, DNS method is based on the reduction of dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid in the presence of reducing sugar and NaOH, while different sugars are reported to produce different intensities in red-brown coloration (Başkan et al., 2016).

Polysaccharides in foodstuff might undergo hydrolysis rendering monosaccharides, which are of utmost importance in total carbohydrate content determination. For this purpose, the following methods were herein applied to determine total carbohydrate content: Anthrone method (Body et al., 2018); phenol-sulfuric method (Dubois et al., 1956; Wang et al., 2017; Le & Stuckey., 2016).

The samples herein used to compare total carbohydrates determination methods were: wheat bran, canjica corn and cassava flour. Wheat bran is the by-product of the manufacture of wheat flour. Its composition includes insoluble fibers (cellulose and lignin), starch, oligosaccharides and traces of reducing sugars and sucrose (Hemdane et al., 2016). Canjica corn is composed of grains or pieces of canjica corn, which present partial or total absence of the germ, depending on the mechanical or manual (degermination) scarification process (Brazil, 1989). In the carbohydrate fraction of canjica, a great quantity of starch is present, followed by fiber and low soluble sugars. (Strazzi, 2015). Dry cassava flour is the product obtained from healthy cassava roots, duly cleaned, peeled, crushed, grated, grounded, pressed, sieved and dried at the appropriate temperature (Brazil, 2011). Dias and Leonel (2006) characterized cassava flour from different Brazilian states and reported variations in the levels of starch, fiber and soluble sugars.

The use of titrimetric and spectrophotometric methods is still present in accredited laboratories for the issuance of food quality reports. Moreover, these tests are also reliable for industrial and scientific purposes, being largely used in foodstuff research.

Spectrophotometric methods for total sugar determination are in current use hence their good sensitivity, low cost, easy operation and accessible equipment. Literature also reports colorimetric tests for sugar determination in varied samples, albeit their analytic features are not as remarkable as those of spectrophotometric assays (Başkan et al., 2016).

Due to the different characteristics of each method, and the importance of determining sugar content in foods, the present work aims to compare titration and spectrophotometric methods used in the determination of soluble sugars and total carbohydrates in foods in order to verify their accuracy in the detection of these nutrients.

2. Materials and methods

2.1. Determination of total sugars (total soluble carbohydrates)

The first stage of this study involved the analysis of reducing sugars, sucrose, and total sugars in three nectar-based products, namely: peach, guava, and mango nectars, as well as three flavors of soft drinks: lemon, guarana, and orange. The samples were purchased in a supermarket located in Goiania, Goias, Brazil. The soft drinks were degassed before analysis. Solution of known concentration of analytic-grade glucose, fructose and sucrose were used as controls. For determination of total sugars was applied the methods Lane-Eynon (Fehling), Luff-Schoorl, DNS and Somogyi – Nelson.

The overall procedure was divided in two steps: quantification of sugars pre-hydrolysis and post-hydrolysis. The calculation of dilutions was based on the linear range of the methods. Therefore, from 0.02 to 0.12 mg of sugar was established for the Somogyi-Nelson according to the methodology described in this study. Regarding DNS, linear range was from 0.10 to 0.54 mg, while Luff-Schoorl was up to 50 mg, and Lane-Eynon up to 100 mg. Another possibility to verify whether the dilution is correct in the case of spectrophotometric methods is to observe whether the absorbances of the samples are close to the reading range of the absorbances of the standard curve.

Whenever possible, it is suggested to proceed with only one (1) dilution to remove aliquots from the sample; spectrophotometric methods are generally more demanding regarding sample clarity, therefore, a filtration was performed to remove solid particles after dilution.

It is important to highlight that colored or turbid samples may interfere with turning point verification or spectrophotometric readings. Thus, they must be clarified with the use of activated carbon or precipitated with lead acetate. Acetate forms insoluble complexes with interfering substances, which may be removed through filtration or centrifugation (McClements, 2014). The methods used are described below:

• *Lane-Eynon (Fehling)*

It was used an adaptation of the method described by Lane & Eynon (1934) and Fehling's solution was standardized using 1% glucose solution. Therefore, the method comprises as it follows: addition of 10 mL of Fehling A solution, 10 mL of Fehling B solution, and 40 mL of water in erlenmeyer followed by boiling of the mixture in a magnetic stirrer under heating (Hotlab, Nalgon). Addition and titration of methylene blue indicator in sample solution until red precipitate appeared. Sucrose after acid hydrolysis is determined considering the relation described by Meade (1967): addition of 10 mL of HCl per 10 mL of solution with 17°Brix for hydrolysis and 20 mL of 6 N

NaOH for neutralization. A ten-minute water bath (Dubnoff NT-232, Novatecnica) at 60°C followed the acid addition, and was neutralized with NaOH 6N, cooled, and the volume re-filled. Calculation of sucrose content occurred through the difference between reducing sugars content pre and post hydrolysis with a conversion factor of 0.95. The content of total sugars is the sum of reducing sugars and sucrose.

Lane-Eynon method requires constant boiling during titration to prevent cuprous oxide (reddish-colored) from being oxidized by the oxygen present in the air and returning to the cupric oxide (blue color) (Tavares et al., 2010). In addition, considering the necessity of heating, titration should continue up to three minutes in order to avoid the degradation of sugars from prolonged exposure to heating.

• *Luff-Schoorl*

The samples were analyzed according to Matissek et al. (1998) followed by a blank assay preparation with 25 mL of Luff-Schoorl reagent, 25 mL of water, 10 mL of 30% potassium iodide, 25 mL of 6 N sulfuric acid solution, and 0.5 mL of 1% starch. The mix was titrated with 0.1 N sodium thiosulfate up to white coloration. The samples were then added with 25 mL of Luff-Schoorl reagent and 25 mL of sample. The solution was heated on magnetic stirrer with heating (Hotlab, Nalgon) coupled to reflux, and subjected to 10-minute boiling. Thereafter, the solution was cooled and added with 10 mL of 30% potassium iodide, 25 mL of 6N sulfuric acid solution and 0.5 mL of 1% starch. Thereafter, the solution was titrated with 0.1 N sodium thiosulfate to white coloration. Calculation of the difference in blank volume and titrated sample volume was followed by the insertion of the amount of reducing sugar in mg in the table cited by Vicente (1994). Sucrose was determined according to Meade (1967) after acid hydrolysis and calculated by the difference between the reducing sugars content pre and post hydrolysis using a conversion factor of 0.95. The content of total sugars is the sum of reducing sugars and sucrose.

For most of Luff-Schoorl method application cases, the calculation of the results should involve interpolation hence the values relating to the titrated volume and to the amount of sugar are tabulated.

• *DNS*

The method described by Miller (1959) was proceeded with an adaptation and was prepared using a standard curve with glucose concentrations ranging from 100 to 540 μg. Sample dilution was followed by aliquots of 0.3 to 1.0 mL pipetted into test tubes, addition of 2.0 mL of DNS reagent, and volume quenched to 4.2 mL with water followed by water bath (Dubnoff NT-232, Novatecnica) for six minutes at 100°C and cooled in running water. The absorbance at 540 nm was read in a spectrophotometer (Rayleigh UV-1601 UV-VIS), sucrose was determined after acid hydrolysis according to Meade (1967) and calculated by the difference between the content of reducing sugars pre and post hydrolysis and multiplication by the conversion factor of 0.95. The content of total sugars was determined by the sum of reducing sugars and sucrose.

• *Somogyi-Nelson*

The methodology herein used was adapted from Somogyi (1945) and made use of a standard curve with concentrations ranging from 20 to 120 μg from a 0.01% glucose solution for reducing sugars determination. Thence, 1.2 mL of water and 1.0 mL of cupric reagent were added for the reaction and underwent boiling water bath (Dubnoff NT-232, Novatecnica) for 10 minutes. The mix was then cooled in running water and added with 1.0 mL of arsenic-molybdic reagent and 6.0 mL of water. Absorbance was read at 510 nm in a spectrophotometer (Rayleigh UV-1601 UV-

VIS). Sucrose was determined after acid hydrolysis according to Meade (1967) and calculated by the difference between the content of reducing sugars pre and post hydrolysis followed by multiplication by the conversion factor of 0.95. Total sugars content is the sum of reducing sugars and sucrose.

2.2. Determination of total carbohydrates

At the second stage, total carbohydrate analysis was performed on samples of wheat bran, cassava flour, and canjica, purchased at a supermarket in the city of Goiania, Goias, Brazil. The samples were milled and screened in a 20 mesh sieve before analysis, and to validate the methodologies, solutions of known concentration containing analytic-grade xanthan gum, pectin citric, starch, and sucrose were used as controls.

In the case of foods containing higher proportion of polysaccharides than soluble sugars, it is not advisable to apply the Brix measurement, which led us to seek to know the food composition and proceed with dilution attempts according to the working range of the chosen method.

Notwithstanding, it is recommended to use a single dilution, since the amount of dilutions multiplies the errors. After dilution, the aliquots should be pipetted under agitation considering the samples insolubility in water as well as to avoid the precipitation of carbohydrates. In the case of starch-rich samples, heating in aqueous solution may benefit the dilution process. For samples which are rich in insoluble fibers, heating has no effect, while for wheat bran, sample preparation consisted of sifting the bran (20 mesh sieve), performing the dilution, and pipetting the aliquots under stirring. The following methods were therefore applied:

• *Phenol-sulfuric*

The analysis described by Dubois et al. (1956) was carried out with a few modifications and proceeded with a curve with glucose concentrations ranging from 10 to 90 μg. After sample preparation, aliquots of 0.2 to 1.5 mL were pipetted into test tubes and the volume was filled until 2.0 mL with 0.8 mL of 5% (w/w) phenol and 5 mL of sulfuric acid. The tubes were then shaken and left to stand for 30 minutes for further reading in a spectrophotometer (Rayleigh UV-1601 UV-VIS) at 490 nm.

• *Anthrone*

Methodology adapted from Morris (1948). Standard curve was prepared with glucose concentrations ranging from 50 to 300 μg. Sample preparation was followed by aliquots of 0.2 to 1.2 mL and pipetted into test tubes. The volume was thereafter filled until 2.0 mL with water. 4 mL of Anthrone reagent was then added to all tubes. The tubes were shaken and allowed to stand for 15 minutes. The absorbance was read in a spectrophotometer (Rayleigh UV-1601 UV-VIS) at 620 nm.

2.3. Statistical analysis

Statistical analysis was performed with at least two replicates in triplicate. Data of reducing sugars, sucrose and total sugars were subjected to analysis of variance (ANOVA) using the program Statistica version 10.0 followed by Tukey test at 5%, while total carbohydrates analysis occurred through Student test at 5%.

3.Results and discussions

3.1. Determination of total sugars

The control solution revealed a difference in the determination of reducing sugars between the results of Somogyi-Nelson, DNS and Luff-Schoorl (Table 1). In general, all methodologies presented satisfactory results with a maximum deviation of 0.79.

Table 1. Total sugars (TS), reducing sugars (RS) and sucrose contents of the control solution and different flavors of nectars

Sample	Analysis	DNS	Eynon	Luff	Somogyi	°Brix
Control	TS	9.74 ± 0.79^{a}	9.85 ± 0.07^{a}	9.51 ± 0.0^{a}	10.17 ± 0.51^{a}	10.0
	RS	7.65 ± 0.16^{b}	7.92 ± 0.0^{ab}	7.76 ± 0.04^{b}	8.40 ± 0.18^{a}	
	Sucrose	2.08 ± 0.63^{a}	1.93 ± 0.07^{a}	1.75 ± 0.03^{a}	1.77 ± 0.33^{a}	
Guava	TS	13.95 ± 0.33^{a}	13.08 ± 0.23^{ab}	12.38 ± 0.11^{b}	12.89 ± 0.29^{b}	13.6
	RS	6.73 ± 0.06^{a}	6.01 ± 0.07^{a}	6.17 ± 0.02^{a}	6.29 ± 0.37^{a}	
	Sucrose	7.22 ± 0.40^{a}	7.07 ± 0.15^{ab}	6.22 ± 0.09^{b}	6.61 ± 0.09^{ab}	
Mango	TS	14.55 ± 0.19^{a}	13.55 ± 0.08^{b}	13.29 ± 0.31^{b}	13.59 ± 0.31^{ab}	14.8
	RS	7.79 ± 0.09^{a}	7.17 ± 0.16^{b}	6.98 ± 0.09^{b}	7.80 ± 0.02^{a}	
	Sucrose	6.76 ± 0.28^{a}	6.38 ± 0.24^{a}	6.32 ± 0.21^{a}	5.80 ± 0.29^{a}	
Peach	TS	12.12 ± 0.15^{a}	11.03 ± 0.23^{b}	11.71 ± 0.01^{ab}	12.34 ± 0.38^{a}	12.0
	RS	3.87 ± 0.04^{a}	3.56 ± 0.19^{a}	3.77 ± 0.01^{a}	3.92 ± 0.02^{a}	
	Sucrose	8.25 ± 0.19^{ab}	7.48 ± 0.04^{b}	7.95 ± 0.02^{ab}	8.43 ± 0.36^{a}	

Expressed as mean ± standard deviation. Different letters on the same line indicate a significant difference ($P \leq 0.05$) by the Tukey test at 5%.

Brix measurement was performed to render reference values. °Brix establishes the amount of total soluble solids in the sample expressed as percentage (% w / w); it usually refers to soluble sugars, but may also be atributed to organic acids, minerals, and water-soluble vitamins as well (Dongare et al., 2015); therefore, Brix provides an approximate value of total sugars.

Caldas et al. (2015) conducted comparative experiments between refractometry, phenol-sulfuric method, and liquid chromatography applied to the determination of total sugars in nectar and concentrated grape juice. For these samples, refractometry was adequate to routine measurements of total sugars and presented the lowest variation among the contents, in addition to an easy and simple use.

In the case of guava nectar, the Brazilian legislation recommends the minimum of: 10°Brix, 7% of total sugars, and 35% of pulp (Brazil, 2003) and according to Ishartani et al. (2018), guava has approximately 7% of soluble sugars, which are largely composed of fructose and glucose as well as a low sucrose amount (< 2%). However, the nectar is known to be added with sucrose. Therefore, the investigation was proceeded with the quantification of total sugars by reducing sugars and sucrose using the methods indicated for such analysis, namelly: Luff-Schoorl, Lane-Eynon, DNS, and Somogyi-Nelson.

The results of guava nectar indicated no difference between the methodologies for reducing sugars while sucrose had a difference between DNS and Luff-Schoorl values, which led to differences in total sugars between both methods as well.

Concerning mango nectar, Brazilian Ministry of Agriculture established the minimum range of 10°Brix, 7% for total sugars, and 40% of pulp (Brazil, 2003). In the study by Dar et al. (2016) mango presents sugar concentrations in descending order, namelly: sucrose, fructose, and glucose, as well as that the aldose content does not exceed 1% and the analysis of reducing sugars of mango nectar indicated that spectrophotometric methods statistically differ from titrimetric methods; however, sucrose had no difference between the results. Regarding the values of total sugars, DNS showed a difference in relation to titrimetric methods, which in turn did not differ from the Somogyi-Nelson results.

Brazilian legislation states that peach nectar must have at least 10°Brix, 7% total sugars, and 40% fruit pulp (Brazil, 2003).

The results of sucrose analysis given by Somogyi-Nelson differed from Lane-Eynon. Sucrose content was verified to be practically double than that of reducing sugars, which, in turn, had no statistical difference between the methods herein used. The content of reducing sugars was below the remaining flavors, probably because, according to Saidani et al. (2017), peach presents about 10% of total sugars, out of which 80% is sucrose and the remaining is glucose, fructose, and others. Regarding total sugars, the values found by DNS and Somogyi-Nelson presented statistical difference regarding the value obtained by Lane-Eynon method.

Concerning the production of soft drinks, sucrose may be partially or totally replaced with inverted sucrose, fructose or glucose. Minimum or maximum quantities of sugars are not defined in the Brazilian legislation.

Lemon soda should contain at least 2.5% lemon juice according to the Brazilian legislation (Brazil, 2009). Regarding total sugars determination, Luff-Schoorl differed from spectrophotometric methods (Table 2) while for reducing sugars, the value found by applying the Somogyi-Nelson technique differed from the others. The sucrose contents of the titrimetric methods were similar to each other, but different from the ones of spectrophotometric tests.

Orange soda should contain at least 10% orange juice, which in turn must have a minimum of 10.5°Brix according to Brazilian legislation (Brazil, 1998). The different methodologies had no distinguishable results for total sugars, reducing sugars, and sucrose.

Guarana soft drink is produced with at least 20 mg / 100 mL of natural guarana extract according to the Brazilian legislation (Brazil, 2009).

Table 2. Total sugars (TS), reducing sugars (RS) and sucrose contents of different flavors of soft drinks

Sample	Analysis	DNS	Eynon	Luff	Somogyi	°Brix
Lemon	TS	10.30 ± 0.12^{bc}	10.60 ± 0.04^{ab}	10.74 ± 0.06^{a}	10.20 ± 0.07^{c}	10.0
	RS	2.90 ± 0.0^{b}	2.60 ± 0.11^{c}	2.75 ± 0.06^{bc}	3.27 ± 0.08^{a}	
	Sucrose	7.40 ± 0.12^{b}	8.00 ± 0.14^{a}	7.99 ± 0.00^{a}	6.94 ± 0.01^{c}	
Orange	TS	10.00 ± 0.30^{a}	10.21 ± 0.14^{a}	9.84 ± 0.27^{a}	9.59 ± 0.22^{a}	11.0
	RS	4.10 ± 0.04^{a}	3.82 ± 0.03^{a}	3.87 ± 0.02^{a}	3.82 ± 0.19^{a}	
	Sucrose	5.80 ± 0.26^{a}	6.39 ± 0.11^{a}	5.97 ± 0.25^{a}	5.78 ± 0.03^{a}	
Guarana	TS	8.41 ± 0.15^{a}	8.40 ± 0.05^{a}	8.40 ± 0.18^{a}	8.19 ± 0.14^{a}	9.0
	RS	1.04 ± 0.01^{a}	0.95 ± 0.02^{ab}	0.91 ± 0.02^{b}	0.93 ± 0.04^{ab}	
	Sucrose	7.37 ± 0.16^{a}	7.40 ± 0.07^{a}	7.50 ± 0.16^{a}	7.26 ± 0.10^{a}	

Expressed as mean ± standard deviation. Different letters on the same line indicate a significant difference ($P \leq 0.05$) by the Tukey test at 5%.

The methods to quantify the total sugars of guarana soft drink did not indicate different results. The content of reducing sugars achieved through the DNS was different from the values obtained through Luff-Schoorl. All methodologies presented similar results for sucrose. Guarana soda presented the lowest reducing sugar content in relation to the remaining assayed flavors. This fact reflected the sample preparation, especially for the Lane-Eynon technique.

Most of the sugars in guarana soft drink are sucrose, which is an important point to emphasize since knowledge on the food composition benefits the satisfactory progress of the analysis. Therefore, Brix measurement revealed that the three flavors of soft drinks have close Brix degrees, but the

sucrose content of guarana soft drink is above that of reducing sugars.

In order to calculate the dilution during the stage without hydrolysis, the proportion of reducing and non-reducing sugars was estimated. Considering the presence of small amounts of reducing sugars in guarana soft drink, it did not require dilution to undergo Lane-Eynon method. In order to proceed with the analysis through such method, the preparation of the sample dilution considers that the solution must contain about 1 g of reducing sugars, a quantity based on the standard solution, which is taken as reference for the calculations. The stage involving hydrolysis uses the Brix value to calculate the dilution as an approximation of the total sugars.

During the execution of the analysis, it was found that the Lane-Eynon method is indicated for samples with amounts of sugars above 1%; in contrast, the pure sample should be directly titrated (*i.e.* without dilution), which may lead to higher sample volume expenditure considering repetitions and results accuracy. Despite being an official method, it was recommended to substitute the Lane-Eynon with another more sensitive method at low concentrations in these cases.

In contrast, the Somogyi-Nelson method is adequate to low concentrations, which led us to understand that samples with higher total sugars (> 10%) should not undergo it, considering its demand for large dilution volumes.

During sucrose hydrolysis process, formation of furfural (in the case of pentoses) or hydroxymethylfurfural (in the case of hexoses) may result from the action of acids and heat. Incorrect hydrolysis (excess acid, heating time) may favor the formation of these compounds; therefore, it is necessary to neutralize the sample after the acid hydrolysis as well. The presence of furfural leads to a lower amount of sugar in relation to the real value, since it has no reducing property and does not react with Fehling or Luff-Schoorl solution. Moreover, an overestimated value is obtained through the DNS method derived from furfural influence on the coloration, and consequently, the absorbance value.

Another important point is that ascorbic acid significantly interferes in the results of reducing sugars and total sugars analysis, either through the Lane-Eynon method or others with the basic principle of oxidation-reduction reaction. Therefore, Tavares et al. (2010) proposed an approach to calculate the error for foods with high concentrations of ascorbic acid (above 500 mg / 100 g) and low concentrations of sugars.

Baskan et al. (2016) also emphasize that polyphenols can interfere in the determination of reducing sugars when using colorimetric methods based on redox reactions.

Silva et al. (2003) compared several methods to establish reducing sugars, sucrose, and total sugars in honeys to verify that the results of sucrose and total sugars presented no difference between the applied methods; however, for reducing sugars, the Luff-Schoorl methodologies and Munson-Walker generated lower values.

Demiate et al. (2002) compared the Somogyi-Nelson and Lane-Eynon method with Phenol-sulfuric acid (which is taken as the reference) in the determination of reducing sugars and total sugars of apple juice and soft drinks. The analysis of variance indicated no significant difference between the studied methodologies considering the significance level of 1%.

3.2. Determination of total carbohydrates

The methods showed no difference regarding the control solution and the results of total carbohydrates. Moreover, the wheat bran presented no significant difference (Table 3).

Table 3. Total carbohydrates contents of wheat bran, cassava flour and canjica

Sample	Phenol-sulfuric	Anthrone
Control	0.53 ± 0.02^{ns}	0.51 ± 0.02^{ns}
Wheat bran	74.5 ± 1.13^{ns}	72.4 ± 2.33^{ns}
Cassava flour	90.6 ± 0.55^{s}	83.8 ± 1.89^{s}
Canjica	76.1 ± 2.97^{ns}	77.5 ± 1.30^{ns}

Expressed as mean ± standard deviation. Averages in the same line do not differ significantly (ns) or differ (s) from each other ($P \leq 0.05$).

Regarding cassava flour, Dias & Leonel (2006) characterized cassava flour from different Brazilian states and reported variations in starch, fiber, and soluble sugars. Indeed, a significant difference appeared in the analysis of total carbohydrates. The application of the Phenol-sulfuric methodology revealed highest content, a value close to the findings of Nepa (2011), 89.2% of total carbohydrates.

White canjica contains 78.1% of carbohydrates according to Nepa (2011) and a value close to those rendered through Phenol-sulfuric and Anthrone methods, with no significant difference.

The methods used in this work to analyze total carbohydrates have different linear ranges: Anthrone method quantifies from 0.05 to 0.3 mg of carbohydrates while Phenol-sulfuric technique ranges between 0.01 and 0.09 mg.

According to Silva et al. (2003), a major disadvantage of the Phenol-sulfuric method is the use of higher amounts of sulfuric acid. Nevertheless, it is a simple method which generates sensitive and reproducible results. The case of Anthrone method involves the use of sulfuric acid as well, but only in the Anthrone reagent preparation. Nonetheless, it was verified that Anthrone method had greater difficulty regarding the reproducibility of the results.

Total carbohydrates are normally calculated by inference, considering the difficulty of analysis or even that the environmentally hazardous potential of the recommended techniques (Phenol-sulfuric and Anthrone). The calculation by inference can lead to erroneous results hence experimental errors in relation to the methods associated with the determination of other nutrients. Therefore, a direct measure of total carbohydrate content should be carried out upon an expressive content.

Method linear range and food composition have high influence on the results. It is important to observe that the choice of method is associated with the sample type, method working range, cost of equipment and reagents, time of analysis, including time to prepare reagents, sample, and reaction time. It is also worth noticing the issue of chemical residues generation and the determination purpose, either for routine analysis or research.

4. Conclusions

The analytical methods were successfully applied in quantitative tests of soluble sugars and total carbohydrates of nectars, soft drinks, wheat bran, cassava flour, and canjica. The spectrophotometric methods are commonly regarded as more accurate than titrimetric. However, the present study showed that no differences were found between most of the results. For the samples which undergone total carbohydrates determination, the analysis through Phenol-Sulfuric method may render more reproducible results than through Anthrone method.

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EVALUATION OF POSTHARVEST BEHAVIOR OF COCONUT (*Cocos nucifera* L.)

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ABSTRACT

Coconut is a Tropical fruit of interest for Colombia; one part of its production is used at industrial level; nonetheless, there is little diversification of products with added value and lack of availing of coconut water, the husk, and peel. The aim of this research was to evaluate the behavior of the physical and physical-chemical properties of coconut pulp (CP) and its coconut water (CW) during storage at 25 °C, to determine the adequate time for use as raw material for its transformation, using a completely random design (CRD) via analysis of variance (ANOVA) and Tukey tests, with 5% significance level. Where the independent variable were the control times at 15, 22, 29, 36, 43, and 50 days after harvest, at the rate of 3 coconuts/lot for each control time. Among the response variables we determined the percentage distribution of the CP, CW, and inner shell (endocarp), as well as properties of Xw, pH, soluble solids, acidity, a_w, color (L*, a*, b*), viscosity, and texture. Results showed general CP and CW deterioration after de 36 days of storage, mainly due to increased acidity, fermentation odors, loss of Xw, lipid oxidation (LO), and CP softening and discoloration, among others.

1.Introduction

Coconut represents a raw material of great interest in the Colombian Caribbean and Pacific. Its production was of 114,773 t in 2013, with the departments of Nariño, Córdoba, and Cauca the principal producers: 33.7, 26.1, and 15.1%, respectively (Agronet, 2013). At agro-industrial level, the kernel is used mainly to produce coconut milk (CM) and dehydrated coconut. In addition, coconut oil is used in the cosmetics sector (Debmandal and Mandal, 2011; Seow and Gwee, 1997). Compositionally, CP has been evaluated with Xw: 41.7±0.5%, fat: 40.2±1.2%, proteins: 4.1±0.3%, sugar: 5.6±0.2%, and raw fiber: 3.5±0.1%; while dehydrated CP present fat content values: 65.5±2.0%, protein: 6.8±0.4%, sugar: 6.5±0.3%, raw fiber: 9.2±0.2%, and carbohydrates: 6.0±0.2%. (Yalegama *et al.*, 2013). Other authors have evaluated the quality of Macapuno coconut in mature state during 42 days of storage, to simulate conditions in retail markets, finding that at 30 °C the fruit undergoes a high respiration rate between 40 and 60 mg CO_2 $kg^{-1}h^{-1}$ with 3-day maximum storage time. At 2 and 5 °C the respiration rate diminishes (4 and 20 mg CO_2 kg^{-1} h^{-1}, respectively) with a low rate of ethylene production (0.6 and 0.8 $Lkg^{-1}h^{-1}$, respectively), permitting a storage life up to six weeks (Luengwilai *et al.*, (2014). This weight loss limitation is also accompanied by darkening of the nucleus and of the degree of fat oxidation (Luengwilai *et al.*, 2014). The aim of this research was to evaluate the behavior of the physical and physical-chemical properties of CP

and CW, Enano Malayo variety (manila), during storage at 25 ºC to determine the adequate time for its processing as raw material for its transformation.

2. Materials and methods

2.1. Materials

The fruit yield was determined as percentage ratios of the CP, CW, and the inner shell (endocarp). The CP and CW characterization was performed in terms of humidity (Xw): AOCW method 930.15/90; water activity (a_w): spray point hygrometer (Aqualab series 3TE, Decagon, Devices, Pullman, WA, USA) (Cortés-Rodríguez *et al*., 2007); ºBrix: AOCW method 932.12/90; LO: the proportional mix (CP+CW) was evaluated by using the spectrophotometric method (Hornero-Méndez *et al.*, 2001) and determining the extractable oil through the method described by Bae & Lee, (2008) modified; viscosity of the CW (μ): rheometer (Brookfield DV-III Ultra (Brookfield Engineering Laboratories, Inc., USA) a 25 °C, ULA spindle and velocity at 250 rpm (Mirhosseini *et al.*, 2008); CP texture: determined the CP average firmness (F_{CP}) through penetration tests in a texture analyzer (TA-TA-XT2i, Stable Microsystems Ltd., United Kingdom), 25-kgf load cell, stainless steel probe (ϕ = 5 mm), rate of penetration: 1.0 mm s^{-1} and penetration distance of 3 mm (Prieto *et al.*, 2011); acidity index (IA): AOCW method 942.05/90, expressed as malic acid for CW and as lauric acid for CP; pH: AOCW method 981.12/90; color: coordinates of the CIELAB (L*:Luminosity, a*: red-green chromaticity, b*: yellow-blue chromaticity), X-Rite spectrophotometer, D_{65} illuminant, and 10° observer (Cortés, 2004). In addition, bromatological characterization was carried out: fat (AOCW method 920.39/90), proteins (AOCW method 955.04/90), total dietary fiber (AOCW method 985.29/90), and ashes (AOCW method 942.05/90). Calcium quantification was performed via atomic absorption spectrophotometry, according to NTC 5151 of 2003. A completely random design (CRD) was used to evaluate the results, with analysis of variance (ANOVA) and Tukey tests, with 5% significance level. The independent variable were the control times at 15, 22, 29, 36, 43, and 50 days after harvest, at the rate of 3 coconuts/lot for each control time and the response variables for CP and CW were: Xw, a_w, pH, color (L, a*, b*), IP, soluble solids. Texture and acidity index were also determined for CP. For CW: density and percentage of acidity and percentage distribution of CP, CW, and peel.

2.2.1. Samples

The study used coconuts (*Cocos nucifera* L.) of the Enano Malayo (Manila), Tumaco, Colombia varieties, with an age from bloom to harvest of 12 months. Three lots were evaluated (three samples/lot) up to 50 days after harvest (dah).

3. Results and discussions

The bromatological composition of CP at 15 dah (date received in the collection center) was the following: Xw: 53.5±6.4%, protein 3.3±0.3%, dietary fiber 12.9±2.5%, fat 19.9±3.0%, ashes 1.1±0.3%; additionally, calcium contents for CP and CW were statistically similar, 146.3±41.3 and 108.0±16.5 mg/kg, respectively. The bibliographic review reports a variability of results on the coconut composition, attributable to diverse factors, such as variety, agronomic management, edafoclimatological conditions of the production zone, state of maturity, among others (Siriphanich *et al.*, 2011). Yalegama *et al.*, (2013) reported for CP values of Xw: 41.7±0.5%, fat 40.2±1.2%, protein: 4.1±0.3, sugar: 5.6±0.2%, and raw fiber 3.5±0.1%; while Appaiah *et al.*, (2015) reported higher values of Xw (51.0±0.3%). Regarding calcium contents in CW, the same variability occurs, reporting in unripe (8.75±0.04 mg/100 mL), ripe (15.19±0.03 mg/100 mL), and over-ripe states (23.98±0.05 mg/100 mL) (Thuan-Chew *et al.*, (2014).

Tables 1, 2 presents the distribution of the coconut parts and the CP and CW properties

during storage. The ANOVA presented statistically significant differences ($p < 0.05$) in the CW and CP percentage ratios and the CW properties (Xw, °Brix, density, IA, pH, μ and b*) with respect to the time factor; while there were no significant differences ($p > 0.05$) in the CP properties, or in the percentage ratio of the inner shell, the IP and in the CW a_w, L* and a*. The CW had a tendency to diminish the mass (30.1→15.4%), which has been observed during the physiological maturation during harvest by other authors and which continues during postharvest (Jackson *et al.,* 2004; Terdwongworakul *et al.,* 2009); while the CP and the peel tend to increase over time: (47.1→55.2%) and (26.0→33.8%), respectively. The CP and CW physiological phenomena are typical during maturation and senescence of this fruit, promoted by respiration, transpiration, and water absorption by the solid endosperm (Thuan-Chew *et al.,* 2014; Siriphanich *et al.,* 2011).

Table 1. Physical composition of CW and CP during storage

T (days)	CW (%)	CP (%)	Inner shell (%)		L*	a*	b*	μ (cP) Texture (N)
15	30.1±5.2c	47.1±3.1a	26.0±3.8a	CW	51.0±1.5c	0.3±1.0a	-1.0±0.3a	0.7±0.1ab
				CP	71.8±4.3b	-1.1±0.2a	3.1±0.8ab	81.1±8.3a
22	22.8±9.1b	49.5±3.8ab	30.4±7.1ab	CW	52.2±0.44bc	-1.08±0.0b	-0.02±0.06b	0.7±0.1a
				CP	72.7±10.6b	-0.8±0.5ab	3.7±1.9ab	75.7±8.5a
29	22.3+5.7b	51.9±4.7ab	30.0±5.3ab	CW	49.0±3.7c	1.5±1.8a	-0.9±0.2a	0.7±0.1ab
				CP	67.4±5.2ab	-0.8±0.3ab	3.7±1.3ab	78.0±13.9a
36	21.1±6.2bc	52.9±8.9bc	29.5±7.1ab	CW	50.9±3.0c	0.6±1.0a	-1.0±0.2a	0.7±0.1ab
				CP	67.5±10.9ab	-1.2±0.2a	2.4±1.5a	71.2±6.3a
42	15.9±6.6ab	53.6±2.4ab	31.0±4.5ab	CW	50.7±2.0c	-0.4±1.9a	-1.1±0.4a	0.8±0.1bc
				CP	69.6±9.0b	-0.3±1.5b	4.2±1.9ab	73.1±15.0a
50	15.4±1.5a	55.2±3.6b	33.8±7.4b	CW	50.8±1.2c	2.4±2.8a	-1.2±0.3a	0.8±0.1bc
				CP	61.7±6.9a	-0.8±0.6ab	3.7±2.0ab	73.5±19.8a

CW: coconut water; CP: coconut pulp; **μ**: viscosity; The values in the same column of the same variable, with the same letters indicate that there are no significant differences ($p > 0.05$).

Table 2. Chemical composition of CW and CP during storage

T (days)		Xw (%)	a_w	°Brix	ÍP (meqH_2O_2/ kg oil)	ÍA (p/v)	pH
15	CW	96.1±0.8c	0.981±0.007b	3.7±0.6a	0.7±0.3a	0.04±0.01a	5.7±0.4cd
	CP	50.4±5.2bc	0.978±0.005a	6.4±3.0bc		0.51±0.24a	6.1±0.2b
22	CW	95.9±0,4c	0.974±0.011ab	4.0±0.4ab	2.1±2.5a	0.04±0.01a	6.0±0.3cd
	CP	48.5±5.3bc	0.978±0.005a	5.1±1.7ab		0.60±0.16ab	6.0±0.3b
29	CW	95.9±1.0c	0.952±0.076a	4.1±1.0ab	2.3±2.0a	0.07±0.02a	6.2±0.2a
	CP	47.9±4.6bc	0.979±0.003a	4.8±1.1ab		0.66±0.11ab	5.6±0.8a

36	CW	95.1±1.2cd	0.971±0.012ab	5.1±1.1bc	2.8±2.4a	0.08±0.01a	5.5±0.6b
	CP	51.2±6.7c	0.979±0.007a	4.0±1.9a		0.68±0.13ab	6.0±0.2b
42	CW	94.0±1.6abc	0.969±0.012ab	5.8±1.6cd	3.5±4.0a	0.08±0.02a	5.7±0.6b
	CP	45.8±3.9ab	0.976±0.005a	5.2±2.3ab		0.66±0.17ab	6.0±0.2b
50	CW	93.5±1.5ab	0.969±0.013ab	7.1±1.8cd	3.9±1.8a	0.14±0.01a	5.6±0.1b
	CP	50.1±6.0bc	0.978±0.005a	4.6±1.8ab		0.72±0.16bc	6.0±0.2b

CW: coconut water; CP: coconut pulp; Xw: % humidity; ÍP: peroxide index; ÍA; acidity index, expressed in expressed as malic acid for AC and as lauric acid for PC. The values in the same column of the same variable, with the same letters indicate that there are no significant differences ($p>0.05$

The bibliographic review reports that on full maturity, changes in percentage distribution of coconut (*Cocos nucifera L.*) present many fluctuations: CP (28→33 %p/p), CW (6→25 %p/p), peel (31→54 %p/p) (Jayalekshmy *et al.*, 1986; Siriphanich *et al.*, 2011; Appaiah *et al.*, 2015). The Xw of the CP had values between 51.2 and 45.8%, with CW showing values of 96.1– 92.5%, corresponding in all cases to high values of a_w (0.980 – 0.952), which makes these matrices perishable and favorable to microbial growth and degradation processes (Appaiah *et al.*, 2015; Haseena *et al.*, 2010). The soluble solids of the CW increase progressively throughout the storage time, which could be attributed to internal dehydration undergone by the fruit and it is coherent with the decrease previously mentioned of the percentage ratio of the CW and of the Xw of the CW (96.1±0.8 →93.5±1.5).

With respect to the CW's μ, it tends to increase mainly because of the higher content of soluble solids in the CW concentrated over time (3.7±0.6 →7.1±1.8). The CP firmness (F_{CP}) provided by the fiber content present and of rigid or hard nature (Yalegama *et al.*, 2013; Raghavendra *et al.*, 2009) behaved as a homogeneous group with high variability, fluctuating between 81.1±8.3 and 73.5±19.8 N. Some authors have reported CP softening during storage because of CP disintegration and deterioration due to fungal growth, where the most frequent found in the coconut peels are Aspergillus spp., Penicillium spp., Fusarium spp., and Curvulria spp. (Haseena *et al.*, 2010; Luengwilai *et al.*, 2014).

The IP displayed similar behavior during storage time (0.74±0.3→3.9±1.8 meqH_2O_2/kg); it is a useful indicator of the degree of oxidation of lipids, fats, and oils that reduces stability and produces the formation of unpleasant taste that affect quality negatively during storage, as well as consumer acceptance due to the rancid odor (Hornero-Méndez *et al.*, 2001). Some authors have defined the start of rancidity in coconut when IP reaches values of 35.5 meq O_2 peroxide/kg oil (Waisundara *et al.*, 2007), a value higher than the maximum reached for the CP+CW mixture at 25 °C (1.6 meq H_2O_2/kg oil) (11.3 meq O_2 peroxide/kg oil); however, at 29 days of storage a strong rancid odor became evident. Now then, changes in IP were low for CP and CW; nevertheless, a tendency is noted to increased acidity and diminished pH over time, which revalidates the fermentation odors and rancidity of the product.

Regarding the color of the CW, L* varied from 51.0±1.5 to 50.8±1.2, showing a translucent liquid phase with a light brown tone and/or yellowing, emitting bad odor that could be attributable to the contraction and discoloration of the skin, the fall of the perianthus, and fungal attack in the soft perianthus region (Haseena *et al.*, 2010); while chromaticities a* and b* had values close to zero in the chromatic plane a*, b*, indicating its achromaticity or placement in the zone of grey tones. The CP was bright white, where L* varied between 71.8±4.3 and 61.7±6.9; while chromaticity a* remained between -1.1±0.2 and -0.8±0.6, and lastly b* from 3.1±0.8 to 3.7±2.0, very similar behavior to that reported by Luengwilai *et al.*, (2014), where no statistically

significant differences were noted in variables L* a*, while b* diminished 1.05 to -0.96.

Up to 29 to 36 dah, coconut does not show appreciable organoleptic changes that demerit its quality, but as of this time (42 dah) deterioration symptoms were observed (in CW and CP), like fermentation odors, fungal problems, softening and discoloration of the pulp, among others. This behavior coincides with that reported by Luengwilai *et al.*, (2014), who worked with Macapuno coconut, where peeled ripe coconut was stored at 2, 5, and 30 °C and its quality was evaluated after three days. During storage at 30 °C, the fruit showed a 3-day useful life. In contrast, with storage at 2 or 5 °C the storage life increased from three days to six weeks

4. Conclusions

The Enano Malayo coconut variety with acceptable quality for use as raw material in food processing must have a storage time below 29 dah. During this time, the physical-chemical and sensory characteristics of the products are within the acceptance limits; however, the fruit had high variability in CP and CW properties, which is due to the poor or almost null traceability available for this fruit.

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A SIMPLE SYSTEM TO DETECT AND MEASURE FORMALIN IN FRUIT BY USING CONDUCTIVITY, pH AND CAPACITANCE MEASUREMENT

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ABSTRACT

Economically motivated adulteration (EMA) of food is becoming common in some developing countries. Formalin is a harmful organic chemical substance which is often used by businessmen to keep their fruits etc. look fresh for longer times. It consists of two substances: formaldehyde and water. Formalin is used for long time preservation for various types of foods and other things from putrefaction. It affects human life as well as environmental ecology. At present, EMA by Formalin is becoming severe in countries like Bangladesh. Formalin detection is a challenging task. Most of the techniques to detect formalin are based on chemical sensors. The paper aims at detecting formalin in fruits by electrical properties such as conductivity and capacitance together with cheap pH sensor. The fruits are immersed in distilled water and conductivity, capacitance and pH of the water are measured. The result is very promising with an average error of 7.18%. For higher concentration the average error is 5.02%. Although the proposed method does not give result instantaneously, but it could lead to a method of detecting formalin mainly based on electrical properties in future.

1. Introduction

Economically motivated adulteration (EMA) is increasing tremendously in some countries in the name of preservatives. The people of developed country do not agree with the use of food additives at all (Zugravu et al., 2017). Detecting adulteration of fruits and fruit-derived products often requires costly equipment and complex process (Sobolev et al., 2015). Formalin is an aqueous solution of formaldehyde that is 37 per cent by weight, usually containing 10 to 15 per cent methanol to prevent polymerization of the formaldehyde. It is used in the manufacture of resins, textiles and as a laboratory fixative or preservative. But formalin is used by some businessman in countries like Bangladesh to preserve fruit for longer time. This practice is widespread now. This practice is severely dangerous (Ali, 2013). So detection of formalin is growing more attention in order to take preventive measures for public health safety.

Formalin detection using chemical and optical properties requires costly sensors and equipment (Möhlmann, 1985; Tang et al., 2016) as well as complex methodology. Detection method of formalin based on electrical properties could be a better solution because it

does not need any chemical sensor or reagents. For this purpose some researchers have tried in different ways. Development of portable electronic reader was proposed (Hashim et al., 2015) for detection of formaldehyde gas sensor. This electronic reader can detect the level of formaldehyde concentration, which later translated into voltage level. Three level of detection is possible such as high, medium and low concentration of gas respectively but exact quantitative measurement is not reported. To detect formalin based on optical characteristic, refractive index (Arif et al., 2016) is chosen for analysing through simulation. They used Photonic Crystal Fiber (PCF) based Formalin sensing technique. Their study reveals the refractive index of Formalin along with a proposal of a PCF structure for Formalin sensing but it was not implemented practically. Conductivity of formalin changes with the change of concentration of formalin (Hasan et al., 2014). This kit performs using only one property. Three level of detection is possible without exact quantitative measurement by this kit. A variable-temperature variable-thickness interferometer (Khan et al., 2007) was assembled to perform dispersive Fourier transform spectroscopy (DFTS) on liquids at millimeter and sub millimeter waves. During testing 10% formalin and 1,4-dioxane, they first used DFTS for environmental and biological applications. If formalin's broadband dielectric properties and signatures are known, spectroscopic analyses of preserved biological tissues can be done to identify potentially malignant or cancerous tissues.

Some methods discussed above are complex, some do not show conclusive results and some are still in simulation level. This led us to endeavor a method which would be simple yet provide conclusive result. We proposed a simple hybrid method of detecting formalin in fruit mainly based on electrical properties and only one chemical property. The primary results showed promising performance. To our knowledge, no studies have done so far in this way to detect formalin adulteration in fruits.

2. Materials and methods

2.1. Materials

Formalin has some distinct characteristic. We have used pH, conductivity and capacitance to detect and measure quantity of formalin in test solution. Conductivity varies for different concentrations of formalin. pH decreases from 7 (pH of distilled water) if formalin exists. At different concentration of formalin, capacitance changes from picofarad to microfarad range.

2.2. Conductivity measurement:

For conductivity measurement we have used Wheatstone bridge circuit (Coughlin et al., 2001).This is a simple technique for conductivity measurement. Actually we transformed change in conductivity to change in voltage using the circuit (Figure 1).

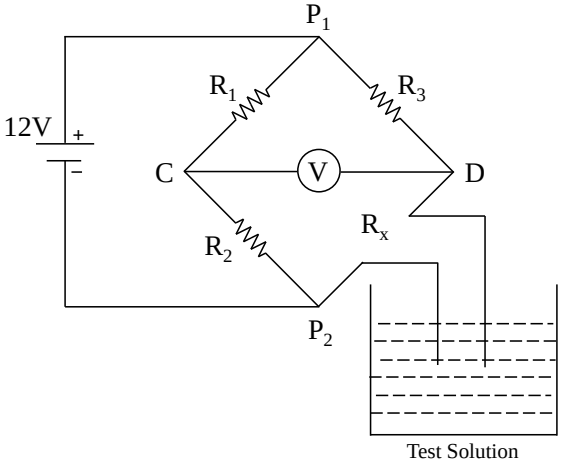

Figure 1. Wheatstone Bridge circuit

There are four resistors in our bridge circuit. They are R_1, R_2, R_3 and R_X. R_1, R_3 are simple 2W, 1MΩ resistor. R_x is the unknown resistance of water. The current through the multimeter depends on the potential difference between C and D. When the potential difference across the multimeter is zero, this is called null condition. It is acquired by adjusting potentiometer R_2.

From the figure,

The voltage at point D,

$$V_D = V \times R_X/(R_3 + R_X) \quad (1)$$

The voltage at point C,

$$V_C = V \times R_2/(R_1 + R_2) \quad (2)$$

The voltage (V) across galvanometer or between

P_1 and P_2 is,

$$V_{DC} = V \times R_X/(R_3 + R_x) - V \times R_2/(R_1 + R_2) \quad (3)$$

When the bridge is balanced i.e. $V_D = V_C$, then $V_{DC} = 0$

There is an almost linear relationship between concentration of formalin and conductivity. If the concentration of formalin increases, it decreases resistance and increases conductivity. This changes RX, which changes V_D, the bridge becomes unbalanced and V_{DC} has some value which is actually a function of concentration of formalin.

2.3. pH measurement:

Formalin has a distinct pH characteristic. So detection of formalin can be performed by using a pH sensor which gives output voltage according to pH of the solution. The pH of formalin is less than water's pH i.e. 7.

At different concentration of formalin, pH of the solution varies from 2 to 6.2. When we added 0.02cc formalin to 100 ml distilled water, we found pH 6.2. When we gradually mixed formalin with 100 ml distilled water, pH value decreases.

Figure 2. pH Sensor

2.4. Capacitance measurement:

Formalin has distinct capacitive characteristic. Normal water shows dielectric constant of 80. But formalin shows dielectric constant of 23. We have used parallel plate used for Printed Circuit Board (PCB) after some modification to measure the capacitance of formalin. Parallel plates are insulated by using color spray to reduce the conductive effect.

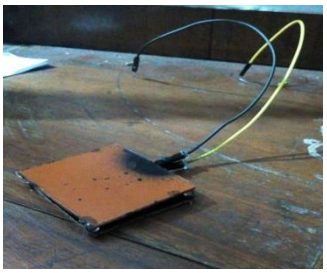

Figure 3. Practical Implementation of parallel plate capacitor using PCB

The detection circuit used for this study used auto range detection schemes for different ranges of capacitors. We used Arduino Mega for measuring capacitance. Each capacitance meter has an RC circuit with known resistor values and an unknown capacitor value. The Arduino measures the voltage at the capacitor and record the time it takes to reach 63.2% of its voltage when fully charged. This time is known as time constant. Since the resistance value is already known, we can easily measure capacitance.

We charged the capacitor through a resistor using one of the Arduino pins. Using the ADC of the Arduino, we measured the voltage that the capacitor reached. Arduino started to measure time to reach the 63.2% of the full voltage. We used the following RC circuit for measuring capacitance.

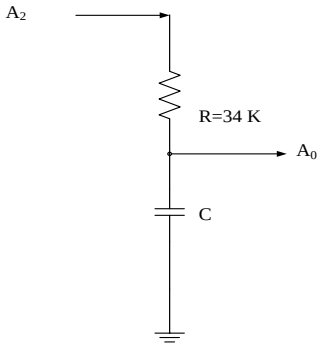

Figure 4. Capacitance measurement Circuit

The internal pull up resistance of the Arduino was used as the resistor of the RC network. It has a value of around 34 kΩ. A 5 volt

supply was placed at A_2 and the voltage at A_0 was sampled along with the time. The value of test capacitance was calculated from (Wahid et al., 2014)

$$C = \frac{-t}{R \ln(1-V_{A0}/V_{A2})} \quad (4)$$

Table 1. Comparison of measured capacitance using Arduino with nameplate value of capacitance

Capacitor (nameplate value)	Capacitor (measured using Arduino)	% of Error
10 pF	11.72 pF	17.2
100 pF	78.5 pF	–21.5
1 nF	0.92 nF	–8
10 nF	11.09 nF	10.9
100 nF	107.50 nF	7.5
1 µF	1.08 µF	8
3.3 µF	3.8 µF	15.1
10 µF	9.74 µF	–2.6

Readings were taken for various capacitors available in market and in each case the readings were within a tolerance limit of 25%. The readings along with errors of the system for various ranges of capacitors are listed in the "Table 1".

2.5. System algorithm:

We immersed the sample fruit in distilled water for 10 minutes. Then we take out the fruit. The remaining water is our test solution. We start our detection procedure by determining pH of the test solution. It indicates the existence of formalin in test solution. If pH is greater than 5.5, then it indicates that there is no formalin. If pH is less than this limit, it demonstrates the existence of formalin in test solution.

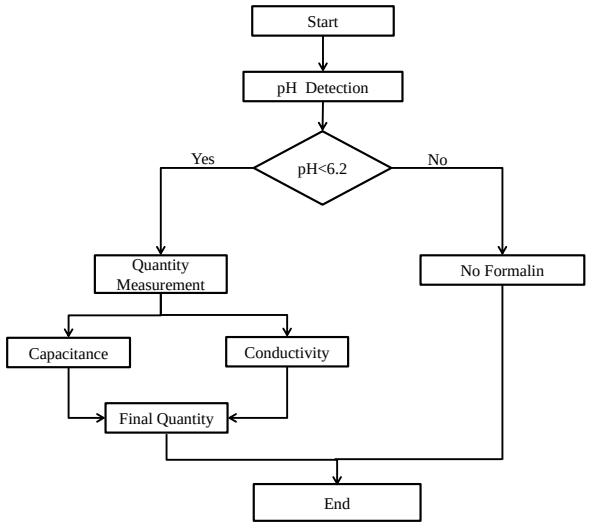

Figure 5. Algorithm of the system

Then it performs the quantity measurement. Our detection algorithm uses two parameters. It analyses quantity using the capacitive and conductive property. Using those properties, our detection system analyzes final value and shows result.

We implemented final circuit by arranging all the three individual parameter-based circuits. For correlating all the three parameter-based circuits, we went through numerous trial and errors. Finally we fine-tuned the program which best correlates the detected formalin concentration with known samples. After calibration, we measured the existence and quantity of the formalin.

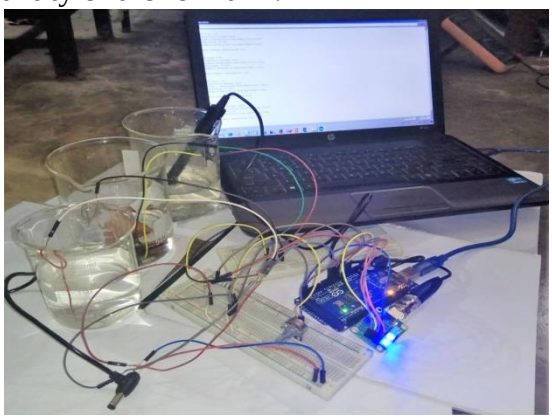

Figure 6. Photo of final implemented system

3. Results and discussions

3.1. Formalin concentration measurement using conductivity circuit:

We used Wheatstone bridge circuit for conductivity measurement. At balance condition, when there was no formalin existed in the solution, the output voltage became zero. Then we added 0.02cc formalin to 100ml distilled water.

Formalin is a conductive solution. So, when formalin was added to distilled water, the resistance of water decreased which made the bridge unbalanced. So the voltage difference of bridge circuit increased. So we got an unbalanced voltage output of 2.19 volt. When formalin was 0.20cc in the solution, the voltage difference of the bridge circuit became 4.85 volt. We further added formalin to the solution but the voltage difference was not increased. The output voltages corresponding to different concentrations are summarized in "Table 2".

We plotted output voltages along x axis and formalin concentration along y axis

Table 2. Output voltage of Wheatstone bridge circuit for different formalin concentration

Formalin Concentration (cc)	Output voltage (Volts)
0	0
0.02	2.19
0.04	2.98
0.06	3.42
0.08	3.60
0.10	3.89
0.12	4.09
0.14	4.38
0.16	4.56
0.18	4.72
0.20	4.85

(Figure 7) in order to find an equation which can give formalin concentration as a function of any output voltage. After adding trend line in formalin concentration versus voltage curve we found the equation of trend line as

$$f_c = 0.0159V^5 - 0.2817V^4 + 1.962V^3 - 6.6481V^2 + 11.147V - 7.2622 \quad (5)$$

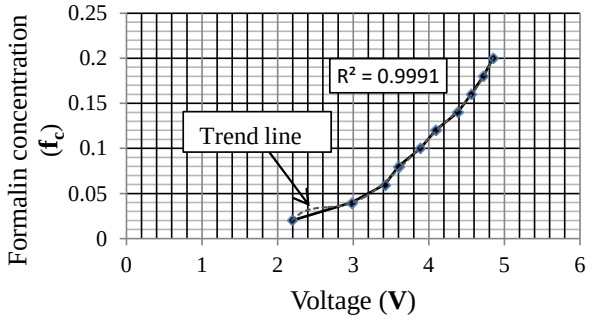

Figure 7. Voltage vs Formalin concentration Curve

From visual inspection we can see that the trend line follows the curve almost exactly. The R^2 value is 0.9991. We measured formalin concentration by following this equation via Arduino code.

3.2. Formalin concentration measurement using capacitance measurement circuit:

We used capacitance measurement probe as discussed earlier to measure capacitance for various formalin concentration. The measured capacitance of the solution varied extremely during measurement of lower concentration. When concentration of formalin was increased, measured capacitance variation became less.

Therefore we termed it as measured capacitance range instead of measured capacitance. The measured capacitance ranges corresponding to different concentrations are summarized in "Table 3". As capacitor measurement varied extremely, we used conditional statements for different capacitance range in Arduino to determine the final concentration of formalin.

Table 3. Capacitance of test solution for different formalin concentration

Formalin Concentration (cc)	Measured Capacitance Range
0	800pF-8nF
0.02	8nF-12nF
0.04	19nF-26nF
0.06	70nF-96nF
0.08	160nF-210nF
0.10	350nF-450nF
0.12	28µF-29µF
0.14	29.1µF-31µF
0.16	31.1µF-35µF

3.3. Final Result

We combined output of three individual circuits and got the final result. We varied formalin concentration from 0.02cc to 0.18cc. Actual and measured values from final circuit were compared and summarized in "Table 4". At first, for no formalin in solution, we got pH 6.46. We designed our detection circuit to show no formalin condition for pH greater than 6.2. So the actual value and measured values are same. For 0.02cc formalin, pH value is 5.14, which is less than 6.2. For this condition, bridge circuit showed output of 2.95 volts. This determined concentration of formalin was 0.04cc. On the other hand; the capacitive circuit gave output of 0cc formalin by showing capacitive output of 13.81 pF. It showed no formalin output as the capacitance output is less than its distinguished range of 8-12 nF. After averaging, our detection circuit shows concentration output of 0.02 without showing any error. Overall average error is 7.18% and for higher concentration of formalin (i.e. greater than 0.08cc) the average error is 5.02%.

At first we got enormous percentage of error because we used distilled water for our experiment. Distilled water is termed as hungry water. It has no salt, no minerals. Probably due to this it was affected by sensor probe and environment while the formalin concentration was low. After adding enough formalin to the solution, the fluctuation decreased. The reason may be because formalin was added as impurity and it reaches more towards equilibrium. It decreased percentage of error too.

Table 4. Actual and Measured value of formalin concentration

Actual formalin concentration(cc)	pH value	Conductivity Voltage (V)	Formalin concentration from Conductivity (cc)	Capacitance	Formalin concentration from Capacitance (cc)	Final Measured Formalin	% of Error	Average % of error
0	6.46	0	0	601.60pF	0	0	0	
0.02	5.14	2.95	0.04	13.81pF	0	0.02	0	
0.04	4.47	2.86	0.04	1018.9pF	0.02	0.03	25	
0.06	4.28	3.32	0.06	12.55nF	0.04	0.05	16.6	
0.08	3.55	3.81	0.10	23.30nF	0.06	0.08	0	

0.10	3.26	4.22	0.13	233.91nF	0.08	0.11	10	7.18
0.12	3.12	4.39	0.15	26.69µF	0.12	0.13	8.33	
0.14	3.60	4.58	0.17	28.23µF	0.12	0.14	0	
0.16	2.79	4.78	0.19	29.40µF	0.14	0.17	6.25	
0.18	1.92	4.91	0.22	31.59µF	0.16	0.19	5.55	

We have plunged an apple having formalin on its surface into distilled water for 10 minutes. Then we have taken the apple from water and tested the remaining water. Tested result showed existence of formalin as well as its concentration. We have picked fresh papaya from tree in the yard of our department and plunged into distilled water for 10 minutes. Then we have taken papaya from water afterward tested the remaining water. Tested result showed no formalin.

To find formalin free fruit for testing is difficult. Fruits available in the market are contaminated by formalin even before coming to the market by farmers. Then it might be affected by formalin used for preserving it for long days. But we plucked papaya from tree right in front of our department which is for sure formalin free. So above test result is conclusive.

4. Conclusions

In this study, a method consisting three parameters to detect formalin in fruits is demonstrated. The process of detection by this system is simple, reliable although the system is bulky and it takes some time. The detection system also enables to know the quantity of formalin in test solution. Proposed system proved to be an encouraging device for the detection and measurement of formalin. Its size can be reduced by combining all the sensors in same probe. Future works may emphasis on the reduction of the time required and build a robust detection mechanism.

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PHYSICAL-CHEMICAL CHARACTERIZATION AND TECHNOLOGICAL AND THERMAL PROPERTIES OF TAMARIND (*TAMARINDUS INDICA* L.) FROM THE CERRADO OF GOIÁS, BRAZIL

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ABSTRACT

Brazil is a country with different biomes and the Cerrado is known for its rich resources and flora. Among the fruits in the Cerrado, we can highlight the tamarindeiro, whose fruit, tamarind, exhibit excellent nutritional quality. Tamarind is enough explored on the continent of origin (Africa), however surveys involving all utilities of the plant are still insignificant. So, the objective of the work was to characterize shells, pulp and tamarind seeds of the Cerrado, Goiás, as to physico-chemical, technological and thermal properties. The collected fruits obtained average proportions of 22,2 ± 1,1% shells, 44,0 ± 2,4% pulp and 14,4 ± 1,6% seeds, and approximately 20% fibers. It presented high carbohydrate content and low water activity for the three portions and lower values of ash, lipids and proteins. The shell and seed flours presented high content of total dietary fiber and fruit pulp presented acid pH (3,02 ± 0,01) and high titratable acidity (29,82 ± 0,24). The seed flour had a water absorption and solubility index greater than the shell flour, and lower oil absorption index. The tamarindo pulp presented 4 peaks in your thermogram, being the first relative to the gelatinization of starch, 2 and 3 peaks suggested the formation of carbohydrate-lipid complexes and protein denaturation and 4 peak the glass transition. Tamarind shell and seed flour showed similar behavior to pulp after 115 °C, with 2 endothermic peaks. Concluded that the integral tamarind fruit has specific physico-chemical, nutritional, thermal and technological characteristics and suitable for use in the food industry.

1.Introduction

Brazil is a country of great dimensions and with a variety of biomes, of which the Cerrado is included. Considered the second largest Brazilian biome, the Cerrado occupies areas in the states of Goiás, Minas Gerais, Maranhão, Tocantins, Mato Grosso, Mato Grosso do Sul, and portions in other states, and stands out as one of the richest savannahs in the world, one of the Brazilian *hotspots*. In addition, it presents a lesser known vegetable heterogeneity, which includes numerous exotic fruit species with peculiar sensorial characteristics (Morzelle *et al*., 2015, Carneiro *et al*., 2014).

Among the fruit species of the Cerrado of Goiás, the tamarind (*Tamarindus indica* L.) is found in dispersed plantations without great agroindustrial interest. The tamarind tree is a multifunctional tropical fruit tree grown mainly for its fruits, but all its parts, such as bark, seeds, leaves may offer some benefit because they present nutritional and therapeutic properties

(Rao, Kumar and Ramana, 2015, Sulieman *et al.*, 2015). Tamarind is characterized by a unique sweet acid taste due to the combination of high levels of tartaric acid and sugars. The fruit has excellent nutritional quality with high levels of carbohydrates, proteins and mineral elements (Adeola and Aworh, 2012, Pereira *et al*., 2011).

In general, fruits and vegetables are sources of macro, micronutrients and dietary fiber, besides being important natural sources of phytochemical compounds (Yahia, 2010). The consumption of raw fruits and also of its by-products brings numerous health benefits, contributes to the development of new foods and, consequently, to the recovery of waste from agro-industrial processes, with greater industrial, economic and environmental impacts (Silva *et al.*, 2014).

In the countries of origin, on the African continent, tamarind is widely exploited and valued as a source of sustainable subsistence of cultural, dietary and economic importance. However, the impact of the research involving all utilities of the plant has been insignificant (Adeola and Aworh, 2012). Studies of the properties of fruits and their parts are important for the knowledge of the nutritional value, to add value and quality to the derived products (Paz *et al.*, 2015). The production of food from exotic or lesser known fruits and, consequently, their trade and consumption have increased due of their attractive sensory properties and nutritional and therapeutic values (Bicas *et al.*, 2011).

Brazil being a country with great potential and diversified production of fruit, and yet there are several species of these little explored and/or known beyond their regions of origin, which have excellent sensory and nutritional characteristics, the objective of this work was to characterize the tamarind fruit of the Cerrado of Goiás in its pulp, bark and seeds.

2. Material and methods

2.1. Raw material collection and sample preparation

The fruits were collected in Rio Verde (latitude 18º01'09,8"S, longitude 50º40'17,7"W) and Ceres cities (latitude 15º18'23,7"S, longitude 49º36'02,6"W), Goiás state, Brasil, in maturation stage suitable for consumption in the months of August and September 2017, and transported to the Agroindustrial Waste Utilization Laboratory, School of Agronomy of the Federal University of Goiás, in plastic bags at room temperature. The fruits were then selected for the presence of insects and breakdowns and separated manually in shells, pulp and seeds, weighing in a semi-analytical balance and calculating the proportions in percentages of whole fruit. The shells and seeds were sanitized in sodium hypochlorite solution 200 ppm, dried in an air circulation oven at 40 °C for 16 hours, then crushed in an industrial blender (Vitalex, LQI-02, Catanduva, Brazil), and ground in a cyclone rotor mill (Tecnal, TE65I/2, Piracicaba, Brazil). The tamarind shell and tamarind seed flour were conditioned in bags of high density polyethylene (HDPE) and stored in a freezer at –18 °C until the analysis. The tamarind pulps were kept in natura, conditioned and stored under the same conditions as flours until the analysis.

2.2. Proximal composition and total energy value

Moisture, ash, protein, lipids and total dietary fiber were determined according to the methods of the Association of Official Analytical Chemistry (2010). Moisture was determined by oven drying with air circulation at 105 ºC until constant weight, ash by weighing after muffle incineration at 550 ºC, the nitrogen content by the Kjeldahl method, considering 5,75 as a conversion factor for the calculation of crude protein of vegetable origin, the total lipid content by hot extraction using petroleum ether by the Soxlet method. The dietary fiber was obtained by enzyme-gravimetric method and the total carbohydrate content by difference. All

analyzes performed in triplicate. The total energy was estimated considering the conversion factors of 4 kcal g^{-1} for protein and carbohydrate, and 9 kcal g^{-1} for lipids (Merrill and Watt, 1973).

2.3. Physical and chemical characterization

The pH measurement was determined using a potentiometer (Tecnal, TEC-51, Piracicaba, Brazil), with electrode insertion directly into 5 g of diluted sample in 100 mL of water. The total titratable acidity was determined by titration with NaOH 0,1 N. The determination of the water activity (a_w) was obtained in digital AquaLab (Series 3 TE, Pullman, Washington, USA), of 25 ºC. Soluble solids content (SS) was determined using a digital refractometer. All according to the AOAC (2010). The instrumental color parameters (L*, a* and b*) were determined in a colorimeter (Bankinh Meter Minolta, BC-10, Ramsey, USA), in which the coordinate L* expresses the degree of luminosity of the color, a* the degree of variation between red and green and b* the degree of variation between blue and yellow. The values a* and b* were used to calculate the coordinate C* (chroma = $(a^{2*}+b^{2*})^{1/2}$) and hue angle (H = tang $^{-1}$(b*/a*)) (Machado *et al.*, 1997). The determination of reducing and total sugar was performed by the 3,5-dinitrosalicylic acid method, according to Miller (1959), with absorbance reading at 540 nm in the spectrophotometer (Ultrospec, 2.000 UV/Visível, Cambridge, Inglaterra), in aqueous extracts with concentration of 0,2 mg mL^{-1}.

2.4. Absorption and solubility in water and oil absorption of tamarind shell an seed flours

The water solubility index (WSI) of the flours was determined according to Anderson *et al.* (1969). Samples of 2 g were weighed into centrifuge tubes, added with 30 mL of distilled water at 25 °C and shaken on a mechanical stirrer for complete homogenization of the samples. The tubes were placed in a water bath for 30 min at 28 °C with shaking and centrifuged for 10 min at 5300 rpm (2500 G) (Best Etetronics, TG-WS, Xangai, China). A 10 mL aliquot of the supernatant was removed and placed in previously tared petri dishes, which remained in an air circulation oven for 2 hours at 105 °C. The plates were weighed and the ISA value was expressed in g of precipitate per g of dry matter. The water absorption index (WAI) of flours equals the weight precipitate in the tube after removal of the supernatant. The result was expressed in g precipitated per g of dry matter.

The oil absorption index (OAI) of the flours was determined according to the methodology described by Castilho, Fontanari and Batistuti (2010). 2 g of sample was weighed into centrifuge tubes, 10 ml of soybean oil was added and homogenized for 2 min on mechanical stirrer. Samples were left standing for 15 minutes at 25 ± 3 °C, and then centrifuged at 8.000 rpm/10 minutes. The volume of the supernatant was measured in graduated cylinder and the OAI was expressed in ml of absorbed oil per gram of sample.

2.5. Thermal properties

The thermal properties of the samples were determined by Differential Scanning Calorimetry (DSC), with calorimeter (TA Instruments, Q20 DCS, New Castle, EUA), based on the methodology described by Weber, Collares-Queiroz and Chang (2009). Samples of 2 mg (b.s) were weighed in aluminum sample port, suitable for DSC equipment. Distilled water (6μL) was added and maintained for 12h at 25 °C to standardize the water distribution. The samples were subjected to a heating cycle of 35 – 160 °C the velocity of 10 ° C min^{-1}, and subsequent cooling at the same speed, in order to determine the initial, peak and final temperature, and the enthalpy change (ΔH) during heating and cooling, according to the manufacturer's manual.

2.6. Statistical analysis

Statistical analysis (Statsoft, Statistica 7.0, Tusla, USA) was performed using statistical analysis of variance (ANOVA) and Tukey's test at 5% of statistical significance.

3. Results and discussions

3.1. Proportions and proximal composition of fruits

The collected tamarind fruits had average proportions of 22,2 ± 1,1%, 44,0 ± 2,4% e 14,4 ± 1,6% for shell, pulp and seeds, respectively, in relation to the whole fruit. About 20% of the fruit is the fibers that involve pulp and seeds. The proportions found differ from the averages reported by Pereira *et al*. (2011) and Favet, Frikart and Potin (2011) which indicated values of approximately 30% of pulp, 30% of shell and fibers and 40% of seeds, as the average of the proportions of tamarinds. The average number of seeds per fruit was 3 seeds, which explains the lower proportion of seeds in relation to the other parts of the fruit. The proportion of pulp was higher than that suggested by Pereira *et al.* (2011), which can be considered an advantage for fruits of this region, because a higher proportion of pulp in relation to residual fractions are preferred by the industries, as they guarantee a higher processing yield according by Rebouças, Gentil and Ferreira (2008).

The proximal composition and the total energy value are presented in Table 1. The tamarind pulp presented low moisture content when compared to other fruits few explored as Cerrado jatobá (*Hymenaea stigonocarpa* Mart.) (83,12±0,03 g 100 g^{-1}) (Batista *et al.,* 2011) and cajarana (*Spondias lutea* L) (96,1±0,2 g 100 g^{-1}) (Canuto *et al.*, 2010). Evaluating tamarinds from different regions of Nigeria, Adeola and Aworh (2012) found moisture contents in the pulp varying from 16,8 at 36,2 g 100 g^{-1}, inferring that these variations may occur due to climatic differences and cultivars. Costa *et al.* (2015) found high moisture content (74±2 g 100 g^{-1}) in tamarind fruits of the northeast region of Brazil, confirming the variation. Moisture content found in the seed flours of this study is similar to those reported by Mohamed, Mohamed and Ahmed (2015) that found value of 11,21 g 100 g-1 in samples of tamarind seeds. The tamarind shell flour presented the lowest moisture content in relation to the other parts of the fruit, being even smaller than other non-edible fruit shell flours such as passion fruit shell (Cazarin *et al*., 2014) and banana peel (Gonçalves *et al*., 2016). Queiroz *et al*. (2015) evaluated peeling and lychee seed flour finding moisture values of 6,10 and 8,7 g 100 g-1, respectively, being of the larger shells and smaller seeds than the flours of the present study.

The three fractions of the analyzed fruit presented significant difference for verified parameters of ash, lipids, proteins and carbohydrates (Table 1). The values of lipids and proteins were higher in the seed flour, followed by pulp and shell flour. The fact that seeds are better sources of protein when compared to other parts of the fruit can be explained by the storage of their proteins in the concentrated form, since the seeds are nutrient reserve organs (Costa *et al*., 2015). The presence of lipids is always higher in oilseeds and seeds than in fruits and other vegetables, which have low amounts of this nutrient (Rocha *et al*., 2008), fact observed in this study. Khairunnuur *et al*. (2009) studied pulp and tamarind seeds in Malaysia and found ash content of 3,30 and 2,15 g 100 g^{-1}, proteins 2,4 and 13,35 g 100 g^{-1} and lipids 0,14 and 2,90 g 100 g^{-1}, respectively, which are close to the values obtained in this study.

Table 1. Mean and standard deviation of the proximal composition, (g 100 g^{-1}), total and reducing sugars (g 100 g^{-1}) and total energetic value (kcal 100 g^{-1}) of the "in natura" pulp, tamarind shell flour and tamarind seed flour.

Proximal Composition	Tamarind (*Tamarindus indica* L.)		
	Pulp	Shell flour	Seeds flour
Moisture	31,22 ± 0,009a	5,96 ± 0,001c	10,20 ± 0,002b
Ash	2,96 ± 0,004b	3,76 ± 0,000a	2,22 ± 0,002c

Lipids	0,99 ± 0,00b	0,54 ± 0,000c	1,77 ± 0,001[a]
Protein	4,12 ± 0,153b	3,22 ± 0,141c	14,56 ± 0,35[a]
Carbohydrates	60,71c	86,62a	71,25b
Total food fiber	5,19c	70,33a	53,89b
Total sugars	23,84 ± 0,50a	10,22 ± 0,82c	12,66 ± 1,59b
Reducing sugars	0,25 ± 0,03c	5,60 ± 0,07b	8,21 ± 0,09[a]
Total energetic value	268,23c	364,22a	359,17b

* Means followed by the same letter, in the same line, did not differ significantly among themselves by the Tukey test, at 5% probability.

The integral fruit presented as a good source of carbohydrates, however with different compositions between the fractions (Table 1). The pulp presented higher total soluble sugars, and the shell and seed flours, higher fiber content. The seed flour had the highest concentration of reducing sugars. In the tamarind pulp, the total dietary fiber content was within the value suggested by USDA – United States Department of Agriculture (2009) of 5,1 g 100 g^{-1}. Tamarind shell and seed flours presented high fiber contents when compared to other flours of fruit residues such as lychee shell and seeds flour (*Litchi chinensis Sonn*) with 19,88 and 4,75 g 100 g^{-1}, respectively (Queiroz *et al*., 2015), and the banana caturras peel flour (*Musa avendish* Lamb.) with total fibers 10,03 g 100 g^{-1} (Santos *et al*., 2015). The inclusion of these flours in products can guarantee a greater functionality to these foods because the presence of fiber in the diet helps in regularization of the intestinal transit, giving greater protection to the colonocytes and improving digestion according by Araújo and Menezes (2010).

The total energy value presented by the tamarind fractions reveals a high energy fruit offering 13,4 %, 18,2 % and 17,9 % of kcal in a 2000 kcal/day diet for pulp, shell and seeds, respectively.

3.2. Physical-chemical characteristics

Regarding the chemical characteristics evaluated (Table 2), the pulp, shell and tamarind seeds fractions showed significant differences by Tukey test at 5% probability.

The pH found in the tamarind pulp was relatively lower than the pH of 3,40 found by Sulieman *et al*. (2015) in tamarind in the eastern part of Sudan, and higher than that Santos *et al*. (2016) of 2,75 in a study with frozen tamarind pulps. Low pH values can guarantee the preservation of fruit pulp without the need for thermal treatment, avoiding nutritional losses and yeast growth (Brasil *et al*., 2016). The presence of organic acids, important components in the formation of various fruit properties, can also contribute to pH variation (Santos *et al*., 2016). Tartaric, malic and citric acids are the main chemical compounds related to tamarind aroma and taste, according to Palomares (2009), being tartaric acid a strong acid, resistant to oxidative respiration, not decreasing with maturation (Pereira *et al*., 2011; Rizzon and Sganzerla, 2007). The tamarind can be considered a non-climacteric fruit, that is, the respiratory pattern decreases after harvesting, being necessary that the fruit stays in the plant until it is in an optimum state of maturity, which would guarantee more sweet fruits (Chitarra and Chitarra, 2005).

The titratable acidity (TA) presented by the pulp was much higher than in the shell and seed flour, a fact explained by Pereira *et al*. (2011) due to the large amount of organic acids present in this fruit (12 to 30% dry matter). Tartaric acid is the main acid present in tamarind pulp and its presence is uncommon in fruits (Hamacek *et al*., 2013). Thus, TA of the tamarind pulp was expressed in grams of tartaric acid per 100 g of pulp. Tamarinds from the State of Goiás were similar in terms of TA observed by Sulieman *et al*. (2015) in tamarinds from Sudan (28,60 g tartaric acid $100g^{-1}$).

In the literature, the value of soluble solids (SS) for the tamarind pulp presents great variation. The values found in this study (Table 2) corroborate those verified by Lima *et al*. (2015) of 7,25 °Brix and by Santos *et al*. (2016) of 7,70 to 12,58 °Brix, analyzing frozen tamarind pulps. However, they were much lower than the values verified by Sulieman *et al*. (2015) of 39,9 to 46,6 °Brix and by Canuto *et al*. (2010) of 24 °Brix. Factors such as climate, irrigation during cultivation and addition of water during the pulp manufacturing process may influence the soluble solids content, which would explain the lack of uniformity between the values presented in different studies (Santos *et al*., 2016).

The SS/TA ratio is related to the quality of the fruit in terms of maturity and taste, evidencing the balance between sugars and organic acids (Chitarra and Chitarra, 2005). The tamarind pulp showed this reduced relationship, influenced by the high acidity, and it was possible that the fruit was harvested before the optimum ripening stage, which justifies the low soluble solids content.

Shell and tamarind seeds flours showed low water activity (Table 2), and below 0,60 the majority of pathogenic microorganisms do not develop (Forsythe, 2013). The a_w is a relevant factor in the quality of a food because it influences the speed of reactions, oxidation of lipids, microbial growth, degradation of compounds such as chlorophyll, anthocyanins, besides interfering directly in the perishability of a food (Damodaran, Parkin and Fennema, 2010).

The tamarind fruit, in all its parts, presented reddish-yellow coloration (Figure 1) indicated by the angle value Hue obtained (H between 60-70) (Table 3).

Table 2. Mean and standard deviation of the chemical characteristics of the "in natura" pulp, tamarind shell flour and tamarind seed flour.

Characteristics	Tamarind (*Tamarindus indica* L.)		
	Pulp	Shell flour	Seeds flour
pH	3,02 ± 0,01c	4,19 ± 0,02b	5,81 ± 0,03a
SS (°Brix)	7,0 ± 0,1a	0,1 ± 0,1c	1,0 ± 0,1b
AT (g tartaric acid 100 g^{-1})	29,82 ± 0,24a	6,53 ± 0,23b	4,26 ± 0,12c
SS/AT ratio	0,23 ± 0,001a	0,02 ± 0,001b	0,23 ± 0,006a
a_w	0,615 ± 0,01a	0,319 ± 0,001c	0,415 ± 0,002b

* Means followed by the same letter, in the same line, did not differ significantly among themselves by the Tukey test, at 5% probability.

Table 3. Mean and standard deviation of the color coordinates L*, a*, b*, C* and Hue angle of the "in natura" pulp, tamarind shell flour and tamarind seed flour.

Tamarind	Color coordinates				
	L*	a*	b*	C*	H
Pulpa	37,54 ± 0,35c	2,56 ± 0,44c	9,26 ± 0,52c	9,60c	74,56a
Shell flour	54,24 ± 1,04b	11,35 ± 0,3a	24,22 ± 0,58a	26,75a	64,88c
Seeds flour	64,14 ± 2,07a	7,13 ± 0,39b	16,12 ± 0,37b	17,63b	66,13b

* Means followed by the same letter, in the same column, did not differ significantly from each other by the Tukey test, at 5% probability. a* and b* represent the coordinates of chromaticity (C*). The color coordinates were converted to a color angle, $H = \tan^{-1b/a}$ indicating the Hue (H) angle of the sample (0º or 360º = red, 90º = yellow, 180º = green, 270º = blue).

Figure 1. Portions of tamarind (*Tamarindus indica* L.): (A) Pulp, (B) Flours of the shell, (C) Flours of the seeds.

With respect to the chroma (C*), the results indicate that the pulp presents a more opaque coloration, in relation to the other portions. The pulp is characterized as the darkest part, and the seeds the clearest, according to the values of L* found, where values closer to zero approximate black. Canuto *et al*. (2010) found in their studies values L* (33,8 ± 0,5) and H (63,1 ± 0,2) in tamarind pulp, close to the present study, describing the same reddish-yellow coloration.

3.3. Absorption and solubility in water and oil absorption of tamarind shell on seed flours

The water absorption (WAI) and oil (OAI) and water solubility (WSI) indices of the tamarind bark and seed meal at 28 °C were evaluated and their results are expressed in Table 4. The flour of tamarind seeds presented higher WAI than the flour of the shells. Increasing the concentration of fiber and protein in flours can raise the rate of water absorption. WAI is a property that is related to the availability of hydrophilic groups in binding to the water molecules, the gel-forming capacity of the starch molecules and the hygroscopic properties of the fibers, which also makes it possible to absorb water (Santillán-Moreno *et al*., 2011, Filli and Nkama, 2007). Seed flour, even with a lower fiber value than the shell flour, has a protein concentration of 4,5 times higher. Santana, Oliveira Filho and Egea (2017) found in their studies 1,15 g g^{-1} WAI for wheat flour and 4,85 g g^{-1} for passion fruit flour, that is, tamarind shell and seed flours have a greater ability to absorb water than wheat flour, the main raw material in baked goods. This property is important in foods that require hydration and moisture retention as meat products, cakes, breads and other baking products improving yield and texture (Porte *et al*., 2011).

Water solubility index (WSI) was lower in tamarind shell flour when compared to seed flour. The solubility of the flours is related to the amount of water soluble molecules, which can be verified by comparing the water solubility values with the total soluble solids contents of the samples (Ferreira *et al*., 2015). Santana, Oliveira Filho and Egea (2017) presented WAI values of 15,33 g g^{-1} for flaxseed flour and 10,0 g g^{-1} for passion fruit flour, values higher than those found in this study. Flours with high WAI values can be used in foods that require lower temperatures to be prepared as instant and liquid foods or as ingredients for the formulation of soups, desserts and sauces (Santana, Oliveira Filho and Egea, 2017).

The OAI found in the flour of tamarind shells was higher than in the seed flour, however, both presented values higher than the value found by Tril *et al*. (2014) for tamarind powder extract (1,35 g g^{-1}). The ability to absorb oil from a flour may be related to the presence and amount of exposed hydrophobic groups of proteins and their interaction with the hydrophobic chains of fat (Santana, Oliveira

Filho and Egea, 2017). This is an important parameter of quality because it improves the palatability of the food, in addition to also influencing the emulsifying capacity of a product (Goldmeyer *et al*., 2014). High oil absorption index determine whether the flour can be used in meat products such as sausages and bologna or emulsified products such as cakes, mayonnaise or sauces (Porte *et al*., 2011).

Table 4. Mean and standard deviation of the water absorption index (WAI), water solubility (WSI) and oil absorption (OAI) of tamarind shell flour and tamarind seed flour.

Index	Tamarind (*Tamarindus indica* L.)	
	Shell flour	Seed flour
WAI (g g^{-1})	2,43 ± 0,06	4,17 ± 0,04
WSI (g g^{-1})	5,19 ± 0,06	8,19 ± 0,27
OAI (mL g^{-1})	2,06 ± 0,03	1,90 ± 0,02

3.4. Thermal properties

Figures 2, 3 and 4 illustrate the DSC graphical analyzes of lyophilized pulp, tamarind shell and tamarind seeds flours, respectively. The thermogram of the pulp (Figure 2), which presented 4 endothermic peaks, was divided into two parts, the first one being from 35 to 105 °C and the second part from 105 to 155 °C for better visualization of the peaks. The first peak (Figure 2A) relates to gelatinization of the starch present in the pulp. The initial gelatinization temperature (To) of the tamarind pulp was 69,98 °C ± 1,55, to peak (Tp) was 78,66 °C ± 0,02 and end (Te) 86,61 °C ± 0,57. The gelatinization temperature range was 16,63 °C with gelatinization enthalpy (ΔH) to 3,27 ± 0,27 J g^{-1}. When the available water is restricted, as in the case of lyophilizates, the gelatinization can be delayed at higher temperatures due to the melting of the remaining amylopectin crystals (Moreira, Chenlo and Arufe, 2015).

The 2 peak (101,19 °C) (Figure 2A) and 3 peak (118,51 °C) (Figure 2B) suggest endothermic peaks related to protein denaturation and the formation of amylose-lipid complexes, according to Santiago-Ramos *et al*., (2018) and Sánchez-Arteaga *et al*. (2015). The tamarind pulp presents a more complex chemical composition (protein, amylose / amylopectin ratio, minerals, etc.), thus indicating a more complex thermal profile than those reported for isolated starch systems. The presence of two peaks in bands close to temperature may be due to different chemical compounds present (Sánchez-Arteaga *et al*., 2015). Evaluating the thermal properties of bean flour of different varieties, Sánchez-Arteaga *et al*. (2015) found the second endothermic peak between 85 and 105 °C, associated with the presence of heat-resistant proteins. The same peak was observed by Moreira, Chenlo and Arufe (2015) with transition varying between 94,6 and 122,2 °C for flours of chestnut and corn starch.

The peak number 4 (Figura 2B) suggests the glass transition peak (Tg) of the tamarind pulp which presented a range of Tg, *onset*: 138,61°C to *endset*: 147,55°C, and peak 138,85 °C. The Tg evaluates the temperature at which the sample exits from an amorphous equilibrium state to a rubbery or gummy state, inferring thermal treatments lower than Tg temperature in the processing of possible products based on lyophilized tamarind pulp. Tg can be used as an indicator of physico-chemical changes in long periods of storage (Alpizar-Reyes *et al*., 2017).

The tamarind shell and seed flours (Figures 3 e 4) presented similar thermograms after 115 °C, with two endothermic peaks and similar behavior to the lyophilized pulp after this temperature, which suggests the same evaluation of the peaks, that is, the peak between 115 and 120 °C corresponds to the formation of carbohydrate-lipid complexes and/or

denaturation of proteins and the peak formed approximately at 135 °C corresponding to the glass transition of the sample. A small peak in the tamarind shell flour thermogram (Figure 3) is also observed at 86,69 °C for gelatinization.

The endothermic peak of the glass transition to tamarind shell flour had an energy of 18,52 J g^{-1} and Tg of 135,93 °C. In the tamarind seed flour thermogram (Figure 4), is possible check the first peak with To 119,80 °C, Tp 119, 94 °C and Te 120,15 °C. Tg was observed at 137,92 °C with transition energy of 23,65 J g^{-1}.

The melting temperature of the carbohydrate-lipid complexes is generally high because they have high thermal stability, so the longer the chain length of the complex the greater the physical stability (Kawai *et al*., 2012). During the thermal sweep of samples of chestnut flour, Ahmed and Al-Attar (2015) detected transitions at various temperatures (104-106 °C, 114-120 °C e 135-142 °C), associating these transitions to the processes of amylose-lipid complex disorders.

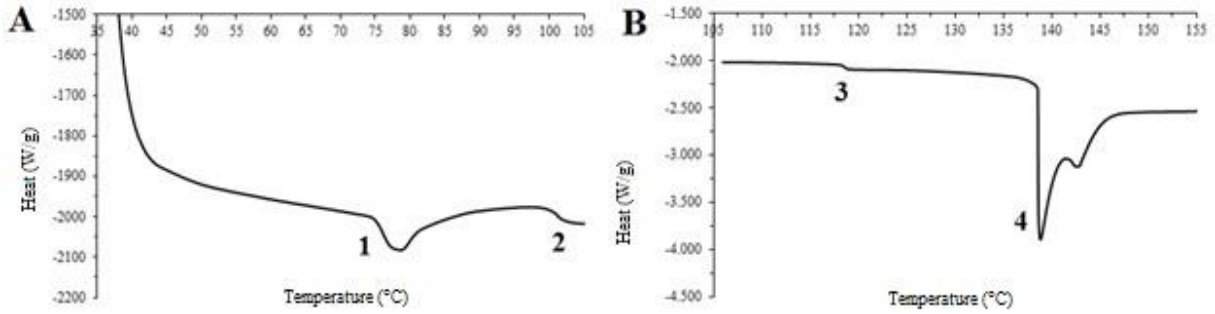

Figure 2. DSC of the freeze-dried tamarind pulp. (A) DSC in the temperature range of 35 to 105 ° C, (B) DSC in the temperature range of 105 to 155 ° C.

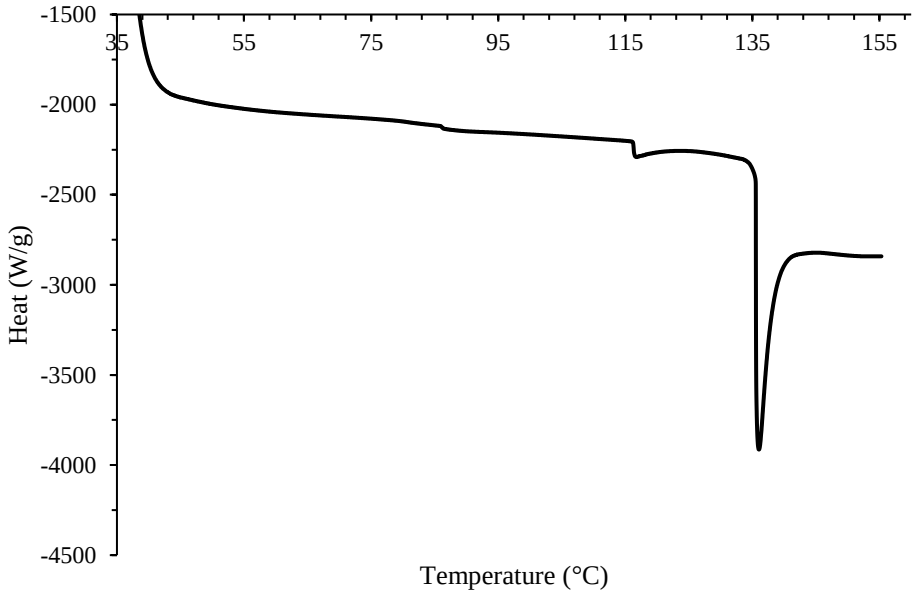

Figure 3. DSC of Tamarind shell flour.

4. Conclusion

The tamarind fruit presents excellent physical-chemical and nutritional qualities in all its portions. Flours derived from tamarind shells and seeds are rich in fiber and have technological properties suitable for the food industry, such as instant products such as soups, and even on products that do not require high temperatures. The thermal properties of the tamarind portions suggest a more detailed investigation because they are complex systems.

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ENGINEERING PROPERTIES AND SHELF LIFE OF FRESHLY HARVESTED INDIAN KIWI CULTIVARS FOR FACILITATING PRIMARY PROCESSING

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ABSTRACT

The present study deals with the engineering properties of three Indian kiwi cultivars. These engineering properties will facilitate the farmers, and industry personals involve in handling, packaging and transportation of fresh harvested fruit. The complete information on physical, mechanical, thermal and biochemical properties of three Indian kiwi cultivars were presented in this paper. This knowledge may be utilized to design and develop modern machineries for primary processing, and packaging of fresh kiwifruit. The shelf life study data also provided in this paper which will help the growers and processors for safe handling, packaging and transportation of the fruit. The physical dimensions viz. length, width and thickness, mean diameters, surface area, volume, sphercity, static coefficient of friction on different materials were measured for all the three Indian cultivars. Significant ($p<0.05$) difference for aspect ratio with Hayward and Monty was observed. Bruno was bigger and heavier than others cultivars. Mean diameters (*GMD, AMD and EMD)* were varying less than 10%. The mechanical properties viz. firmness, hardness, adhesiveness, adhesive force and total positive area for peeled and unpeeled Hayward, Bruno and Monty. Thermal properties i.e. thermal conductivity, specific heat capacity, thermal diffusivity and latent heat of fusion and biochemical properties i.e. moisture, pH, titrable acidity and total soluble solids were also measured in this study. Significant ($p<0.05$) for total positive area was observed for Bruno with Hayward and Monty was observed. No significant ($p>0.05$) difference for moisture and sphericity was observed between Hayward and Monty.

1. Introduction

Kiwifruit (*Actinidia delicosa*) originated from China (Pal et al., 2015) and is mostly grown in many parts of the world including India (Bhardwaj et al., 2014; Ferguson, 1999). It is considered as one of the most important horticultural crops with high medicinal and nutritional value. These fruits are rich in bioactive compounds such as ascorbic acid, polyphenols and flavonoids which have major beneficial health effects i.e. mainly due to their antioxidant properties Amodio et al., 2007; Chandel and Rana, 2002. World-wide production of kiwi fruit was $3.2x10^6$ MT (FAO, 2013). In India, due to its exotic introduction the cultivation area under this fruit is very less. Commercial cultivation of kiwi fruit has been drawn out to the mid-hills of Jammu-Kashmir, Himachal Pradesh, and Arunachal Pradesh with comprehensive research and developmental support in India Singh et al., 2012.

Postharvest management of kiwifruit determines fruit quality and safety, competitiveness in the market, and the profits earned by producers. The postharvest management of kiwifruit in most developing countries like in India is, however, far from satisfactory. The major constraints include inefficient handling and transportation; poor technologies for storage, processing, and packaging; involvement of too many diverse factors; and poor infrastructure. Most of the fruit available to consumers undergo primary processing, commencing at the point of origin and including the transporting, cleaning and sorting. Hence proper information on physical, mechanical, thermal and biochemical properties are important in reducing the loss of kiwifruit during primary processing (Vaidya et al., 2006).

Physical, mechanical, thermal and biochemical properties of kiwi fruits are necessary for appropriate design of equipment for harvesting, storage, handling, transporting, conveying, separation, and other processes (Kilickan and Guner, 2008). The porosity, bulk and true density are needed in air flow studies, heat studies, design of silos, grading, separation, drying and storage from undesirable materials (Goswami, 1996).

Mechanical properties play a major role in post-harvest handling of fruits. The major forces experienced are compression and puncture by a specific point on the fruit. Excessive damage i.e. compression may lead to bruising and breakage of fruit (Sirisomboon et al 2012), while puncture may lead to increase in wound respiration which enhances normal deterioration and visual aspects (Allende *et al*., 2004). Texture is ~~a~~ very important mechanical properties and is necessary quality factor of kiwifruits for acceptance in the quality control and post-harvest handling and processing (Batu, 2004). The Soft kiwifruits can be easily marketable, but if the kiwifruits are too soft, they are very difficult to slice and to sell in the market or further processing (Razavi and Parvar 2007). Hence firmness and hardness are the two most important textural properties considered in the quality characterization of kiwifruit processing in industry. These are also related to ripeness rate and the fruit susceptibility to damage during harvesting and processing (Arazuri et al., 2007).

Thermal properties of foods and beverages must be known to perform the various heat transfer calculations involved in designing storage and refrigeration equipment and estimating process times for refrigerating, freezing, heating, or drying of foods and beverages. Because the thermal properties of foods and beverages strongly depend on chemical composition and temperature, and because many types of food are available, it is nearly impossible to experimentally determine and tabulate the thermal properties of foods and beverages for all possible conditions and compositions.

Kiwi fruit production is still limited in India i.e. cultivated only in Himalayan range hence long transportation and storage is necessary, for this reason knowledge of physical, thermal, mechanical and biochemical properties of kiwifruit are essential in every stage of handling and primary processing of kiwifruit. Many studies have been recorded on the physical and mechanical properties of fruits such as anola (Goyal *et al.*, 2007, mango Jha *et al*., 2006 orange Topuz et al., 2005, kumquat Jaliliantabar et al., 2013, date fruit Jahromi et al., 2008, sweet cherry Vursavus et al., 20, cider apple Guillermin et al., 2006). The material properties vary with the cultivar, soil and geographical location (Goyal et al., 2007). However, there is limited information available about physical, biochemical and mechanical properties of kiwi fruit grown in India, hence the main objective of this study is to inform the engineering properties (physical, mechanical, thermal), biochemical properties and shelf life of three Indian kiwi fruit cultivars to gain the knowledge for optimizing handling, fresh storage and facilitating the design of modern machineries in primary processing.

2. Materials and methods

2.1. Materials

Three kiwi cultivars, viz. 'Hayward', 'Bruno' and 'Monty' (Fig 1, Fig 2 and Fig 3) were harvested from the Dirang valley (Arunachal Pradesh, India) in the month of late November, 2015 and then stored in refrigerated condition (4±1°C) before conducting the experiments. All the experiments were conducted at room temperature (25 ± 2 °C). All the physical, biochemical and mechanical properties were studied for 50 fruits from each cultivar.

(a) (b) (c)

Figure 1. (a) Hayward; (b) Bruno and (c) Monty

2.1.1. Physical properties

Fifty Samples from each cultivar were taken and their physical properties viz. mass, mass of peel and flesh were measured using digital electronic balance (CPA 225D, Sartorious AG, Germany) with an accuracy of 0.001g. Other physical properties like principle linear dimensions namely length (L), width (W), and thickness (T) were measured using a Vernier caliper with an accuracy of 0.02 mm (Athmaselvi et al., 2014; Razavi and Parvar 2007; Ercisli et al., 2012). Geometric mean diameter (D_g), Arithmetic diameter (D_a), Equivalent dimeter (D_e) and sphericity (Φ), aspect ratio (R_a) values were also found using the following Eq.1 to 5 (Mohsenin, 1970; Razavi and Parvar 2007; Maduako and Faborocde 1990).

$$Da = \frac{(L+W+T)}{3} \qquad \text{(Eq. 1)}$$

$$Dg = (LWT)^{1/3} \qquad \text{(Eq. 2)}$$

$$D_e = \left[\frac{(L(W+T)^2)^{\frac{1}{3}}}{1.56}\right] \qquad \text{(Eq. 3)}$$

$$\Phi = \frac{(LWT)^{\frac{1}{3}}}{L} \qquad \text{(Eq. 4)}$$

$$Ra = \frac{W}{L} X100 \qquad \text{(Eq. 5)}$$

The surface area (S) were determined using the relationship given by McCabe et al., 1993. Bulk density of whole fruit was calculated using a container with known mass and volume filled with the samples to the top. Then the fruits were poured to the container with a constant rate. After filling the container, it was weighted and bulk density (ρ_b) was calculated from the ratio of fruit mass in the container to its volume (Athmaselvi et al., 2014). The true density (ρ_t) and volume (V) of whole fruits were determined by using toluene displacement method Kilickan and Guner 2008; Kabas et al., 2006. The porosity (ε) was calculated from the eq.6 (Mohsenin, 1970; Singh and Goswami, 1996).

$$\varepsilon = \left\{\left(1 - \frac{\rho_b}{\rho_t}\right) X\ 100\right\} \qquad (6)$$

The peel ratio (R_s) was determined according to Athmaselvi et al., 2014 Athmaselvi et al., 2014 i.e. dividing the Peel mass (M_s) to the fruit mass (M_f). The packing coefficient (λ) was determined by the ratio of the volume of fruit (V) packed to the total volume of carton

(V_0) (Topuz *et al.,* 2005). The coefficient of static friction of kiwi fruits was measured on four frictional surfaces namely plywood, glass, fiberglass and galvanized iron sheets in accordance with Izli *et al*., 2009; Alibas and Koksal 2015. Packing coefficient was determined by the ratio of the volume of fruit packed to the total volume (Topuz et al., 2005).

2.1.2. Mechanical properties

Mechanical properties were measured according to Razavi and Parvar (2007) with slight modification using Texture Analyzer (TA-HD-plus, Stable Micro Systems, UK l). Analyses were performed in the orientation of thickness when the fruits were kept at natural rest position. 5 mm cylindrical flat probe was forced into the fruit at a constant rate of 20 mm/min for unpeeled fruits and 10mm/min for peeled fruits with a depth of 22.5 mm and 8 mm respectively. Texture profile analysis test was performed and the operating conditions maintained during analyses were pre-test speed: 1.5 mm/s, post-test speed: 10.0 mm/s and trigger force: 0.1 N. Mechanical properties such as firmness, hardness, adhesiveness, adhesive force and total positive area were also extracted.

2.1.3. Thermal properties

Thermal properties viz. specific heat, thermal conductivity, thermal diffusivity and latent heat of fusion were calculated with the help of moisture content of kiwifruits. Sweat, 1974 had adopted the standard mathematical formula for calculating the thermal properties of fruits having moisture content higher than 60% were shown in Eq (7, 8, 9 and 10).

The mathematical formula connecting the various engineering properties with moisture content of the kiwifruits are as follows:

Specific heat Capacity (Cp)

Specific heat capacity is defined as the quantity of heat gained or lost by a unit mass of fruits to accomplish unit change in temperature (Dickerson., 1968).

$$Cp = 1.675 + 0.025W \qquad \text{Eqn. 7}$$

Where C_p= The specific heat capacity (KJ kg^{-1} °C)

W= Moisture content (%)

Thermal conductivity (K)

It is the degree to which a specified material conducts electricity (Ikegwu and Ekwu., 2009)

$$K = 0.148 + 0.00493W \qquad \text{Eqn. 8}$$

Where K= Thermal conductivity (J sm^{-1} °C),

W= Moisture content (%)

Thermal diffusivity (α) and latent heat (λ)

Thermal diffusivity is the thermal conductivity of a substance divided by the product of its density and its specific heat capacity (Lewis., 1990). While the latent heat is the heat required to convert a solid into a liquid or vapour, or a liquid into a vapour, without change of temperature.

$$\alpha = K/\rho Cp \qquad \text{Eqn. 9}$$

Where α = The thermal diffusivity (x10^{-7} M^2/s)

K= The thermal conductivity (J sm^{-1} °C)

P =Thermal density (kg m^{-3})

Cp= The specific heat (KJ kg^{-1} °C)

$$\lambda = 335W \qquad \text{Eqn. 10}$$

Where λ = The latent heat of fusion (Jkg^{-1})

2.1.4. Biochemical properties

The moisture content of kiwi fruits was determined in accordance with standard procedures (AOAC, 1990). Soluble solids were determined using hand held Refractometer, expressed as °Brix. The pH of the kiwi fruit pulp was determined by using digital pH meter. Acidity was determined by titration method and expressed in citric acid equivalent (Ranganna, 2004).

2.1.5. Shelf life study of whole kiwifruit

Freshly harvested Indian kiwifruit cultivars (Hayward, Bruno and Monty) were stored at ambient temperature (25±2°C and 60% RH) to study the shelf life. Microbial analysis (Cao et al., 2010), firmness (Meng et al., 2012) and respiration rate (Wang et al., 2015) were observed till the fruit gets spoiled.

2.1.6. Statistical analysis

The experimental design was randomized with three replications. Data were analysed using one-way analyses of variance (ANOVA) by SPSS v 16.0 and significant difference ($p<0.05$). The differences between means were compared with Duncan's multiple range tests.

3.Results and discussions

3.1. Engineering properties

These properties (physical, mechanical, thermal and biochemical properties) are needed in process design, for estimating other properties, and for product characterization or quality determination.

3.1.1. Physical properties

The physical properties viz. physical dimensions, mean diameters, mass, bulk density, true density, sphericity, aspect ratio, peel ratio, surface area, true volume, porosity; of three Indian cultivars of kiwi fruits (Hayward, Bruno and Monty) were estimated and their mean value with SD were presented in Table 1. The packaging coefficient of all three cultivars were also presented in Table1.

Physical dimension: length, width and thickness

The length, width and thickness of fruits and vegetables are useful in designing of cleaning, sorting and grading machineries and their operations. A Significant ($p<0.05$) difference for length, width and thickness was observed between all the three cultivars of present study. The Bruno was longer compared to Hayward and Monty. The width and thickness of Hayward were higher than that of the Bruno and Monty. The physical dimension of Iranian Hayward reported by Razavi and Parvar, (2007) were higher as compared to the results obtained for all three Indian cultivars. Whereas the physical dimensions of Iranian kiwi fruit as reported by Lorestani and Tabatabaeefar, (2006) were similar to the Indian Hayward cultivar.

Mean diameters: GMD, AMD and EMD

The average diameters were calculated by the geometric mean diameter (GMD) arithmetic mean diameter (AMD) and the equivalent mean diameter (EMD) as shown in Eq. 1 to 5. The GMD, AMD and EMD for Hayward cultivar was found higher when compared with Bruno and Monty. Among these mean diameters AMD was higher when compared with the GMD and the EMD for all the three cultivars. Significant ($p<0.05$) difference for AMD was observed between Bruno and Monty. Razavi and Parvar (2007) reported the mean diameters of Iranian Hayward which was very much close to the Indian Hayward cultivar. The GMD reported by Lorestani and Tabatabaeefar, (2006) for Iranian kiwi were lower than the results obtained for Indian Hayward cultivar but higher than the results obtained for Indian Bruno and Monty cultivars.

Other physical properties

Bulk density, true density, true volume and mass are useful in designing the processing equipment's, for knowing the tendency of kiwifruit to be partly submerged in water and also useful in the transportation of the fruit by hydrodynamic means. The highest bulk density was found for Bruno. No significant ($p>0.05$) difference for bulk density was observed between all the three cultivars. The value of bulk density reported by Razavi and Parvar (2007) was lower than the results obtained for all the Indian cultivars. The mean true density of Hayward, Bruno and Monty were shown in Table1. The true density of Bruno and Monty was resulted higher when compared with the Hayward. The value of true density reported by Razavi and Parvar (2007) was lower than the results obtained for Indian Bruno and Monty cultivars.

Hayward showed maximum true volume when compared with Bruno and Monty. The

value of true volume for Iranian kiwifruit reported by Razavi and Parvar (2007) was higher than the results obtained for all the Indian cultivars.

Bruno was found to be heavier compared to Hayward and Monty (Table 1). Significant ($p<0.05$) difference for mass was observed between Bruno and Monty. The values of mass reported by Razavi and Parvar, (2007) was higher than the results obtained for Indian cultivars. While the value of mass reported by Lorestani and Tabatabaeefar, (2006) was almost same as the Indian Hayward cultivar.

Sphericity values of kiwifruit indicates the fruit shape towards a sphere. The aspect ratio values close to the sphericity values may also mean that the kiwifruit will undergo a combination of rolling and sliding action on the flat surface. Highest sphericity values were obtained for Monty and the highest aspect ratio values were obtained for Hayward. No significant ($p>0.05$) difference for sphericity was observed between Hayward and Monty. Bruno showed significant ($p<0.05$) difference for aspect ratio with Hayward and Monty. Razavi and Parvar (2007) reported the sphericity and aspect ratio of Iranian Hayward were higher than the results obtained for all the Indian cultivars. While the Lorestani and Tabatabaeefar, (2006) reported the sphericity of Iranian Hayward was higher than the results obtained for Indian cultivars. Hence results indicates that Hayward has a higher tendency to have its shape towards a sphere than that of the Bruno and Monty (Omobuwajo *et al*., 2000).

The surface area is a very important tool in determining the shape of fruit and indicates the behaviour of fruit on oscillating surfaces during processing in manufacturing plants and also important when expressing transfer of heat, gases, water vapour, pesticides and foliar nutrients into or out of fruits (Oyelade et al., 2005). Greater surface area was found for Hayward. The value of surface area reported by Razavi and Parvar (2007) were higher than the results obtained for Indian Bruno and Monty cultivars. Significant ($p<0.05$) difference for surface area was observed between all the three cultivars i.e. Hayward, Bruno and Monty. While the value of surface area reported by Lorestani and Tabatabaeefar, (2006) were lesser than the results obtained for all the Indian cultivars.

Porosity is useful for designing the processing equipment's, for knowing the tendency of kiwifruit to be partly submerged in water and also useful in the transportation of the fruit by hydrodynamic means. The mean porosity of Hayward, Bruno and Monty were shown in Table 1. The highest porosity was obtained for Monty. Significant ($p<0.05$) difference for porosity was observed between Bruno and Monty. The value of bulk density for Iranian kiwifruit reported by Razavi and Parvar (2007) was higher than the results obtained for the Indian Bruno and Monty cultivars. The lower porosity in the Hayward may be due to the higher sphericity and aspect ratio, which ensure more compact arrangement of the kiwifruit.

The mean peel ratio value of Hayward, Bruno and Monty were found to be 11%, 9.81% and 10.78% respectively. No significant ($p>0.05$) difference for peel ratio was observed between all the three cultivars i.e. Hayward, Bruno and Monty. The value of peel ratio for Iranian kiwifruit reported by Razavi and Parvar (2007) was lower than the results obtained for the Indian Hayward cultivar but is higher than the Bruno cultivar. Similarly, Topuz et al. (2005) had reported the peel ratio of orange varieties ranges from 22.95% to 32.88%.

also indicates the void spaces inside the pack and provides necessary information about the size of pack and the number of probable bruising points. The packaging coefficient was found to be equal for Hayward and Bruno which was higher than the Monty shown in Table 1. No significant ($p>0.05$) difference for packaging coefficient was observed between all the three cultivars (Hayward, Bruno and Monty). The value of packaging coefficient reported by Naderiboldaji et al., (2008) was lower than the results obtained for the Indian Bruno and Monty cultivars.

The coefficient of static friction is very useful in conveying fruits and vegetables during processing on conveyor belts. It was found to be high for Bruno i.e. on plywood, glass, fibre glass and galvanized iron sheets when compared with Hayward and Monty. No significant ($p>0.05$) difference was observed between all the three cultivars. Similar kind of results were showed for Iranian kiwifruits by Razavi and Parvar (2007). Coefficient of static friction for kiwifruits was higher than the other fruits like apricot, orange and cactus pear where the static coefficient of friction of cactus pear was found to be 0.181 and for apricot it was 0.281 over different surfaces (Haciseferogullari et al., 2007.

3.1.2. Mechanical properties

The mechanical properties viz. Firmness, hardness, adhesiveness, adhesive force and total positive area of three Indian kiwi cultivars (Hayward, Bruno and Monty)
were estimated and their mean value with SD were presented in Table 2.

Unpeeled kiwifruit

Fruit quality during storage can be efficiently controlled by knowing the packaging coefficient Jaliliantabar 2013. It

The highest firmness and hardness value was obtained for Monty. The value of firmness and hardness for Iranian kiwifruit reported by Razavi and Parvar (2007) was lesser than the results obtained for Indian Hayward and Monty cultivars. The highest adhesiveness and adhesive force was obtained for Monty. Significant ($p<0.05$) difference for total positive area was observed for Bruno with Hayward and Monty. The value of adhesiveness and adhesive force for Iranian kiwifruit reported by Razavi and Parvar (2007) was lower than the results obtained for Indian Hayward and Bruno cultivars. Adhesiveness is very important parameter in measuring the work necessary to overcome the attractive forces between the surface of the probe and surface of the fruit with which the fruit comes into contact. The mean total positive area of Hayward, Bruno and Monty were shown in Table 3. The highest total positive area was obtained for Hayward. The value of total positive area reported by Razavi and Parvar (2007) was lesser than the results obtained for Indian Hayward and Monty cultivars. As it can be seen, all the mechanical properties had resulted higher for unpeeled samples when compared with the peeled kiwifruits.

Peeled kiwi fruit

The Firmness and hardness are considered as an important quality parameter when assessing functional performance or ripeness. The highest firmness value was obtained for Hayward when compared with Bruno and Monty. The value of firmness for Iranian kiwifruit reported by Razavi and Parvar (2007) was lower than the results obtained for Indian Hayward and Monty cultivars. The highest hardness, adhesiveness and adhesive force values were obtained for Monty. The value of hardness, adhesiveness and adhesive force for Iranian kiwifruit reported by Razavi and Parvar (2007) were lesser than the results obtained for all the Indian cultivars. Adhesive force indicates the peak negative load attained in full cycle and force required to pull probe from the sample. Highest total positive area was found to be for Hayward. Significant ($p<0.05$) difference for total positive area was observed for Bruno with Hayward and Monty. The value of total positive area for Iranian kiwifruit reported by Razavi and Parvar (2007) was lesser than the results obtained for Indian Hayward and Monty cultivars. The total positive area indicates the work required to attain deformation indicative of internal strength of bonds within product.

Table 1. Physical properties of three kiwifruit cultivars

Property	Unit	Hayward	Bruno	Monty
		Mean value ± SD		
Length (L)	cm	6.79 ± 0.30[a]	7.67 ± 0.47[b]	6.29 ± 0.25[c]
Width (W)	cm	4.63 ± 0.24[a]	4.33 ± 0.22[b]	4.12 ± 0.29[c]
Thickness(K)	cm	4.19 ± 0.26[a]	3.35 ± 0.12[b]	3.96 ± 0.16[c]
Mass (M)	g	74.70 ± 6.35[a]	76.04±18.58[a]	63.22± 11.80[b]
Geometric mean diameter (D_g)	cm	5.09 ± 0.19[a]	4.80 ± 0.16[b]	4.68 ± 0.15[c]
Arithmetic mean diameter (D_a)	cm	5.20 ± 0.19[a]	5.11 ± 0.21[a]	4.79 ± 0.16[c]
Equivalent mean diameter (D_e)	cm	5.02 ± 0.18[a]	4.83 ± 0.12[b]	4.68 ± 0.24[c]
Sphericity (Φ)	%	73.73 ± 3.58[a]	62.77 ± 2.04[b]	74.49 ± 3.67[a]
Aspect ratio (R_a)	%	67.06 ± 5.36[a]	56.60 ± 2.91[b]	65.72 ± 5.32[a]
Surface area (S)	cm^2	79.81 ± 5.74[a]	72.52 ± 5.36[b]	68.74 ± 5.43[c]
True volume (V_t)	cm^3	82.09 ± 5.88[a]	70.41 ± 4.90[b]	58.53 ± 3.58[c]
Bulk density (ρ_b)	g/cm^3	0.59 ± 0.06[a]	0.67 ± 0.04[a]	0.61 ± 0.02[a]
True density (ρ_t)	g/cm^3	0.91 ± 0.07[a]	1.08 ± 0.04[b]	1.08 ± 0.06[b]
Porosity (Ɛ)	%	35.50 ± 2.40[a]	38.43 ± 3.58[a]	43.80 ± 2.04[b]
Peel ratio (R_s)	%	11.00 ± 1.24[a]	9.81 ± 2.40[a]	10.78 ± 1.67[a]
Packaging coefficient (P)		0.64 ± 0.10[a]	0.64 ± 0.17[a]	0.58 ± 0.11[a]
Coefficient of static friction (μ_s)	PW	0.49 ± 0.01[a]	0.57 ± 0.02[b]	0.45 ± 0.02[c]
	G	0.40 ± 0.01[a]	0.48 ± 0.02[b]	0.36 ± 0.01[c]
	FG	0.45 ± 0.02[a]	0.50 ± 0.02[b]	0.40 ± 0.01[c]
	GIS	0.42 ± 0.01[a]	0.48 ± 0.01[b]	0.38 ± 0.05[c]

[a-b-c]= Different letters in the same row indicates the mean values are significantly different ($p<0.05$)

Table 2. Mechanical properties

Property	Unit	Hayward	Bruno	Monty
		Mean value ± SD		
Peeled sample				
Firmness	g	240.03 ± 0.45[a]	210.25 ± 1.19[b]	250.65 ± 0.54[c]
Hardness	g	408.12 ± 2.45[a]	378.36 ± 5.02[b]	490.74 ± 3.12[c]
Adhesiveness	g.s	-963.23 ± 6.23[a]	-1080 ± 3.48[b]	-950 ± 1.67[a]
Adhesive force	g	-42.685 ± 0.01[a]	-48.00 ± 0.02[b]	-40.00 ± 0.02[a]
Total positive area	g.s	12414.83 ± 30.50[a]	10569.00 ± 54.58[b]	12782.00 ± 35.48[a]
Unpeeled sample				
Firmness	g	428.24 ± 0.11[a]	394.00 ± 0.19[b]	440.00 ± 0.05[a]
Hardness	g	4100.36 ± 2.02[a]	3891.00 ± 3.48[b]	4156.00 ± 1.67[a]
Adhesiveness	g.s	-3206.23 ± 6.23[a]	-3502.00 ± 2.87[b]	-3178 ± 5.71[a]
Adhesive force	g	-177.89 ± 1.23[a]	-187.00 ± 0.91[b]	-163.00 ± 1.23[a]

Total positive area	g.s	41868.05 ± 48.70[a]	37586.00 ± 27.30[b]	408563.00 ± 54.50[a]

[a-b-c]= Different letters in the same row indicates the mean values are significantly different ($p<0.05$)

3.1.3. Thermal properties

The thermal properties viz. Thermal conductivity, specific heat capacity, thermal diffusion and latent heat of fusion of three Indian kiwi cultivars (Hayward, Bruno and Monty) were measured and their mean value with SD were presented in Table 3.

3.1.3.1. Specific heat capacity, thermal conductivity, diffusivity and latent heat of fusion

The specific heat capacity was found to be high for Hayward variety followed by Monty and Bruno varieties. Insignificant difference ($p> 0.05$) was observed for all the varieties i.e. between Hayward and Bruno; Bruno and Monty varieties (Table 3). The result translates that these fruits hold their temperature for long time. But requires lot of energy to heat or cool the fruits. This may be due to the high moisture content of these fruits (McCabe et al., 1993). Various fruits like water melon also hold temperature for long time because of its high specific heat capacity value. Sweat, 1974 had informed that beside moisture content of the fruit the specific heat capacity also influenced by the composition of the fruits such as protein, fat, etc.

The thermal conductivities and diffusivities values of all the kiwifruit cultivars resulted low compared to pineapple, pawpaw, sour sop, cashew (Ikegwu and Ekwu., 2009). Significance difference ($p> 0.05$) was observed for all the cultivars i.e between Hayward, Bruno and Monty varieties. While significant difference ($p<0.05$) was observed between Hayward and Bruno and Insignificant difference ($p>0.05$) was observed between Bruno and Monty. This may be due to the total solids in the fruits. The low thermal diffusivities for these fruits may probably explain their low conductivities. Therefore, during thermal processing the movement or diffusion of heat energy from one point to another would generally be very low. These results translated that these fruits are poor conductors of heat, also the heat energy diffusion or transfer through these fruits and their juices during refrigeration, drying, freezing are very slow (Sweat, 1974). The latent heat of fusion for all the kiwifruit cultivars were very high just like other fruits viz. orange, pineapple, sour sop, mango, guava (Ikegwu and Ekwu., 2009). The values calculated were low with water melon, while banana fruit showed the least mean value. No significant ($p> 0.05$) difference was observed between all the varieties of kiwifruits (Table 3). This means that the amount of energy required for these foods to be frozen in a freezer would be high (McCabe et al., 1993)

Table 3. Thermal properties of three cultivar of Kiwi fruit

Variety	**Unit**	**Hayward**	**Bruno**	**Monty**
		Mean value ± SD		
Thermal Conductivity (K)	J sm^{-1}	0.554 ± 0.56[a]	0.561 ± 0.31[a]	0.547 ± 0.67[a]
Thermal diffusivity (α)	m^2 s^{-1}	1.43 ± 0.049 X 10^{-7a}	1.41 ± 0.06 X 10^{-7a}	1.49 ± 0.03 X 10^{-7b}
Specific heat capacity (C_p)	kJ kg^{-1} °C	3.732 ± 0.40[a]	3.598 ± 0.48[b]	3.697 ± 0.33[c]

Latent Heat of Fusion (ƛ)	kJ kg^{-1}	27560 ± 0.19[a]	27603 ± 0.22[a]	27095 ± 0.26[a]
[a-b-c]= Different letters in the same column indicates the mean values are significantly different ($p<0.05$)				

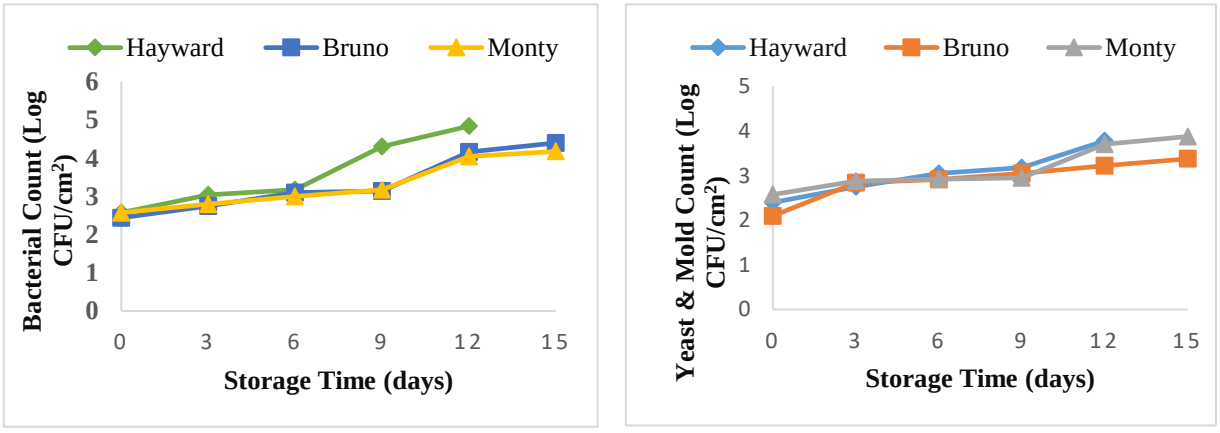
3.1.4. Biochemical properties

The biochemical properties viz. moisture, pH, titrable acidity (TA), total soluble solids (TSS) of three Indian kiwi cultivars (Hayward, Bruno and Monty) were measured and their mean value with SD were presented in Table 4.

The results showed that the Bruno had obtained highest moisture content when compared with Hayward and Monty. No significant ($p>0.05$) difference for moisture was observed between Hayward and Monty. In case of drying and evaporation moisture content can help to suggest the stability in storage of the fruits. The mean value of pH for Hayward, Bruno and Monty were found to be 3.51, 3.68 and 3.57 respectively. While the TA of Bruno is high when compared with Hayward and Monty cultivars. Acidity gives the characteristic flavour to guava (Jagtiani et al., 1988). Acids and sugars are important components for kiwi fruit, because these biochemical properties provide characteristic taste and flavour to the fruit. The mean value of TSS for Hayward, Bruno and Monty during experiment was found to be 10, 10 and 10 °Brix respectively. Similar kind of results was observed by Pal et al. (2015). Sweetness-to-sourness (°Brix/acid) expresses the quality of the fruit juice and is expressed in terms of the flavour quality of the commodity which regulates the fruit value to consumers depends on the content of sugars, organic acids, etc. (Sharma, 2013).

Table 4. Biochemical properties of three cultivar of Kiwi fruit

Variety	**Unit**	**Hayward**	**Bruno**	**Monty**
		Mean value ± SD		
Moisture	%	82.6 ± 1.24[a]	84 ± 1.08[b]	82.9 ± 0.60[a]
pH		3.51 ± 0.01[a]	3.68 ± 0.01[b]	3.57 ± 0.01[c]
TA	%	0.42 ± 0.08[a]	0.40 ± 0.07[b]	0.48 ± 0.02[c]
TSS	°Brix	10 ± 0.01[a]	10 ± 0.01[a]	10 ± 0.01[a]
[a-b-c]= Different letters in the same column indicates the mean values are significantly different ($p<0.05$)				



(a) (b)

Figure 2. (a) and (b). Total bacterial count of kiwifruits under ambient temperature

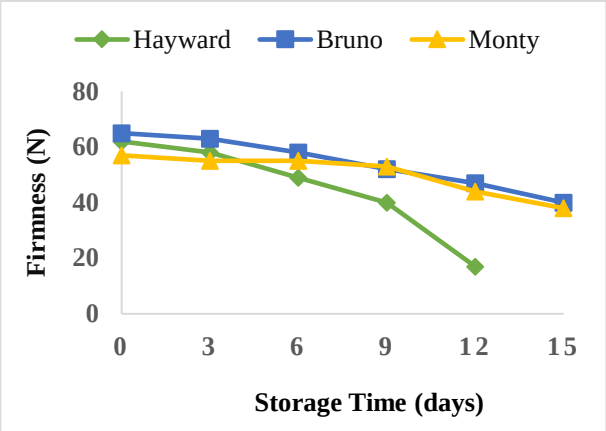


Figure 3. Firmness of kiwifruits under ambient temperature

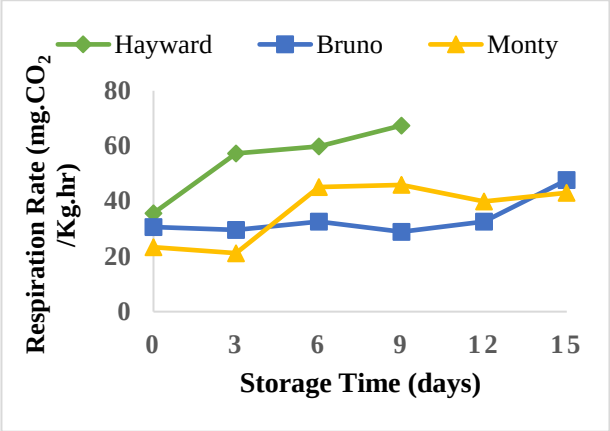


Figure 4. Respiration Rate of kiwifruits under ambient temperature

3.1.5. Shelf life of kiwifruit stored under room temperature

Shelf life study is useful for designing the storage facilities, transportation, refrigeration etc. From this study it was clearly identified that the Hayward cultivar had short shelf life compared with other two varieties i.e. Bruno and Monty. Bacteria, yeast and mold showed significant ($p<0.05$) difference till 12th day of storage and also between the three varieties (Hayward, Bruno and Monty) at ambient temperature. Bruno and Monty cultivars started to spoil from day 15th day hence rejected from the study while the Hayward cultivar spoils after 12th day of storage. Firmness of the Hayward variety reduced significantly ($p<0.05$) between 9th and 12th day of storage for Hayward. Significant difference was seen between Hayward and other two varieties i.e. Bruno and Monty on 9th day of storage under ambient temperature for respiration rate (Fig. 2a, 2b, 3 and 4).

4. Conclusions

The length (6.79cm) width (4.63cm), thickness (4.19cm), Geometric mean diameter (5.09cm), arithmetic mean diameter(5.20cm), equivalent mean diameter (5.02cm), aspect ratio (67.06%), surface area (79.81cm^2), true volume (82.09cm^3) and peel ratio (11), values were found to be higher for Hayward variety when compared with Bruno and Monty. The length (7.67cm), mass (76.04g), bulk density (0.67g/cm^3), moisture (84%) and pH (4.40) values were higher for Bruno when compared with Hayward and Monty. The sphericity (74.49%), porosity (43.80%) and TA (0.50%) values were higher for Monty when compared with Hayward and Bruno. True density (1.08g/cm^3) is equal for Bruno and Monty while packaging coefficient (0.64) values were equal for Hayward and Bruno cultivars. Monty was more spherical thus it can easily roll on the surfaces. Coefficient of static friction values was higher for plywood for all the varieties i.e. 0.49, 0.57 and 0.45 for Hayward, Bruno and Monty cultivars respectively. Mechanical properties like firmness (428.24), hardness (4100.36g), adhesiveness (-3206.23g.s), adhesive force (-177.886g) and total positive area (41868.05g.s) values were high for unpeeled samples when compared with peeled samples. Thermal conductivity and specific heat capacity was higher for Bruno. These physical, mechanical properties, thermal and bio chemical are very important in facilitating the design of modern machinery and processing equipment with altered quality specifications for minimizing the losses during processing. This study concluded that the Hayward cultivar has less shelf life compared with Bruno and Monty cultivars.

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METAL AND LEAD-STRONTIUM ISOTOPE CHARACTERIZATION OF RED AND WHITE WINES FROM BUJORU, SMULTI AND OANCEA WINE CENTER, ROMANIA

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ABSTRACT

The goal of this research was to assess the potential of Sr and Pb composition and also isotopic signature of lead ($^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ and $^{204}Pb/^{206}Pb$), strontium ($^{87}Sr/^{86}Sr$) of wines from Bujoru, Smulți and Oancea wine-growing centers from Dealu Bujorului vineyard. In this study 162 wine samples were investigated. The wine samples were obtained from micro-wine production under conditions of 2014-2016 from Dealu Bujorului vineyard. For all tested wine samples, the toxic metals contents were found in quantities below the limits established by legislation. The highest values were registered to wine obtained from Feteasca Neagra(2016) variety (0.74275±0.00261) from Smulți wine-growing center, the lowest value of $^{87}Sr/^{86}Sr$ isotope ratio was recorded to wine obtained from Muscat Ottonel (2014) variety (0.70165±0.00058) Oancea wine-growing center. A possible explanation for the higher mean of $^{87}Sr/^{86}Sr$ isotopic ration for wine can be the mineral consistency of the vineyard soil and its different eco-climatic conditions. Regarding $^{206}Pb/^{207}Pb$ isotopic ratios, we can say that the analyzed wine samples show shows traces of pollution comes from cars (automobile emissions) (if $^{206}Pb/^{207}Pb$=1.1000-1.1400 [automobile emissions]). The Pb isotope ratio from wines varies in range between 1.12305-1.18597 ($^{206}Pb/^{207}Pb$), 2.09404-2.14190 ($^{208}Pb/^{206}Pb$) and 17.21089-17.70857 ($^{206}Pb/^{204}Pb$) with average 1.15202 ($^{206}Pb/^{207}Pb$), 2.10878 ($^{208}Pb/^{206}Pb$) and 17.42240 ($^{206}Pb/^{204}Pb$). Heat map was discovered a separation of wine varieties for white of this red depending on elemental contents and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$ isotope ratios.

1. Introduction

Among the different criteria adopted to protect and promote food quality, the European Union (UE) has introduced, with the use of quality schemes, the link between territory and food (Sighinolfi *et al.*, 2018). In this context, the protected designation of origin (PDO) represents the highest award that can be attributed to an aliment, and its implies that the entire food chain is within a delimited territory (Bora *et al.*, 2018). Although there are many paper certifications for each food chain process that state for authenticity and quality arising from a particular geographical origin, none of these is based on objective criteria. In recent years, several attempts to develop tracking and tracing models for food processes have been made (Voerkelius *et al.*, 2010; Baroni *et al.*, 2011; Danezis *et al.*, 2016).

Geographical origin and authenticity are factors affecting he overall perception of wines in terms of their quality and price, hence, being

of great important to consumers and wine producers. We refer here to the origin certified products for which elevated prices are justified by the strict relationship which exist between the product quality and the areas of origin (Bora *et al.*, 2018).

Starting from the fact that de term origin has a considerable importance, which correlates to the quality of wines also wine classification in terms of geographical and varietal origins became an argument of significant interest both for consumer and producers. In this respect, various analytical and statistical tools have been proposed for identifying wine are of production, form largest (e.g., country) (Almeida *et al.*, 2016; Aoyama *et al.*, 2017) (e.g., ward, district, wine centers) (Larcher *et al.*, 2003; Avram *et al.*, 2014; Coetzee *et al.*, 2005; Bora *et al.*, 2018).

When dealing with traceability models, it is of utmost importance to determine the identify of geographical indicator that is used to monitor the food chain from the field to the final product. For geographical traceability issues, one of the indicators that can be used is the primary or direct type, such as metal concentration or isotope ratios as bio-elements ($^{13}C/^{12}C$, D/H, $^{18}O/^{16}O$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$) or radiogenic heavy elements ($^{87}Sr/^{86}Sr$, $^{145}N/^{143}Nd$, and $^{207}Pb/^{206}Pb$) (Horn *et al.*, 1993; Walker *et al.*, 1989; Price *et al.*, 2002; Fortunato *et al.*, 2004; Trincherini *et al.*, 2014; Marchionni *et al.*, 2016; Aoyama *et al.*, 2017).

Evaluation of natural abundance isotope ratios provides information on plant type or animal diet (carbon rations) and geographical origin (lead, strontium, deuterium and oxygen isotopic ratios) (Kelly *et al.*, 2005). However, the $^{13}C/^{12}C$ ratios of plants are affected not only by the botanical origin (C3 and C4 plants) but also by physiological and environmental factors that influence water used efficiently in the leaves. Stomatal conductance and intercellular and ambient CO_2 concentrations are influenced by humidity, precipitation, temperature, water stress plant age, and maturation. Strontium is found in nature as three abundant isotopes: ^{86}Sr (9.75-9.99%), ^{87}Sr (6.94-7.14%), ^{88}Sr (82.29-82.77%) and ^{84}Sr (0.55-0.58%) as less abundant isotope (Berglund *et al.*, 2011). The ^{87}Sr is radiogenic and therefore the ^{87}Sr content increases with time due to radioactive decay of ^{87}Rb (Petrini *et al.*, 2015). Since the content of ^{87}Sr in soil varies with geological age and geographical location, the $^{87}Sr/^{86}Sr$ isotopic ratio can be used as a tracer for determining the geographical origin of wine (Vorster *et al.*, 2010). Lead is found in nature as four abundant isotopes: ^{206}Pb (20.84-27.48%), ^{207}Pb (17.62-23.65%), ^{208}Pb (51.28-56.21%) and ^{204}Pb (1.04-1.65%) as less abundant isotope (Rosman *et al.*, 1998). Their abundance extensively varies because of different decay pathways from ^{238}U, ^{235}U and ^{232}Th to ^{206}Pb, ^{207}Pb, ^{208} Pb respectively (Bora *et al.*, 2018). The Pb isotope of ore deposits and anthropogenic sources has their distinct isotopic ratios or signatures (Cheng *et al.*, 2010). The Pb isotope ratio did not change in industrial or environmental processing and retained its retained its characteristic ratio from source ore (Ault *et al.*, 1970).

Each geologic substratum of vineyards is liable to heave its own Sr isotope composition, which can potentially represent a fingerprint to trace the wine production provenance (Marchionni *et al.*, 2016). The use of $^{87}Sr/^{86}Sr$ in tracking wine regional provenances was among the most pioneering application of isotope geology to other sciences (Barbaste *et al.*, 2002). In most of the cases, however, the analytical uncertainty observed in Sr isotopes analyses of wines from literature is larger than most of the soil/rock isotopic variability, giving strong difficulties in matching data of wines with those from geologic substrata of the vineyards. Recently, high precision analytical method for determining $^{87}Sr/^{86}Sr$ has been provide enabling then direct comparison between data on wines with those of the pedological and geological substrata (Durante *et al.*, 2015; Petrini *et al.*, 2015).

Long lived isotope ratios of heavy metals of geological interest, such as $^{87}Sr/^{86}Sr$, $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, have in the last decades gained importance in tackling the issues of geographical food traceability as well as in solving issues related with archaeological,

environmental, medical and also forensic sciences (Hoogewerff *et al.*, 2001; Voerkelius *et al.*, 2010). This increasing consideration is mainly based on the fact that radiogenic isotopic ratios are extensively used either for tracking geological and environmental processes or dating Earth's materials and cosmological (Stewart *et al.*, 1998). In addition, radiogenic isotope ratios are fractionated by biogenic processes or by low-temperature, and then their abundance in geological materials (minerals and rocks) depends upon: i) the initial radiogenic isotopic abundance, ii) on the age of the mineral/rock and iii) on their parent/daughter isotope ration (Tommasini *et al.*, 2009).

The presence of lead in wine is associated with two major sources as follows: natural sources, which are due to the weathering of rocks, and human activity, which results from the use of fertilizers, pesticides and agricultural and food additives and environmental pollution (Larcher *et al.*, 2003).

The goal of this study is to determine the elemental composition (Pb and Sr) of wine from Bujoru, Smulți and Oancea wine-growing centers from Dealu Bujorului vineyard and to assess their ability to discriminate between geographical origin of wines. Also, the study enhances the knowledge of the large-scale distribution of strontium ($^{87}Sr/^{86}Sr$) and lead ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$) isotope ratios in wine from Dealu Bujorului vineyard. The wines from Smulți and Oancea wine-growing centers have not been analyzed yet regarding concentration of the elemental composition and distribution of strontium ($^{87}Sr/^{86}Sr$) and lead ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$) isotope ratio.

2. Materials and methods

2.1. Experimental section

2.1.1. Study area

For this study, a total of 162 wine samples (81 white wines and 3 red wines), samples originated from Bujoru, Smulți and Oancea wine-growing centers part of Dealu Bujorului vineyard (45°52′10″ N, 27°55′8″E). The Dealu Bujorului vineyard is characterized by an alternate landscape, from flat to hilly areas, with altitude between 100 and 230 m and the predominant soil is levigated chernozem having a clayey sand texture with pH between values 7.0 and 8.0. Although they have moisture deficit, natural conditions (ecoclimatic and ecopedological) offer viable ecosystem for the development of vineyard. The vineyard is crossed by the parallel 46° latitude north, intersected by the 28° longitude meridian. Dealu Bujorului vineyard belongs to Galati country. The specificity of the transition area is highlighted by the predominance of deposits of clays and sands. Versants were made from clay deposits and sandy sands.

The wines were selected from three consecutive vintages (2015, 2016 and 2017) were obtained by microvinification based on EC Regulation No. 2729/2000, consolidated with EC Regulation No. 2031/2006. Of these, 30 samples were from Bujoru wine-growing center, 56 from Smulți wine-growing center and 76 from Oancea wine-growing center. From the harvest of 2015, 2016 and 2017 were collected 54 wine samples.

2.1.2. Climatological data

The climate data used in this research was recorded through AgroExpert system at RSVE Bujoru and also from weather forecasting center. In experimental years the thermal balance values obtained are higher than multiannual average: global thermal balance (Σt°g) was 3560.9 °C (3484.0 °C multiannual average), active thermal balance (Σt°a) was 3526.6°C (3387.5 °C multiannual average) and beneficial thermal balance (Σt°u) was 1736.6°C (1700.1 °C multiannual average). The precipitation quantity was lower (405.4 mm) then average of the last ten years (505.7 mm). During the growing season, the recorded precipitations values were 257.6 mm, much lower than the multiannual average of 291.5 mm for Bujoru Wine Centre. The ecoclimatic conditions of Dealu Bujorului vineyard highlighted the exceptional viticultural characters of the Dealu Bujorului vineyard. These characters were found in the authenticity

and specificity of a wide assortment of wine obtained in the studied area.

2.1.3. Sample collection and microvinification process

The white cultivars consisted of Muscat Ottonel, Feteasca alba ad Feteasca regala, while red cultivars were Feteasca neagra, Merlot and Cabernet Sauvignon under the conditions of 2015, 2016 and 2017. The wine samples resulted from micro-wine production. Micro-wine production was done according to the methodology describe by Bora *et al.*, 2016; Bora *et al.*, 2018, Donici *et al.*, 2019. All wines were providing by the wineries as finished wines in 750 mL bottles with cork stoppers and were stored at 3-4°C before analysis. All vines were planted since 1979, and the vine plantation was organized with 2.2 x 1 m distance between rows and plants. Vines were pruned according to the Guyot system and were grown on speliers.

2.2. Material and methods used

2.2.1. Reagents and solution

2.2.1.1. Blanks

An appropriately diluted indium intern standard stock solution was prepared from 1000 mg/L In ICP-MS standard solution (Alpha Aeser).

The method blank for the 1:2 diluted wine samples was prepared to contain 7.6% ethanol in 1% nitric acid. The blank for the digested wine samples was prepared by subjecting the solution used for digestion (7 mL 65% nitric acid plus 1 mL H_2O_2) to the microwave digestion program and diluting to 50 mL with Milli-Q water.

2.2.1.2. Physical and chemical analysis

The physical and chemical analysis of young wine were performed in the Winemaking Laboratory of the RSDVV Bujoru and were applied in accordance to the methods of analysis described in the Compendium of international methods of analysis of wines and musts and to the Romanian STAS methods (Bora *et al.*, 2016; Bora *et al.*, 2018). During this analysis the following parameters were determined: alcohol (% vol.) – was determined using the ebulliometric method, STAS 6182/6-70; total acidity (g/L $C_4H_6O^6$) - titrimetric method, STAS 6182-1:2008; volatile acidity (g/L CH_3COOH) - according to STAS 6182-2:2008; residual sugar (mg/L) – according STAS 6182/17-81. The next parameters: acetic acid (g/L); amino nitrogen (mg/L); tartaric acid (g/L); L-lactic acid (g/L); D-malic acid (g L) were determinate using spectrophotometric method.

2.2.1.3. Standards

For analysis of the main quality of parameters of wine, all reagents used for calibration were of analytical grade (TDI - Tecnología Difusión Ibérica, S.L. Fr.).

Two elements (Pb and Sr) were determined in order to assess their ability to discriminate wines by geographical origin. The analysis was made using multielement analysis and ICP-MS technique, after an appropriate dilution, using external standard calibration method. The calibration was performed using XXICertiPUR multielement standard. The working standards and the control sample were prepared daily from the intermediate standards that were prepared from the stock solution. The intermediate solutions stored in polyethylene bottles and glassware was cleaned by soaking in 10% v/v nitric acid for 24 hours and rinsing at least ten rimes with ultrapure water (18.2 MΩ cm^{-1} ultrapure water-Types 1). The accuracy of the methods was evaluated by replicate analyses of fortified samples (10 µL-10 mL concentrations) and the obtained values ranged between 0.8-13.1 percent, depending on the element. The global recovery for each element was estimated and the obtained values were between 84.6-100.9% (Bora *et al.*, 2016; Bora *et al.*, 2018).

Table 1. Instrumental conditions for the determination of each element (ICP-MS)

Element	Correlation coefficient	LoD* (µg/L)	LoQ*** (µg/L)	BEC** (µg/L)
Sr	0.9999	0.1434	0.4775	0.955
Pb	0.9999	0.0003	0.0010	0.002

*Detection limit; **Background equivalent concentration; ***Quantification limit.

For quality control purpose, blanks and triplicates samples (n = 3) were analyzed during the procedure. The variation coefficient was under 5% and detection limits (ppb) were determined by the calibration curve method. Limit of detection (LoD) and Limit of quantification (LoQ) limits were calculated according to the next mathematical formulas: LoD = 3SD/s and LoQ = 10 SD/s (SD = estimation of the standard deviation of the regression line; s = slope of the calibration curve) (Table 1).

For calibration and to verify the achieved accuracy and precision, ten NIST-SRM 987 and NIST-SRM 982 analysis results were pooled together with the calculated relative standard deviation presented in Table 2.

Table 2. Lead isotopic ration and Lead isotopic ration determination precision and accuracy based on the NIST SRM 982 (Lead) NIST SRM 987 (Strontium) (n=10)

$^{207}Pb/^{206}Pb$ (a)	$^{208}Pb/^{206}Pb$ (b)	$^{204}Pb/^{206}Pb$ (c)	$^{87}Sr/^{86}Sr$ (d)
0.46179	0.99736	0.02270	0.71117

[a]Certified value = $^{207}Pb/^{206}Pb$ (0.46707 ± 0.00020); [b]Certified value = $^{208}Pb/^{206}Pb$ (1.00016 ± 0.00036); [c]Certified value = $^{204}Pb/^{206}Pb$ (0.027219 ± 0.00027); [d]Certified value = $^{87}Sr/^{86}Sr$ (0.71034 ± 0.00026).

Based on the obtained results, it was verified that, applying quadrupole ICP-MS, relative standard deviation and reproducibility of approximately 0.5% for $^{87}Sr/^{86}Sr$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ are feasible. The results were in agreement with those reported by Avram *et al.*, 2014; Bora *et al.*, 2016; Bora *et al.*, 2018.

2.2.4. Sample preparation for determination of heavy metals and isotopic ration from wine using ICP-MS

For the determination of elements from wine samples were used an amount of 0.5 mL wine and adjust 8 mL (7 mL HNO_3 65%+1 mL H_2O_2) were placed in a clean Teflon digestion vessel, after 15-30 minutes the mineralization was performed using a microwave system Milestone START D Microwave Digestion System set in three steps: step I (time 10 min., temperature 200ºC), step II (time 15 min., temperature 200ºC) and step III (time 40 min., ventilation - temperature 32ºC). After mineralization, samples were filtered through a 0.45 mm filter and brought to a volume of 50 mL. The Pb and Sr isotope ration in the analysed wine samples ($^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$,) were determined according to the methodology indicated by Bora *et al.*, 2016; Bora *et al.*, 2018.

Table 3. Standard additions for checking accuracy of the microwave digestion ICP-MS method (n = 3) (SRM 1643e)

Element	Certified Concentration (mg/L)	Measured Concentration (mg/L)
Sr	314.00±19.00	314.09±09.06
Pb	19.63±0.21	19.13±0.09

In order to confirm the best-chosen conditions for wine digestion standard additions for checking accuracy of the microwave digestion and recoveries were calculated (Table 3). The digestion seemed visually completed in all of the combinations, but the spiked recoveries showed significant differences for total elements content (*p* - Value = *0.005*).

2.2.5.Instrumentation

In order to get a wider range of data about the quality of the tested wine and to determine the acetic acid, amino acid, tartaric acid, L-lactic acid, and M-malic acid the MIURA ONE I.S.E. S.r.l., Rome, Italy device was used.

All analyses were carried out with iCAP Q Thermo scientific model Coupled Plasma Mass Spectrometer (ICP-MS) equipped with nikel cones, a peristaltic sample delivery pump and a Cetac auto sampler. Instrumental conditions for the ICP-MS were optimized, after completing the mass calibration and detector cross-calibration, by following a manual tuning procedure using Thermo Tuning Solution A containing a manual tuning procedure using Ni, In, Ba, Ce, Pb, Bi and U at 10 µg/L, and As and Se at 100 µg/L. For data acquisition the ICP-MS was operated in peak pump mode, with a dwell time of 20 ms, 100 sweeps. Five replicate measurements were made. The isotopes ^{86}Sr,

^{87}Sr, ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb. The elements selected are mostly metals and are considered to be useful as possible indicators of geographical origin, since they are not generally affected by vinification and are therefore in principle providing a link with the soil composition. Most of them have been tested in a number of previous studies (Almeida *et al.*, 2003; Coetzee *et al.*, 2005; Moreno *et al.*, 2008; van der Linde *et al.*, 2010).

The argon used was of 99.99% purity (Messer, Austria). The instrument was daily optimized to give maximum sensitivity for M^+ ions and the double ionization and oxides monitored by the means of the rations between Ba^{2+}/Ba^+ and Ce^{2+}/CeO^+, respectively, these always being less than 2%. The experimental conditions were: argon flow on nebulizer (0.82 L/min.), auxiliary gas flow 0.80 L/min., argon flow in plasma 15 L/min., lens voltage 7.30 V; RF power in plasma 1100 W, spray chamber temperature (2.42±1.00^{o}C). Accuracy was calculated for the elements taken into consideration (0.5-5.0%).

To reduce molecular interferences, the data of ^{86}Sr, ^{87}Sr, ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb were collected using Collision Cell Technology (CCT). Tuning in CCT mode using 7% H/He gas was carried oud using Autotune function and Thermo Tuning Solution A. To enhance the stability of the analyte signal, the sample uptake of the peristaltic pump was regularly replaced.

2.2.6.Statistical analysis

Data analysis was performed to test if significant differences can be highlighted between the geographical origin of wines samples by district. The statistical interpretation of the results was performed using the Duncan test, SPSS Version 24 (SPSS Inc., Chicago, IL., USA). The statistical processing of the results was primarily performed in order to calculate the following statistical parameters: average and standard deviation. The statistical analysis was performed by using the analysis of variance (ANOVA) followed by multiple comparisons tests in which the level of significance was set at $p \leq 0.05$. ANOVA determined, for each parameter, the mai effect of the region and of the year of harvest on the multiisotopic and multielement composition of samples. Secondly, the data were processed using the Linear Discriminant Analysis (LDA).

Multivariate chemometric method was used as a supervised learning technique for the differentiation of wines intro groups on the basis of grape variety and year of production and finding markers which showed a significant discrimination value (variables with Wilk's lambda near zero, p value <0.05). Stepwise linear discriminant analysis (LDA) was used to identify significant tracers for classification to the geographical discrimination of the wines samples. At each step, the variable with the best discriminating power, as described by an F-statistic and $p < 0.0001$, was entered into the model and then several discriminant functions were obtained. Stepwise Discriminant Analysis (LDA) was used to designate suitable variables for classification of the samples, eliminating the variables that do not contribute to discrimination of the wine. In order to validate the proposed statistic model, based on variables which showed higher significance in first LDA assessment, we performed a second Linear Discriminant Analysis (LDA) for the test set consisting of wines used to build statistical model (training set) together with data from other wine samples that are not included in the first LDA (control-set). Cross-validation was applied to determine the optimal number of variables required to obtain robust models. Linear discriminant analysis (LDA) was performed using Microsoft Excel 2016 and XLSTAT Addinsoft version 15.5.03.3707.

3.Results and discussions

3.1. Analysis of the main quality of parameters of wine

Based on the results regarding the qualitative assessment of the tested varieties, they have a very good suitability in the studied areas. In terms of quality rating, they display particular characters of the varieties, as well as the ecoclimatic conditions and ecopedological influence on the quality of wine (Table 4).

The physico-chemical analysis of wines showed that the highest alcohol content was recorded at the Merlot variety (15.40 % vol.), followed by Feteasca neagra (15.17 % vol.). The highest level of acidity was recorded at the Muscat Ottonel (6.37 g/L $C_4H_6O_6$) and the lowest acidity in the Feteasca regala (4.36 g/L $C_4H_6O_6$).

3.2. $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratio in wine samples Bujoru, Smulți and Oancea Wine Center

Lead isotopic analysis of wines from France (Bordeaux) showed that lead in the wines changed over time and reflect the dominant source of atmospheric lead pollution in southern of France (Médina *et al.*, 2000). Other researchers have found that lead isotopic compositions in wine may not always reflect those of leaded petrol, but reflect the isotopic signature of local, dominant metallurgical industries (Galani-Nikolakaki *et al.*, 2002). These studies confirm atmospheric deposition as being the dominant contributor to the lead total concentration and isotopic composition from wines. Some studies have shown that contamination from tin-lead foil capsules in the presence of corrosion and cork disintegration can dominate the source of lead from wines (Gulson *et al.*, 1992). Other researchers have attributed the lead in wine to machinery or additives used during the vinification process where environmental contamination in this case is low (Almeida *et al.*, 2003). In Central Europe, the lead isotopic ratio, as signatures of pollution sources, ranges from relatively high $^{206}Pb/^{207}Pb$ ratios (natural Pb, coals, fly ashes, $^{206}Pb/^{207}Pb$ = 1.1700 - 1.2200) to low $^{206}Pb/^{207}Pb$ values (gasoline, petrol combustion, $^{206}Pb/^{207}Pb$ = 1.0600 - 1.1400) [30].

The original composition of soil samples retains its chemical composition from the geographical area it belongs to (Shirahata *et al.*, 1980; Gulson *et al.*, 1981; Elbaz-Poulichet *et al.*, 1984; Bora *et al.*, 2013). This property is useful in order to identify of the source of lead in a subjected wine sample provided that the measurements of the isotope ratio is precise and accurate.

The Pb isotope ratio from wines (Table 5) varies in range between 1.12305 - 1.18597 ($^{206}Pb/^{207}Pb$), 2.09404 - 2.14190 ($^{208}Pb/^{206}Pb$) and 17.21089 - 17.70857 ($^{206}Pb/^{204}Pb$) with average 1.15202 ($^{206}Pb/^{207}Pb$), 2.10878 ($^{208}Pb/^{206}Pb$) and 17.42240 ($^{206}Pb/^{204}Pb$). Regarding $^{206}Pb/^{207}Pb$ isotope ratios based on analyses it can be concluded that the wine obtained from vine varieties grown in the Bujoru wine-growing centers how's traces of pollution comes from cars (automobile emissions) (if $^{206}Pb/^{207}Pb$ = 1.1000 - 1.1400 [automobile emissions]).

The obtained isotope ratio values are comparable with Avram *et al.*, 2014 (1.1100 to 1.2000 Romania wines) and Almeida *et al.*, 2016 (1.1440 to 1.1820 Brazilian wines).

The highest values of $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ were registered to wine obtained from Muscat Ottonel (2015) variety from Bujoru wine-growing center (2.14190 ± 0.00743 $^{208}Pb/^{206}Pb$) and Cabernet Sauvignon (2014) variety from Oancea wine-growing center (17.70857 ± 0.00394 [0.02238] $^{208}Pb/^{206}Pb$) while Cabernet Sauvignon (2014) from Oancea wine-growing center (2.09205 ± 0.00576 $^{208}Pb/^{206}Pb$) and Feteasca Regala (2014) from Oancea wine-growing center (17.21089 ± 0.00116 $^{206}Pb/^{204}Pb$) recorded the lowest isotope ration. The results are comparable with Almeida *et al.*, 2016 (2.0700 to 2.1570 Brazilian wines $^{208}Pb/^{206}Pb$; 16.6670 to 17.9960 Brazilian wines $^{204}Pb/^{206}Pb$) and also with Barbaste *et al.*, 2002 (2.0990 to 2.1030 Italian wines $^{208}Pb/^{206}Pb$; 17.544 to 18.3210 Italian wines $^{204}Pb/^{206}Pb$).

The abundance of the lead isotopes ^{204}Pb (non-radiogenic), ^{206}Pb, ^{207}Pb and ^{208}Pb (radiogenic) originated from the genesis of the substrate varies with geological ages. The original composition of the rock upon its formation and consequently, with geographical areas (Gulson *et al.*, 1992), this property is useful in order to identify of the source of lead in a subjected wine sample provided that the measurements of the isotope ratio is precise and accurate.

Concerning $^{87}Sr/^{86}Sr$ isotope ratio, the values are between the ranges from 0.70112 to 0.74275 with an average value of 0.71909. The highest values were registered to wine obtained from Fetească neagră (2016) variety (0.74275 ± 0.00261) from Smulți wine-growing center, the lowest value of $^{87}Sr/^{86}Sr$ isotope ratio was recorded to wine obtained from Muscat Ottonel (2014) variety (0.70165±0.00058) Oancea wine-growing center.

The values of $^{87}Sr/^{86}Sr$ isotope ratio obtained are comparable with Geana *et al.*, 2017 (0.71015 to 0.72311 Romanian wines); Avram *et al.*, 2014 (0.7600 to 0.9300).

Variation of the $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratio and heavy metals concentration from wines with different geographical origins confirm the link with geological substratum of the production territory, making the $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ration and heavy metals concentration a robust instrument for tracing the geographical provenance of wines.

3.3. Combining multielement analysis and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope ratio from wine samples for discrimination analysis

Multivariate techniques are methods of analysis generally recognized as very all know to study environmental problems (Tauler *et al.*, 1995). From this kind of methods, Linear Discriminant Analysis (LDA) has been selected one of the most advantageous to have a close look of our system. LDA belongs to supervised pattern recognition methods (Vončina, 2009) and has the aim to assign object to several predetermined classes.

Linear Discriminant Analysis (LDA) was used as a supervised learning technique for the differentiation of wines intro groups on the basis of grape variety and year of production and finding markers which showed a significant discrimination value (variables with Wilk's lambda near zero, p value <0.05 and higher F coefficients). Stepwise linear discriminant analysis (LDA) was used to identify significant tracers for classification to the geographical discrimination of the wines samples. Stepwise Discriminant Analysis (LDA) was used to designate suitable variables for classification of the samples, eliminating the variables that do not contribute to discrimination of the wine. In order to validate the proposed statistic model, based on variables which showed higher significance in first LDA assessment, we performed a second Linear Discriminant Analysis (LDA) for the test set consisting of wines used to build statistical model (training set) together with data from other wine samples that are not included in the first LDA (control-set).

Cross-validation was applied to determine the optimal number of variables required to obtain robust models.

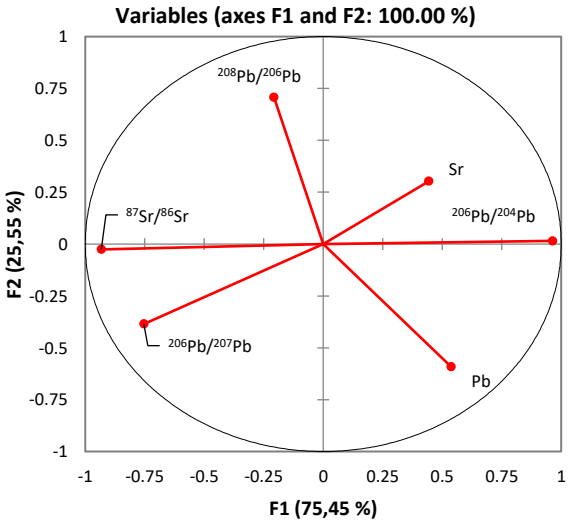

Figure 1. Correlation between analyzed parameters and the factors in discriminant analysis the origin of the wine

In this study, the content of certain wines shows high concentration of metals, but not exceeding the maximum recommended by International Organisation of Vine and Wine (O.I.V., 2015), and this mostly due to agricultural practices, fertilizers and technological winemaking processes.

Elements like Pb, Cu, Ni, Cd, U and Hg showed a high discriminatory power for geographic origin of Romanian wines, but additional new elements (Mn, Cr) and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio have been investigate in order to identify new tracers for geographical traceability of Romanian wines (Geana *et al.*, 2017; Bora *et al.*, 2016; Bora *et al.*, 2018).

The wines obtained in the three wine-growing centers can be geographical fingerprints based on the concentration of Pb, Cu, Ni, Cd, Hg, Mn, Cr, U and also based on the $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio (Bora *et al.*, 2016).

Based on the elemental contents and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio, the cross-validation technique provided a 100 % percentage of predicted membership according to the origin of the wine (F1 = 75.45 % and F2 = 25.55 %). The linear correction revealed acceptable scores for the two defined discriminant factors (F1 and F2) (Figure 1).

A significant differentiation of wines according to wine-growing centers and year of wine production was carried out for wines samples, which demonstrates the importance of elemental profile for the geographical traceability of wines (Figure 2).

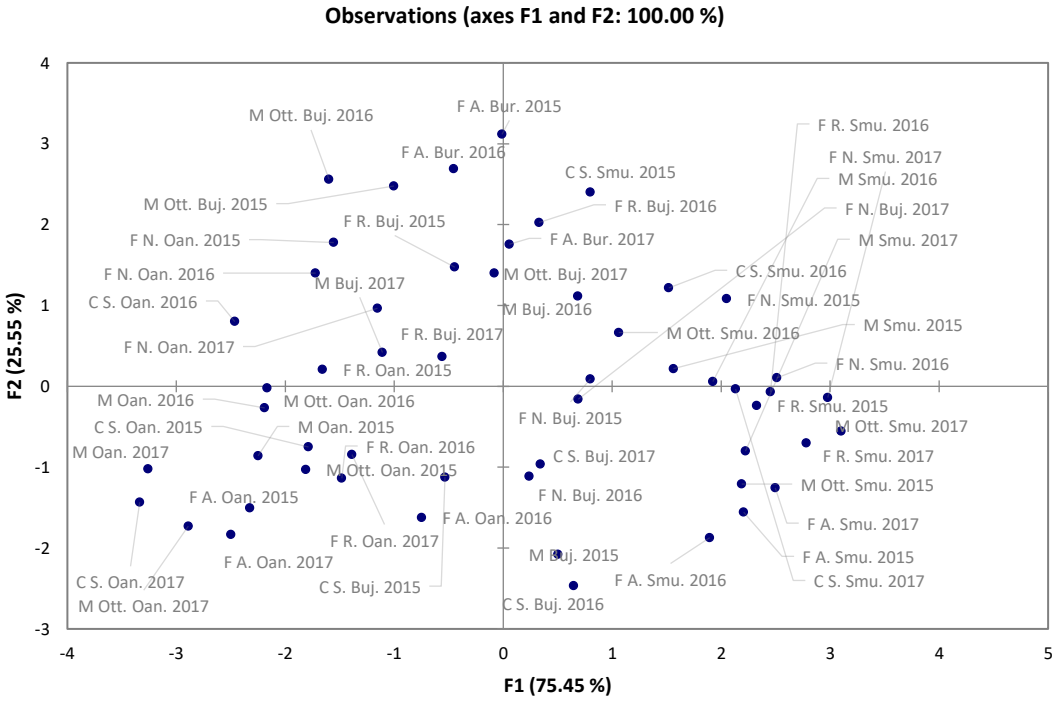

Figure 2. Differentiation of wines based on element contents and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$ isotope ratios

Table 4. The analysis of the main quality parameters in wine samples from Dealu Bujorului Vineyard (Bujoru, Smulți and Oancea Wine Centre)

Wine Centre	Variety	Year	Alcohol (% vol.)	Total acidity (g/L $C_4H_4O_6$)	Volatile acidity (g/L CH_3COOH)	Residual Sugar (g/L)	Non reducing extract (g/L)	Acetic acid (g/L)	Amino acid (mg/L)	Tartaric acid (g/L)	L-Malic acid (g/L)	L-Lactic acid (g/L)
Bujoru Wine Center	M Ott.	2014	14.46 ± 0.12 abcde	4.68 ± 0.10 lmnop	0.32 ± 0.04 mnop	6.47 ± 0.25 îjklm	24.75 ± 0.53 cdefg	0.33 ± 0.01 ab	22.52 ± 0.95 ab	1.29 ± 0.05 jkl	1.12 ± 0.10 i	ULD
		2015	14.41 ± 0.45 abcdef	4.91 ± 0.06 iîjkl	0.46 ± 0.03 cdefghiîjkl	4.59 ± 0.07 opqrsșt	21.88 ± 0.76 hiîjk	0.34 ± 0.05 ab	17.37 ± 3.44 efghiîjklmnop	1.34 ± 0.12 jkl	1.23 ± 0.12 i	ULD
		2016	13.89 ± 0.59 cdefghiî	4.70 ± 0.07 lmnop	0.50 ± 0.03 bcdefgh	11.19 ± 0.75 a	22.45 ± 1.00 fghiîjk	0.31 ± 0.02 abc	23.03 ± 1.53 a	1.67 ± 0.19 efghiîjkl	2.07 ± 0.08 abcd	ULD
	F A.	2014	13.26 ± 0.34 fghiîjklmno	5.31 ± 0.31 efgh	0.46 ± 0.08 cdehiîjkl	3.63 ± 0.17 ștț	21.81 ± 0.84 hiîjk	0.34 ± 0.11 ab	22.10 ± 0.95 a	1.59 ± 0.63 ghiîjkl	1.18 ± 0.08 i	ULD
		2015	13.11 ± 0.59 hiîjklmno	5.25 ± 0.06 fghi	0.54 ± 0.03 abcd	3.85 ± 0.46 rsștț	22.86 ± 1.03 ghiîjk	0.30 ± 0.02 abc	23.04 ± 1.53 a	1.19 ± 0.07 l	1.12 ± 0.10 i	ULD
		2016	14.39 ± 0.47 abcdefg	5.41 ± 0.42 defgh	0.46 ± 0.03 cdefghiîjkl	5.03 ± 0.43 nopqrs	20.11 ± 1.13 k	0.19 ± 0.05 efghiîjklmno	20.96 ± 1.57 abcdefg	2.14 ± 0.23 cdefghi	1.23 ± 0.01 i	ULD
	F R.	2014	13.59 ± 0.26 cdefghiîjk	4.55 ± 0.02 mnop	0.60 ± 0.06 ab	5.51 ± 0.33 jklmnopq	22.56 ± 1.13 ghiîjk	0.12 ± 0.02 mnopq	13.13 ± 1.58 rs	2.33 ± 0.01 bcdef	1.22 ± 0.11 i	ULD
		2015	14.03 ± 0.55 cdefghi	4.36 ± 0.15 p	0.61 ± 0.05 a	2.45 ± 0.19 ț	20.96 ± 1.33 îjk	0.12 ± 0.03 nopq	19.77 ± 1.37 abcdefghiîj	1.77 ± 0.48 efghiîjkl	1.34 ± 0.12 fghi	ULD
		2016	12.76 ± 0.91 îjklmnop	4.48 ± 0.36 nop	0.52 ± 0.04 abcde	3.25 ± 0.04 tț	20.15 ± 1.18 k	0.19 ± 0.02 efghiîjklmno	20.84 ± 1.66 abcdefgh	1.37 ± 0.07 îjkl	1.07 ± 0.04 i	ULD
	F N.	2014	14.55 ± 0.23 abcd	5.33 ± 0.02 efgh	0.43 ± 0.04 defghiîjklm	0.30 ± 0.06 u	26.22 ± 0.63 abcde	0.18 ± 0.05 ghiîjklmnop	22.26 ± 1.71 ab	1.62 ± 0.26 fghiîjkl	1.92 ± 0.23 abcdefg	ULD
		2015	15.17 ± 0.53 ab	5.43 ± 0.21 defgh	0.42 ± 0.06 defghiîjklmn	3.63 ± 0.62 ștț	22.11 ± 1.16 ghiîjk	0.36 ± 0.05 a	20.81 ± 0.80 abcdefgh	1.37 ± 0.05 îjkl	1.30 ± 0.12 ghi	ULD
		2016	14.73 ± 1.00 abc	5.25 ± 0.06 fghi	0.48 ± 0.05 cdefghiîj	3.76 ± 0.33 sștț	21.68 ± 1.67 hiîjk	0.21 ± 0.03 defghijkl	17.45 ± 3.38 efghiîjklmnop	2.04 ± 0.13 cdefghiîjk	1.28 ± 0.06 ghi	ULD
	M	2014	14.52 ± 0.27 abcde	4.83 ± 0.13 klmn	0.31 ± 0.03 op	3.59 ± 0.35 ștț	27.19 ± 1.40 a	0.31 ± 0.09 abc	13.89 ± 3.78 pqrs	1.27 ± 0.06 l	1.21 ± 0.11 i	ULD
		2015	15.40 ± 0.63 a	5.31 ± 0.11 efgh	0.41 ± 0.03 efghiîjklmno	6.60 ± 1.08 îjkl	24.33 ± 1.12 defgh	0.16 ± 0.05 klmnopq	18.23 ± 2.70 cdefghiîjklmn	1.23 ± 0.12 l	1.29 ± 0.12 ghi	ULD
		2016	15.44 ± 0.48 a	4.66 ± 0.18 lmnop	0.52 ± 0.04 abcdef	6.65 ± 0.59 îjk	21.69 ± 0.67 hiîjk	0.18 ± 0.02 fghiîjklmnop	17.08 ± 3.66 ghiîjklmnopq	1.78 ± 0.19 efghiîjkl	1.22 ± 0.01 i	ULD
	C S.	2014	13.47 ± 0.54 defghiîjklmn	5.29 ± 0.06 fgh	0.50 ± 0.06 bcdefgh	3.59 ± 1.33 ștț	26.18 ± 1.46 abcde	0.16 ± 0.04 îjklmnopq	22.36 ± 1.88 ab	2.06 ± 0.16 cdefghiî	1.23 ± 0.01 i	ULD
		2015	13.00 ± 0.46 giîjklmnop	4.67 ± 0.12 lmnop	0.50 ± 0.04 bcdefghi	4.37 ± 1.67 pqrsșt	22.45 ± 0.95 fghiîjk	0.12 ± 0.01 nopq	21.40 ± 0.46 abcd	1.78 ± 0.19 efghiîjkl	1.29 ± 0.12 ghi	ULD
		2016	13.76 ± 0.85 cdefghiîj	5.32 ± 0.11 efgh	0.45 ± 0.03 cdefghiîjkl	5.67 ± 0.49 jklmnop	23.40 ± 1.79 fghiîj	0.31 ± 0.03 abc	21.52 ± 1.80 abcd	2.06 ± 0.15 cdefghiî	1.92 ± 0.56 abcdefg	ULD

Wine Centre	Variety	Year	Alcohol (% vol.)	Total acidity (g/L $C_4H_4O_6$)	Volatile acidity (g/L CH_3COOH)	Residual Sugar (g/L)	Non reducing extract (g/L)	Acetic acid (g/L)	Amino acid (mg/L)	Tartaric acid (g/L)	L-Malic acid (g/L)	L-Lactic acid (g/L)
Smulți Wine Center	M Ott.	2014	13.52 ± 0.51 defghiîjkl	4.48 ± 0.12 nop	0.52 ± 0.03 abcdef	10.78 ± 0.51 ab	22.41 ± 1.05 fghiîjk	0.24 ± 0.07 cdefghiî	9.61 ± 2.45 ș	1.23 ± 0.12 l	1.29 ± 0.12 ghi	ULD
		2015	11.88 ± 0.90 opqrs	4.51 ± 0.18 nop	0.46 ± 0.03 cdefghiîjkl	7.37 ± 1.55 ghiî	23.22 ± 1.52 fghiîj	0.20 ± 0.02 defghiîjklmn	12.96 ± 1.10 rs	1.34 ± 0.12 jkl	1.23 ± 0.01 i	ULD
		2016	13.36 ± 0.43 efghiîjklmno	5.08 ± 0.06 hiîjk	0.48 ± 0.03 cdefghiîj	8.87 ± 1.12 defg	22.78 ± 1.41 ghiîjk	0.09 ± 0.03 pq	16.21 ± 1.70 îjklmnopqr	2.19 ± 0.26 cdefgh	2.28 ± 0.10 abc	ULD
	F A.	2014	12.28 ± 0.74 mnopqr	4.73 ± 0.06 lmop	0.56 ± 0.15 abc	7.48 ± 0.79 ghiî	26.60 ± 2.02 abcd	0.16 ± 0.04 îjklmnop	19.34 ± 1.71 abcdefghiîjk	1.70 ± 0.13 efghiîjkl	2.26 ± 0.12 abcd	ULD
		2015	12.33 ± 0.44 lmnopqr	4.64 ± 0.27 lmnop	0.49 ± 0.03 cdefghiîj	11.37 ± 0.96 a	27.88 ± 1.25 a	0.14 ± 0.03 lmnopq	18.11 ± 0.49 defghiîjklmno	1.44 ± 0.11 iîjkl	2.07 ± 0.55 abcd	ULD
		2016	13.22 ± 0.43 ghiîjklmno	4.59 ± 0.23 lmnop	0.47 ± 0.04 cdefghiîjk	10.06 ± 0.14 abc	23.45 ± 1.11 fghiîj	0.17 ± 0.05 hiîjklmnop	22.99 ± 2.13 a	2.93 ± 0.22 ab	1.37 ± 0.17 efghi	ULD
	F R.	2014	11.52 ± 0.34 qrs	5.18 ± 0.11 ghiîj	0.45 ± 0.02 cdefghiîjkl	8.95 ± 0.95 cdef	21.20 ± 1.00 îjk	0.28 ± 0.05 abcd	13.40 ± 1.08 qrs	1.44 ± 0.47 iîjkl	1.06 ± 0.14 i	ULD
		2015	11.07 ± 0.17 s	5.26 ± 0.05 fghi	0.36 ± 0.03 klmnop	5.36 ± 0.12 klmnopq	22.08 ± 1.59 ghiîjk	0.18 ± 0.05 ghiîjklmnop	19.56 ± 1.44 abcdefghiîj	1.88 ± 0.45 defghiîjkl	1.27 ± 0.06 hi	ULD
		2016	12.18 ± 0.37 nopqrs	5.15 ± 0.07 ghiîjk	0.42 ± 0.09 defghiîjklmn	10.01 ± 1.00 abcd	21.18 ± 1.98 hiîjk	0.24 ± 0.04 cdefghiî	19.47 ± 1.99 abcdefghiîjk	1.48 ± 0.17 hiîjkl	2.12 ± 0.02 abcd	ULD
	F N.	2014	14.15 ± 0.78 bcdefgh	5.74 ± 0.07 bcd	0.50 ± 0.06 bcdefghi	11.04 ± 0.51 a	23.01 ± 2.08 fghiîj	0.15 ± 0.06 jklmnopq	17.17 ± 1.65 fghiîjklmnopq	2.65 ± 0.33 abc	2.52 ± 0.52 ab	ULD
		2015	12.96 ± 0.63 hiîjklmnop	5.29 ± 0.13 fgh	0.33 ± 0.04 lmnop	8.08 ± 1.32 fghi	28.76 ± 0.19 a	0.10 ± 0.01 q	17.40 ± 0.65 efghiîjklmnop	1.60 ± 0.25 ghiîjkl	2.00 ± 0.67 abcd	ULD
		2016	13.08 ± 0.58 hiîjklmno	5.17 ± 0.04 ghiîj	0.45 ± 0.07 cdefghiîjkl	6.26 ± 0.93 îjklmn	22.71 ± 1.51 ghiîjk	0.21 ± 0.02 defghiîjkl	21.10 ± 2.01 abcdef	2.12 ± 0.01 cdefghi	2.13 ± 0.03 abcd	ULD
	M	2014	13.10 ± 0.58 hiîjklmno	5.25 ± 0.06 fghi	0.37 ± 0.02 jklmnop	4.19 ± 0.29 qrsșt	21.52 ± 1.80 hiîjk	0.26 ± 0.06 bcdefgh	21.08 ± 1.12 abcdef	1.33 ± 0.01 kl	1.29 ± 0.12 ghi	ULD
		2015	13.95 ± 0.52 cdefghiî	4.88 ± 0.10 îjklm	0.38 ± 0.06 îjklmnop	6.85 ± 0.60 iîj	22.49 ± 2.09 ghiîjk	0.28 ± 0.05 abcde	21.19 ± 1.23 abcde	2.49 ± 0.25 bcd	1.26 ± 0.06 hi	ULD
		2016	14.03 ± 0.43 cdefghi	4.48 ± 0.32 op	0.40 ± 0.06 ghiîjklmnop	8.29 ± 0.54 fgh	21.22 ± 0.23 hiîjk	0.21 ± 0.02 defghiîjkl	20.13 ± 1.41 abcdefghiî	1.37 ± 0.16 îjkl	1.22 ± 0.01 i	ULD
	C S.	2014	11.74 ± 0.31 pqrs	5.38 ± 0.28 efgh	0.31 ± 0.03 op	3.74 ± 0.52 sștț	27.95 ± 1.00 a	0.20 ± 0.02 defghiîjklmn	19.92 ± 1.28 abcdefghiî	1.85 ± 0.44 defghiîjkl	2.56 ± 0.58 a	ULD
		2015	13.07 ± 0.84 hiîjklmno	5.18 ± 0.06 ghiîj	0.34 ± 0.01 lmnop	5.22 ± 0.58 lmnopqr	27.63 ± 1.03 ab	0.21 ± 0.02 defghiîjkl	16.62 ± 2.00 hiîjklmnopqr	1.34 ± 0.11 jkl	1.29 ± 0.11 ghi	ULD
		2016	12.03 ± 0.34	5.22 ± 0.01 ghiî	0.44 ± 0.01 defghiîjklm	6.51 ± 0.84 îjklm	21.34 ± 1.00 hiîjk	0.16 ± 0.03 îjklmnopq	14.32 ± 2.21 opqrs	2.37 ± 0.07 bcde	2.19 ± 0.13 abcd	ULD
Oancea Wine Center	M Ott.	2014	12.18 ± 0.64 nopqrs	6.37 ± 0.16 a	0.47 ± 0.02 cdefghiîjk	3.28 ± 0.36 ștț	22.00 ± 2.11 ghiîjk	0.16 ± 0.07 îjklmnopq	2.66 ± 1.52 t	1.70 ± 0.56 efghiîjkl	1.34 ± 0.10 fghi	ULD
		2015	11.52 ± 0.34 qrs	5.44 ± 0.18 defgh	0.50 ± 0.06 bcdefgh	3.60 ± 0.17 ștț	23.45 ± 2.21 fghiîj	0.20 ± 0.02 defghiîjklmn	10.81 ± 1.33 șș	1.71 ± 0.55 efghiîjkl	1.26 ± 0.07 hi	ULD

Wine Centre	Variety	Year	Alcohol (% vol.)	Total acidity (g/L $C_4H_4O_6$)	Volatile acidity (g/L CH_3COOH)	Residual Sugar (g/L)	Non reducing extract (g/L)	Acetic acid (g/L)	Amino acid (mg/L)	Tartaric acid (g/L)	L-Malic acid (g/L)	L-Lactic acid (g/L)
		2016	13.08 ± 0.68 hiîjklmno	5.51 ± 0.15 cdefg	0.42 ± 0.08 defghiîjklmn	8.74 ± 0.17 defg	26.14 ± 1.44 abcde	0.26 ± 0.04 bcdefg	15.59 ± 3.01 klmnopqr	2.96 ± 0.23 a	1.66 ± 0.59 cdefghi	ULD
	F A.	2014	12.25 ± 0.45 nopqrs	5.33 ± 0.01 efgh	0.39 ± 0.05 hiîjklmnop	5.12 ± 0.49 mnopqrs	25.10 ± 1.06 bcdef	0.21 ± 0.02 defghiîjk	15.14 ± 1.50 lmnopqr	1.67 ± 0.54 efghiîjkl	1.95 ± 0.55 abcdef	ULD
		2015	13.36 ± 0.34 efghiîjklmno	5.19 ± 0.19 ghiîj	0.39 ± 0.05 iîjklmnop	5.52 ± 0.13 jklmnopq	23.01 ± 2.09 fghiîj	0.19 ± 0.02 efghiîjklmno	18.85 ± 3.03 bcdefghiîjkl	3.27 ± 0.16 a	1.06 ± 0.14 i	ULD
		2016	12.98 ± 0.55 hiîjklmnop	5.41 ± 0.17 defgh	0.43 ± 0.02 defghiîjklm	9.63 ± 0.52 bcde	23.36 ± 2.39 fghiîj	0.27 ± 0.06 abcdef	17.46 ± 0.53 efghiîjklmnop	1.70 ± 0.55 efghiîjkl	1.33 ± 0.10 fghi	ULD
	F R.	2014	11.88 ± 0.57 opqrs	5.25 ± 0.06 fghi	0.51 ± 0.06 abcdefg	10.48 ± 0.66 ab	21.40 ± 1.40 hiîjk	0.17 ± 0.05 hiîklmnop	17.37 ± 3.47 efghiîjklmnop	2.52 ± 0.88 bcd	2.51 ± 0.12 ab	ULD
		2015	11.29 ± 1.11 rs	4.37 ± 0.12 p	0.45 ± 0.07 cdefgiîjkl	5.74 ± 1.03 jklmnop	23.34 ± 1.13 fghiîj	0.17 ± 0.06 iîjklmnopq	18.85 ± 3.02 bcdefghiîjkl	1.63 ± 0.61 fghiîjkl	1.63 ± 0.61 cdefghi	ULD
		2016	12.52 ± 0.33 klmnopq	4.99 ± 0.11 iîjklm	0.52 ± 0.07 abcdefg	10.59 ± 0.55 ab	21.45 ± 0.95 iîjk	0.19 ± 0.02 efghiîjklmno	19.28 ± 1.74 abcdefghiîjk	1.22 ± 0.18 l	1.93 ± 1.12 abcdefg	ULD
	F N.	2014	13.26 ± 0.72 fghiîjklmno	4.70 ± 0.49 lmnop	0.39 ± 0.06 ghiîjklmnop	4.70 ± 0.63 opqrsș	22.73 ± 1.00 ghiîjk	0.27 ± 0.05 abcde	20.15 ± 1.96 abcdefghi	2.19 ± 0.13 cdefgh	1.23 ± 0.01 i	ULD
		2015	13.92 ± 0.53 cdefghiî	5.10 ± 0.07 hiîjk	0.37 ± 0.06 jklmnop	8.52 ± 0.74 efgh	27.08 ± 0.44 abc	0.16 ± 0.04 îjklmnopq	19.55 ± 1.52 abcdefghiîj	2.03 ± 0.52 cdefghiîjk	1.38 ± 0.25 efghi	ULD
		2016	13.18 ± 0.29 hiîjklmo	5.24 ± 0.11 fghi	0.43 ± 0.03 defghiîjklm	5.81 ± 0.47 jklmno	22.44 ± 0.99 ghiîjk	0.19 ± 0.02 efghiîjklmno	14.63 ± 0.70 nopqrs	1.86 ± 0.42 defghiîjkl	1.26 ± 0.07 hi	ULD
	M	2014	13.52 ± 1.11 defghiîjkl	4.55 ± 0.01 mnop	0.30 ± 0.06 p	7.33 ± 0.55 hiî	20.84 ± 0.90 jk	0.16 ± 0.06 îjklmnopq	15.95 ± 1.41 jklmnopqrs	1.60 ± 0.64 ghiîjkl	1.62 ± 0.01 defghi	ULD
		2015	12.37 ± 0.07 lmnopqrs	5.82 ± 0.40 bc	0.33 ± 0.05 lmnop	5.29 ± 0.56 klmnopq	23.77 ± 3.23 efghiî	0.20 ± 0.02 defghiîjklmn	14.92 ± 3.01 mnopqrs	2.54 ± 1.15 bcd	1.90 ± 0.60 bcdefgh	ULD
		2016	12.59 ± 1.28 jklmnopq	5.66 ± 0.29 cde	0.32 ± 0.03 mnop	7.41 ± 1.80 ghiî	22.67 ± 1.52 ghiîjk	0.19 ± 0.07 efghiîjklmno	20.48 ± 1.38 abcdefgh	2.23 ± 0.19 cdefg	1.99 ± 0.47 abcde	ULD
	C S.	2014	12.92 ± 0.74 iîjklmno	6.04 ± 0.13 b	0.39 ± 0.05 ghiîjklmnop	2.45 ± 0.20 ț	24.19 ± 2.21 defghi	0.21 ± 0.03 defghiîjkl	18.74 ± 0.39 bcdefghiîjklm	1.34 ± 0.01 jkl	1.26 ± 0.06 hi	ULD
		2015	12.19 ± 0.26 nopqrs	5.32 ± 0.04 efgh	0.43 ± 0.11 defghiîjklmn	4.67 ± 0.57 opqrsșt	22.07 ± 0.44 ghiîjk	0.23 ± 0.06 cdefghiîjk	20.22 ± 0.77 abcdefghiî	1.30 ± 0.07 kl	1.97 ± 0.64 abcde	ULD
		2016	11.63 ± 0.59 pqrs	5.59 ± 0.13 cdef	0.41 ± 0.03 efghiîjklmno	5.71 ± 0.55 jklmnop	21.67 ± 0.59 hiîjk	0.20 ± 0.02 defghiîjklmn	16.26 ± 1.67 iîjklmnopqr	3.20 ± 0.05 a	2.20 ± 0.11 abcd	ULD

Average value ± standard deviation (n = 3). Romans letters represent the significance of the variety difference ($p \leq 0.05$). The difference between any two values, followed by at least one common letter, is = insignificant; Ott. = Muscat Ottonel; F A. = Feteasca Alba; F R. = Feteasca Regala; F N. = Feteasca Neagra; M = Merlot; C S. = Cabernet Sauvignon. ULD = under the limit of detection.

Table 5. The $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$, isotope rations obtained from wine samples from Dealu Bujorului Vineyard (Bujoru, Smulți and Oancea Wine Centre)

Wine Centre	Variety	Year	$^{206}Pb/^{207}Pb$	SD	$^{208}Pb/^{206}Pb$	SD	RSD (%)	$^{206}Pb/^{204}Pb$	RSD (%)	$^{87}Sr/^{86}Sr$	RSD (%)	Pb (µg/L) M.A.L.* 0.15 mg/L	Sr (µg/L) M.A.L.** -
Bujoru Wine Center	M Ott.	2014	$1.13254^{ijk}\beta\gamma\delta$	0.00036	$2.12629^{cde}\beta\gamma$	0.00053	0.09456	$17.46918^{d}\alpha$	0.09456	$0.71240^{klm}\varepsilon$	0.01725	39.93 ± 1.93^{lmno} $\alpha\beta\gamma$	$133.64\pm3.49^{p}\iota$
		2015	$1.12757^{klm}\gamma\delta\varepsilon$	0.00452	$2.14190^{a}\alpha$	0.00743	0.34696	$17.43110^{de}\alpha\beta\gamma$	0.12536	$0.71572^{ijk}\delta\varepsilon$	0.44613	$41.95\pm1.60^{klm}\alpha$	$152.57\pm4.74^{n}\theta$
		2016	$1.12391^{m}\varepsilon$	0.00133	$2.12926^{bcd}\beta\gamma$	0.00799	0.37512	$17.41786^{de}\beta\gamma$	0.03015	$0.72304^{gh}\alpha\beta$	0.41614	$26.22\pm1.16^{yw}\zeta$	$326.81\pm2.05^{b}\alpha$
	F A.	2014	$1.14202^{efg}\alpha$	0.00822	$2.12488^{cde}\beta\gamma$	0.00288	0.13531	$17.43660^{de}\alpha\beta\gamma$	0.07738	$0.72135^{ghi}\alpha\beta$	0.04199	$36.29\pm1.63^{opr}\beta\gamma\delta$	$273.79\pm5.29^{d}\gamma$
		2015	$1.13349^{ijk}\beta\gamma$	0.00188	$2.12629^{cde}\beta\gamma$	0.00539	0.25334	$17.39880^{e}\beta\gamma$	0.20089	$0.71227^{klm}\varepsilon$	0.03451	$41.73\pm3.38^{klm}\alpha$	$214.67\pm2.41^{hi}\zeta$
		2016	$1.12772^{klm}\gamma\delta\varepsilon$	0.00423	$2.12378^{cde}\gamma$	0.00190	0.08933	$17.31524^{f}\delta$	0.03479	$0.71810^{hij}\gamma\delta$	0.83133	$40.50\pm2.08^{lmn}\alpha\beta$	$109.39\pm1.32^{tu}\kappa$
		2014	$1.13487^{ijh}\beta\gamma$	0.00233	$2.12289^{de}\gamma$	0.00058	0.02748	$17.39163^{e}\gamma$	0.41595	$0.71844^{hij}\beta\gamma\delta$	0.69694	$26.90\pm2.41^{xy}\zeta$	$214.94\pm0.87^{hi}\zeta$
	F R.	2015	$1.13349^{ijk}\beta\gamma$	0.00188	$2.12228^{e}\gamma$	0.00081	0.03830	$17.42611^{de}\alpha\beta\gamma$	0.12511	$0.72156^{gh}\alpha\beta$	0.01270	$33.20\pm2.56^{rst}\delta\varepsilon$	$217.20\pm1.79^{h}\zeta$
		2016	$1.12776^{klm}\gamma\delta\varepsilon$	0.00422	$2.12258^{e}\gamma$	0.00234	0.11034	$17.43908^{de}\alpha\beta\gamma$	0.13200	$0.72208^{gh}\alpha\beta$	0.15312	$35.78\pm3.33^{prs}\gamma\delta$	$181.55\pm3.05^{l}\eta$
		2014	$1.13469^{hij}\beta\gamma$	0.00219	$2.12569^{cde}\beta\gamma$	0.00040	0.01862	$17.42780^{de}\alpha\beta\gamma$	0.11945	$0.72602^{defg}\alpha$	0.57532	$30.29\pm0.78^{utvx}\varepsilon\zeta$	$234.15\pm5.53^{g}\varepsilon$
	F N.	2015	$1.13547^{ghij}\alpha\beta$	0.00083	$2.12975^{bc}\beta\gamma$	0.00448	0.21026	$17.42310^{de}\alpha\beta\gamma$	0.05384	$0.72529^{fg}\alpha$	0.05395	$26.18\pm3.10^{yw}\zeta$	$325.16\pm4.00^{b}\alpha$
		2016	$1.12776^{klm}\gamma\delta\varepsilon$	0.00339	$2.12600^{cde}\beta\gamma$	0.00640	0.30112	$17.42147^{de}\beta\gamma$	0.00080	$0.72568^{efg}\alpha$	0.11648	$32.79\pm2.67^{rst}\delta\varepsilon$	$300.14\pm1.96^{c}\beta$
		2014	$1.12938^{jklm}\beta\gamma\delta\varepsilon$	0.00559	$2.12790^{cde}\beta\gamma$	0.00379	0.17816	$17.40896^{e}\beta\gamma$	0.03385	$0.72298^{gh}\alpha\beta$	0.34219	$33.43\pm1.03^{rst}\delta\varepsilon$	$107.49\pm2.08^{u}\kappa$
	M	2015	$1.12305^{m}\varepsilon$	0.00266	$2.13343^{b}\beta$	0.00150	0.07040	$17.41815^{de}\beta\gamma$	0.03441	$0.72523^{fg}\alpha$	0.07722	$35.89\pm2.42^{prs}\gamma\delta$	$261.40\pm6.37^{e}\delta$
		2016	$1.12773^{klm}\gamma\delta\varepsilon$	0.00333	$2.12418^{cde}\gamma$	0.00093	0.04382	$17.39969^{e}\beta\gamma$	0.17041	$0.71430^{jkl}\delta\varepsilon$	0.21700	$41.20\pm1.81^{klmn}\alpha$	$276.10\pm2.00^{d}\gamma$
		2014	$1.13161^{ijkl}\beta\gamma\delta$	0.00560	$2.13011^{bc}\beta\gamma$	0.00619	0.29060	$17.42141^{de}\beta\gamma$	0.02771	$0.72339^{gh}\alpha$	0.28125	$41.78\pm2.24^{klm}\alpha$	$130.23\pm1.62^{pr}\iota$
	C S.	2015	$1.12391^{m}\varepsilon$	0.00133	$2.12701^{cde}\beta\gamma$	0.00652	0.30661	$17.41199^{de}\beta\gamma$	0.06782	$0.72254^{gh}\alpha\beta$	0.23652	$42.06\pm3.12^{kl}\alpha$	$109.72\pm0.83^{tu}\kappa$
		2016	$1.13320^{ijk}\beta\gamma$	0.00203	$2.12657^{ecd}\beta\gamma$	0.00431	0.20260	$17.44611^{de}\alpha\beta$	0.12788	$0.72137^{ghi}\alpha\beta$	0.03197	$40.99\pm3.71^{klmn}\alpha$	$216.08\pm2.56^{h}\zeta$
Smulți Wine Center	M Ott.	2014	$1.18229^{abc}\alpha\beta\gamma$	0.00028	$2.10364^{fg}\alpha\beta$	0.00108	0.05110	$17.21643^{g}\gamma\delta$	0.02526	$0.73086^{bcdef}\beta\gamma$	0.15148	$22.80\pm1.75^{wz}\zeta\eta$	$116.56\pm2.75^{s}\lambda$
		2015	$1.18415^{ab}\alpha\beta\gamma$	0.00210	$2.10266^{fgh}\alpha\beta$	0.00041	0.01973	$17.21351^{g}\gamma\delta$	0.01071	$0.73455^{b}\beta$	0.51894	$37.79\pm1.10^{nop}\delta$	$245.14\pm4.02^{f}\beta$
		2016	$1.18523^{a}\alpha\beta$	0.00107	$2.10723^{fg}\alpha\beta$	0.00501	0.23773	$17.22768^{g}\alpha$	0.03155	$0.73304^{bc}\beta\gamma$	0.41046	$32.89\pm2.03^{rst}\varepsilon$	$258.09\pm1.29^{e}\alpha$
	F A.	2014	$1.18502^{a}\alpha\beta$	0.00235	$2.10382^{fg}\alpha\beta$	0.00206	0.09777	$17.21852^{g}\beta\gamma\delta$	0.03237	$0.73127^{bcde}\beta\gamma^{f}$	0.14192	$28.13\pm3.16^{vxy}\eta$	$127.65\pm1.80^{r}\kappa$
		2015	$1.17677^{cd}\gamma\delta$	0.00741	$2.10191^{hijk}\alpha\beta$	0.00040	0.01895	$17.21930^{g}\beta\gamma$	0.03086	$0.73224^{bc}\beta\gamma$	0.11604	$30.78\pm1.63^{utv}\varepsilon\zeta\eta$	$106.62\pm1.35^{u}\xi$
		2016	$1.17765^{bcd}\beta\gamma\delta$	0.00741	$2.10192^{hijk}\alpha\beta$	0.00054	0.02553	$17.21549^{g}\gamma\delta$	0.03216	$0.73144^{bcde}\beta\gamma$	0.02684	$32.30\pm0.82^{stu}\varepsilon\zeta$	$114.33\pm2.15^{st}\lambda$
		2014	$1.18504^{a}\alpha\beta$	0.00207	$2.10868^{f}\alpha$	0.00592	0.28051	$17.21089^{g}\delta$	0.00677	$0.73177^{bcde}\beta\gamma$	010621	$53.16\pm1.00^{cd}\alpha$	$199.66\pm2.60^{j}\varepsilon$
	F R.	2015	$1.18312^{abc}\alpha\beta\gamma$	0.00146	$2.10741^{fg}\alpha\beta$	0.00457	0.21674	$17.21584^{g}\gamma\delta$	0.02606	$0.73265^{bc}\beta\gamma$	0.22273	$51.62\pm0.55^{de}\alpha$	$176.73\pm1.47^{lm}\eta$
		2016	$1.18452^{a}\alpha\beta$	0.00196	$2.10153^{hijk}\beta$	0.00053	0.02529	$17.21536^{g}\gamma\delta$	0.03313	$0.73195^{bcd}\beta\gamma$	0.16894	$50.29\pm0.67^{defg}\alpha$	$218.82\pm2.67^{h}\gamma$
		2014	$1.17462^{d}\delta$	0.00216	$2.10756^{fg}\alpha\beta$	0.00471	0.22341	$17.21444^{g}\gamma\delta$	0.00703	$0.73258^{bc}\beta\gamma$	0.91340	$43.40\pm2.65^{jkl}\beta\gamma$	$124.29\pm7.59^{r}\kappa$
	F N.	2015	$1.18532^{a}\alpha\beta$	0.00109	$2.10225^{ghij}\alpha\beta$	0.00010	0.00467	$17.21707^{g}\gamma\delta$	0.02226	$0.73076^{bcde}\beta\gamma^{f}$	0.69139	$44.73\pm3.32^{ijk}\beta$	$129.00\pm3.76^{pr}\kappa$
		2016	$1.17784^{bcd}\beta\gamma\delta$	0.00336	$2.10473^{fg}\alpha\beta$	0.00672	0.31908	$17.21688^{g}\gamma\delta$	0.02298	$0.74275^{a}\alpha$	0.35191	$37.59\pm1.60^{nop}\delta$	$106.86\pm2.34^{u}\xi$
		2014	$1.18597^{a}\alpha$	0.00059	$2.10190^{hijk}\alpha\beta$	0.0058	0.02736	$17.21491^{g}\gamma\delta$	0.03356	$0.73152^{bcde}\beta\gamma$	0.00832	$28.88\pm5.32^{tvxy}\zeta\eta$	$140.97\pm1.56^{o}\iota$
	M	2015	$1.18303^{abc}\alpha\beta\gamma$	0.00268	$2.10130^{hijk}\beta$	0.00013	0.00639	$17.23090^{g}\alpha$	0.00410	$0.73077^{bcdef}\beta\gamma$	0.68614	$22.49\pm1.20^{z}\theta$	$179.19\pm0.88^{lm}\eta$
		2016	$1.18098^{abcd}\alpha\beta\gamma\delta$	0.00839	$2.10242^{ifgh}\alpha\beta$	0.00187	0.08896	$17.22479^{g}\alpha\beta$	0.02298	$0.73402^{b}\beta$	0.20095	$27.29\pm1.16^{vxy}\eta$	$153.22\pm6.73^{n}\theta$
		2014	$1.18515^{a}\alpha\beta$	0.00020	$2.10206^{hijk}\alpha\beta$	0.00055	0.02615	$17.21491^{g}\gamma\delta$	0.03356	$0.72750^{cdefg}\gamma$	0.57915	$43.44\pm2.33^{jkl}\beta\gamma$	$216.46\pm1.81^{h}\gamma$
	C S.	2015	$1.18396^{ab}\alpha\beta\gamma$	0.00130	$2.10483^{fg}\alpha\beta$	0.00623	0.29610	$17.21343^{g}\gamma\delta$	0.01220	$0.73239^{bc}\beta\gamma$	0.25200	$40.47\pm0.80^{lmn}\gamma\delta$	$209.50\pm1.54^{i}\delta$
		2016	$1.17964^{abcd}\alpha\beta\gamma\delta$	0.00654	$2.10397^{fg}\alpha\beta$	0.00546	0.25930	$17.21474^{g}\gamma\delta$	0.00203	$0.73120^{bcdef}\beta\gamma$	0.02999	$39.47\pm1.10^{lmnop}\delta$	$192.29\pm6.86^{k}\zeta$

Wine Centre	Variety	Year	$^{206}Pb/^{207}Pb$	SD	$^{208}Pb/^{206}Pb$	SD	RSD (%)	$^{206}Pb/^{204}Pb$	RSD (%)	$^{87}Sr/^{86}Sr$	RSD (%)	Pb (µg/L) M.A.L.* 0.15 mg/L	Sr (µg/L) M.A.L.** -
		2014	$1.14230^{ef}\alpha\beta\gamma$	0.00187	$2.09557^{jkl}\alpha$	0.00015	0.00729	$17.61229^{c}\beta\gamma$	0.05492	$0.70165^{p}\beta\gamma$	0.08200	$55.78\pm0.81^{bc}\beta$	$174.80\pm5.33^{m}\theta$
	M Ott.	2015	$1.14184^{efg}\alpha\beta\gamma$	0.00286	$2.09531^{kl}\alpha$	0.00026	0.01218	$17.61543^{c}\beta\gamma$	0.02934	$0.70788^{mno}\alpha\beta\gamma$	1.35361	$57.36\pm1.38^{b}\beta$	$192.81\pm3.20^{k}\eta$
		2016	$1.14537^{e}\alpha\beta$	0.00268	$2.09557^{jkl}\alpha$	0.00010	0.00482	$17.61396^{c}\beta\gamma$	0.08560	$0.70326^{op}\beta\gamma$	0.10740	$48.46\pm0.85^{efg}\delta\varepsilon$	$187.54\pm2.93^{k}\eta$
		2014	$1.14212^{efg}\alpha\beta\gamma$	0.00166	$2.09541^{kl}\alpha$	0.00001	0.00028	$17.70222^{ab}\alpha\beta$	0.15455	$0.70221^{op}\beta\gamma$	0.01809	$57.28\pm0.81^{b}\beta$	$193.09\pm3.07^{k}\eta$
	F A.	2015	$1.14018^{efgh}\gamma$	0.00003	$2.09404^{l}\alpha\beta$	0.00230	0.10978	$17.64307^{c}\alpha\beta\gamma$	0.24352	$0.70247^{op}\beta\gamma$	0.08219	$27.32\pm1.52^{vxy}\eta$	$273.46\pm4.00^{d}\gamma$
		2016	$1.14605^{e}\alpha$	0.00169	$2.09651^{ijkl}\alpha$	0.00168	0.02529	$17.64396^{c}\beta\gamma$	0.08560	$0.70175^{op}\beta\gamma$	0.04854	$42.63\pm1.05^{kl}\zeta$	$216.14\pm2.66^{h}\zeta$
		2014	$1.14121^{efgh}\beta\gamma$	0.00173	$2.09412^{l}\alpha\beta$	0.00171	0.22341	$17.61823^{c}\beta\gamma$	0.06862	$0.70171^{p}\beta\gamma$	0.11789	$47.57\pm2.25^{fgh}\varepsilon$	$260.66\pm3.04^{e}\delta$
	F R.	2015	$1.14435^{e}\alpha\beta\gamma$	0.00390	$2.09528^{kl}\alpha$	0.00028	0.01323	$17.61146^{c}\beta\gamma$	0.06004	$0.70288^{op}\beta\gamma$	0.20128	$51.54\pm2.90^{de}\gamma$	$244.49\pm3.51^{f}\varepsilon$
		2016	$1.14629^{e}\alpha$	000142	$2.09411^{l}\alpha\beta$	0.00105	0.04990	$17.6127^{c}\beta\gamma$	0.05553	$0.70542^{nop}\beta\gamma$	0.97519	$51.20\pm1.44^{def}\gamma$	$215.68\pm0.78^{h}\zeta$
		2014	$1.14114^{efgh}\beta\gamma$	0.00163	$2.09576^{jkl}\alpha$	0.00076	0.03433	$17.62720^{c}\alpha\beta\gamma$	0.11928	$0.70560^{nop}\beta\gamma$	0.68843	$50.18\pm2.18^{defg}\gamma\delta$	$326.16\pm2.66^{b}\beta$
	F N.	2015	$1.14435^{e}\alpha\beta\gamma$	0.00390	$2.09528^{kl}\alpha$	0.00028	0.01323	$17.63062^{c}\alpha\beta\gamma$	0.11989	$0.71448^{jkl}\alpha$	0.25095	$51.66\pm1.87^{de}\gamma$	$249.15\pm4.62^{f}\varepsilon$
		2016	$1.14500^{e}\alpha\beta$	0.00317	$2.09577^{jkl}\alpha$	0.00074	0.03537	$17.60973^{c}\gamma$	0.04787	$0.70916^{lmn}\alpha\beta$	1.03156	$61.33\pm1.21^{a}\alpha$	$270.16\pm1.00^{d}\gamma$
		2014	$1.14121^{efgh}\beta\gamma$	0.00177	$2.09612^{ijkl}\alpha$	0.00149	0.07126	$17.70857^{a}\beta\gamma$	0.94776	$0.70112^{p}\gamma$	0.10738	$36.59\pm1.13^{opr}\eta$	$249.43\pm2.40^{f}\varepsilon$
	M	2015	$1.14287^{ef}\alpha\beta\gamma$	0.00435	$2.09556^{jkl}\alpha$	0.00010	0.00468	$17.63746^{c}\alpha\beta\gamma$	0.06768	$0.70412^{nop}\beta\gamma$	0.35258	$40.93\pm1.81^{klmn}\zeta$	$362.15\pm2.90^{a}\alpha$
		2016	$1.14550^{e}\alpha\beta$	0.00034	$2.09532^{kl}\alpha$	0.00028	0.01338	$17.61914^{c}\beta\gamma$	0.02278	$0.70317^{op}\beta\gamma$	0.29783	$42.13\pm0.55^{kl}\zeta$	$118.42\pm1.12^{s}\lambda$
		2014	$1.14184^{efg}\alpha\beta\gamma$	0.00286	$2.09205^{l}\beta$	0.00576	0.27513	$17.70857^{a}\alpha$	0.02238	$0.70203^{op}\beta\gamma$	0.13326	$55.60\pm0.89^{bc}\beta$	$148.54\pm3.16^{n}\iota\kappa$
	C S.	2015	$1.14200^{efg}\alpha\beta\gamma$	0.00313	$2.09565^{jkl}\alpha$	0.00003	0.00146	$17.63840^{c}\alpha\beta\gamma$	0.25064	$0.70573^{nop}\beta\gamma$	0.69427	$38.10\pm0.79^{mnop}\eta$	$114.09\pm4.41^{st}\lambda$
		2016	$1.14407^{e}\alpha\beta\gamma$	0.00224	$2.09592^{ijkl}\alpha$	0.00052	0.02479	$17.65393^{bc}\alpha\beta\gamma$	0.30105	$0.70120^{p}\gamma$	0.03128	$46.66\pm0.78^{ghi}\varepsilon$	$212.78\pm3.86^{hi}\zeta$
Average			1.15202	0.00279	2.10878	0.00238	0.11248	17.42240	0.09244	0.71909	0.31542	40.64±1.85	187.36±3.15
Minimum Values			1.12305	0.00003	2.09404	0.00001	0.00467	17.21089	0.00080	0.70112	0.00832	22.49±1.20	107.49±2.08
Maximum Values			1.18597	0.00839	2.14190	0.00799	0.37512	17.70857	0.94776	0.74275	1.35361	61.33±1.21	326.81±2.05
Almeida *et al.*, 2016			1.14400		2.15700		1.00000					76.00±13.00	
Avram *et al.*, 2014			1.14000	0.10000	2.10000	0.15000	7.10000			0.76000	1.30000	35.90	171.40
Mihaljevnié *et al.*, 2006			1.74000	0.00300	2.09200	0.00700						11.11±5.28	
Barbaste *et al.*, 2002			1.14200	0.00200	2.12500	0.00300	0.16000	17.60000	1.70000			42.90±0.05	
Geana *et al.*, 2017													905.00

Average value ± standard deviation (n = 3). Romans letters represent the significance of the variety difference ($p \leq 0.05$). The difference between any two values, followed by at least one common letter, is = insignificant; Ott. = Muscat Ottonel; F A. = Feteasca Alba; F R. = Feteasca Regala; F N. = Feteasca Neagra; M = Merlot; C S. = Cabernet Sauvignon.
****M.A.L.** for Sr = -.

The cluster heat map is a rectangular tiling of a data matrix cluster trees appended to its margins. Within a relatively compact display area, it facilitates inspection of row, column, and joint cluster structure. Moderately large data matrices (several thousand rows/columns) can be displayed effectively on a high-resolution color monitor and even larger matrices can be handled in print or in megapixel displays.

Heat map was used to discover sample groups, discover groups and also to discover related sample/feature groups. In of elemental contents and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$ isotope ratios from wine (horizontal dendrogram) the dendrogram clearly show two cluster, first cluster is formed from the Sr and second cluster was formatted from Pb, $^{206}Pb/^{204}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{207}Pb$, and $^{87}Sr/^{86}Sr$.

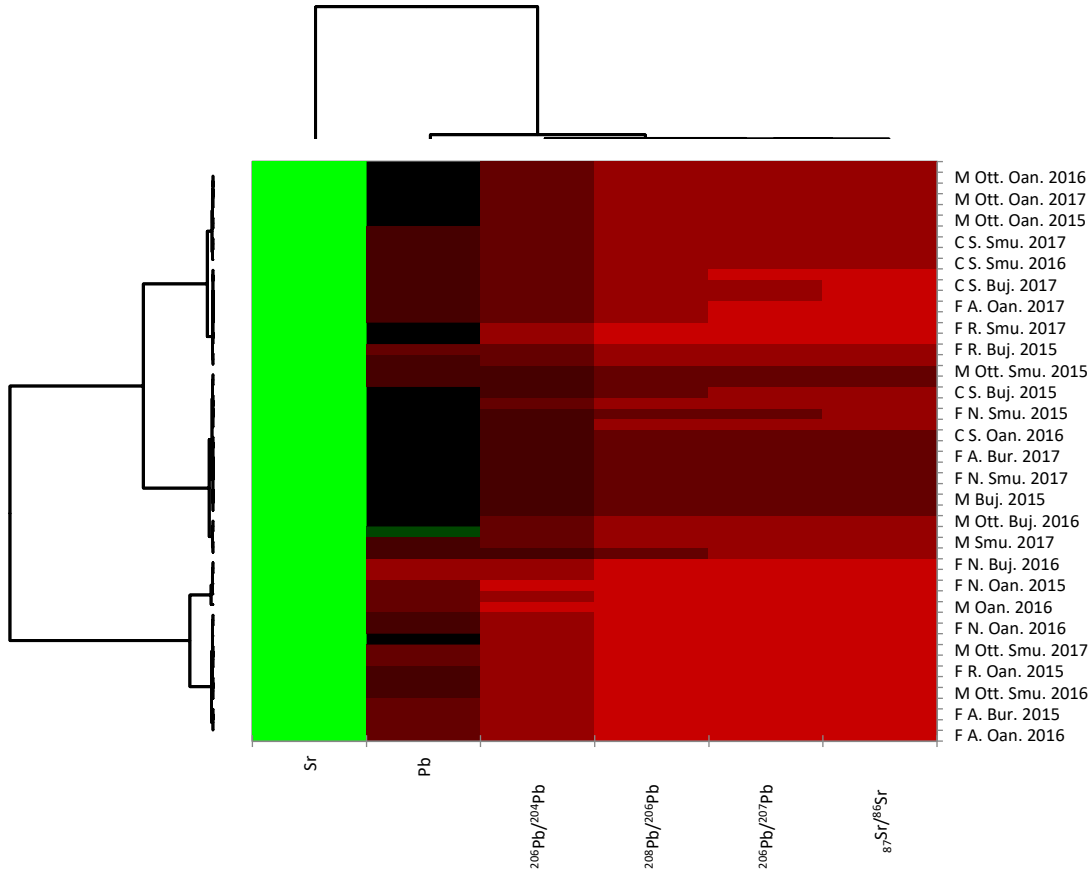

Figure 3. Heat map obtained by cluster analysis of the element contents and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$ isotope ratios

Based on this distribution in can be seen that the Sr recorded the highest values

followed by Pb. The vertical dendrogram show also two cluster, the first M Smu. 2015, C S. Buj. 2015, F R. Buj. 2015, M Buj. 2017, M Oan. 2016, M Oan. 2015, F R. Smu. 2015, F R. Buj. 2017, C S. Smu. 2017, F A. Bur. 2017, F A. Bur. 2015, C S. Buj. 2017, C S. Smu. 2016, F N. Oan. 2016, F N. Oan. 2015 and second cluster was formed from F A. Smu. 2017, M Ott. Smu. 2017, M Ott. Smu. 2016, F R. Oan. 2015, M Ott. Oan. 2017, F R. Oan. 2016, M Buj. 2015, F A. Oan.

2016, F R. Smu. 2017, C S. Oan. 2016, F A. Smu. 2016, M Ott. Buj. 2017. Based on this distribution in can be seen that there is a separation of wine varieties for white of these red depending on elemental contents and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$ isotope ratios, except for a few varieties that do not fit into this rule (F R. Buj. 2015, F R. Smu. 2016, F R. Buj. 2017, F A. Buj. 2017, F A. Buj. 2015 (which have been introduced in red wine cluster)) and M Buj. 2015, C S. Oan. 2016, 2015 (which have been introduced in white wine cluster)) (Figure 3).

4. Conclusions

In this work the Sr and Pb composition and $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio of white wines (Muscat Ottonel, Feteasca Alba, Feteasca Regala) and red wines (Feteasca Neagra, Merlot, Cabernet Sauvignon) production years 2014-2016 from Bujoru, Smulți and Oancea wine-growing centers was studied in order to highlight geographical traceability of elemental composition and isotope ratio for fingerprints of the wines.

Concentration of Pb in analysed wine samples were under Maximum Limit Allowed (M.L.A.), respectively as published by the Organization of Vine and Wine. The content of potentially toxic elements such as Pb are lower than the recommended values found in literature, highlighting the safety and quality of the analysed Romanian wines.

From discrimination analysis of wine samples, was found additional new elements Mn and Cr to tracers for geographical traceability of Romanian wines. Our results confirm that the $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ and $^{87}Sr/^{86}Sr$ isotope ratio can be used to track the origin of wine, discriminate between the wine produced in different years, and be used to characterize wine terroirs for forensic purpose. The wines obtained in the three wine-growing centers can be geographical fingerprints based on the concentration of Sr, Pb and also based on the $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$, $^{87}Sr/^{86}Sr$ isotope ratio. The proposed methodology allowed 100 % successful classification of wines according to the region of provenance and also the years of wine obtaining.

Heat map was discovering a separation of wine varieties for white of this red depending on elemental contents and $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$, $^{206}Pb/^{204}Pb$, $^{87}Sr/^{86}Sr$ isotope ratios.

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RESPONSE SURFACE OPTIMIZATION OF FERMENTING PARAMETERS FOR THE PRODUCTION OF BEER FROM FINGER MILLET AND APPLE JUICE BY USING BOX-BEHNKEN DESIGN

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ABSTRACT

The experiments were planned using Response Surface Methodology, Box Behnken design was used and total seventeen designed experiments were conducted to produce beer from finger millet and apple juice. Effects of independent variables with three levels for each i.e. blend ratios ((Finger millet: Apple Juice) (90:10, 85:15, 80:20)), yeast concentration (6%, 8%, 10%) and malted grain to water ratios (1:8, 1:9, 1:10) were investigated on beer quality. During study it was observed that all the independent parameters i.e. blend ratio, yeast concentration and malted grain to water ratios affected the responses (pH, Titrable acidity, colour, bitterness and alcohol content) significantly. Optimization was done using Design Expert 10.0.1 software, for free beer production. The optimum values were found to be 80.24:19.76 blend ratio, 10% enzyme concentration and 1:8 slurry ratio. The model F-value was found to be highly significant at 1% level of significance for all the responses. The values for pH, titrable acidity, colour, bitterness and alcohol content at optimum conditions were found to be 5.12, 0.12, 17.312, 18.95 and 9.25 respectively all the responses could be predicted by fitting the second order mathematical model and adequacy checked by R2.

1.Introduction

India leads the world in production of millets. In Uttarakhand traditional crops like, finger millet, barnyard millet, black soyabean, horse gram etc. are cultivated in a wide range of soils and under diverse climate conditions. In India, finger millet (*Eleusinecoracana*) (locally called by various name including ragi and nachani) is mostly grown and consumed in Karnataka, Rajasthan, Andhra Pradesh, Tamil Nadu, Orissa, Maharashtra, Kumaon (Uttarakhand) and Goa. Finger millet, one of the major underutilized crops of Uttarakhand, grows well in tropical countries and contains a good amount of reducing sugars (Kumar*et al.*, 2015).It is mainly grown for food grain for human consumption (Upadhyaya *et al.,* 2006).It is rich in calcium and protein and also has good amount of iron and other minerals (Khandelwal *et al.,*2012).Because of its good nutritional value finger millets can be used for brewing process. Malting of finger millet improves its digestibility, sensory and nutritional quality as well as pronounced effect in lowering the anti-

nutrients (Desai *et al.*, 2010).In the past, these cereals were used locally both in malted and unmalted forms for the production of some types of alcoholic beverages in the tropics (Odunfa 1985).The grain is made into a fermented drink (or beer) in Nepal and in many parts of Africa. The use of cereals offers great advantages in brewing (Okafor and Aniche, 1980; Glennie *et al.,* 1983; Ugboaja*et al.,* 1991).So it is estimated that finger millets can be used for the preparation of Beer single handly because of its rich carbohydrates content.

Fruits wines are prepared from fruits namely apples, peaches, oranges, bananas, blackberries, mangoes pumpkin etc.Apple (*Pyrus malus*) belongs to the family Rosaceae. Apple according to scientists is a miracle fruit because of the several health benefits it offers (Kaur *et al.,* 2004). Apple fruits are consumed directly as whole or in form of juice, jams and jellies etc. Apple juice is one of the most widely consumed juices in temperate regions. Apple contains high levels of antioxidants, vitamins, minerals and phenolic compounds (Yazdanshenas*et al.,* 2010). It is rich source of phytochemicals; these phytochemicals are unaffected or affected by some extent during storage (Boyer and Liu, 2004).

Alcoholic beverages like beers are legally consumed in most countries. Beer is an alcoholic beverage made from cereals like barley, corn, rice, oat, sorghum, etc. and tuber crops like cassavaand most widely consumed. Use of finger millets with apple Juice for making beer to increase versatility and add to novelty is not reported.The aspect of blending desired and nutritive fruit juices in such alcoholic beverages could add more acceptability with nutrition. With these considerations, the aim of present studies related to value addition of underutilized crops using fermentation technology and was focused on utilization of underutilized millets namely finger millet (ragi) and apple fruit juiceto develop a new variety of alcoholic beverages like beer.An attempt has been made to explore the underutilized crops utilization (finger millet) using fermentation technology.

2. Materials and methods

2.1.Materials

Raw finger millet (*Eleusinecoracana*) and Apple fruit of good quality was purchased from the local market of Dehradun, Uttrakhand, India. Hop (*Humuluslupulus*) species were procured from the DVKSP Impex Pvt. Ltd. Yeast strain (*Saccharomyces cerevisiae*) was taken from food microbiology lab, UCALS, Uttaranchal University.

2.2. Experimental design

A total of seventeen sets of experiments by using Box Behenken design a total 17 experiments having three factorial points, three levels of each were conducted. Blend ratio (finger millet : apple juice), yeast concentration, malted grain to water ratio (slurry ratio) were selected as independent variables with three levels which were -1, 0, and +1 (Table 1). pH, Titrable acidity, colour, bitterness and alcohol content were selected as the responses. 3D curves were drawn with the help of Design Expert 10.0.1 to get the range of independent variables for product development.

Table 1. Independent variables levels and experimental design

Independent variables		**Coded Levels**		
Name	**Code**	-1	0	1
		Actual Levels		
Blend ratio (Finger millet: Apple Juice)	X_1	90:10	85:15	80:20
Yeast Concentration (%)	X_2	6	8	10
Malted grain : water	X_3	1:8	1:9	1:10

2.3. Procedure

All the experiments were conducted in three steps.

2.3.1. Malting

After cleaning , finger millet were soaked in water at room temperature (28±2°C) for 24 h. for proper aeration of grain the water was changed after every 6 to 8 h over a period of 24 h. After soaking the water was drained off and the grains were left on stainless steel sieves for germination process for a period of 24-36 h. After germination, the germinated grains were dried at 90°C for 12 h in Integrated Malting Unit developed by (Sanjay *et al.*, 2016).

Afterdryingrootlets were removed manually and cleaned malt was stored air tightly for further experiments.

2.3.2. Brewing

Before the preparation of wort, the malt was crushed coarsely in mechanical grinder, after that malted grain and water (1:8, 1:9 and 1:10 slurry ratios) for 100 ml of beer was boiled for 40 min at slow fire. In another ware 100 mL tap water was heated at 68-70°C for and sparging repeat the sparging process 2-3 time for maximum extraction of carbohydrates from finger millet malt. Again boil the wort at 70-80°C for 1 h, as soon as the wort started boiling, 1 g of hops were added to enhance the flavor and colour of the final product. Hops were separated by using strainer and muslin cloth and the wort was cooled to a temperature of 18-20°C for yeast growth during fermentation (Logan *et al.,* 1999).Apple juice at different concentration was added as per the experimental designshown in table 2.

Table 2. Experimental Design

Expt. No.	Coded Levels			Real Levels		
	X_1	X_2	X_3	Blend ratio finger millet: apple juice	Yeast concentration (%)	Slurry ratio (malted grain : water)
1	-1	-1	0	90:10	6	1:9
2	1	-1	0	80:20	6	1:9
3	-1	1	0	90:10	10	1:9
4	1	1	0	80:20	10	1:9
5	-1	0	-1	90:10	8	1:8
6	1	0	-1	80:20	8	1:8
7	-1	0	1	90:10	8	1:10
8	1	0	1	80:20	8	1:10
9	0	-1	-1	85:15	6	1:8
10	0	1	-1	85:15	10	1:8
11	0	-1	1	85:15	6	1:10
12	0	1	1	85:15	10	1:10
13	0	0	0	85:15	8	1:9
14	0	0	0	85:15	8	1:9
15	0	0	0	85:15	8	1:9
16	0	0	0	85:15	8	1:9
17	0	0	0	85:15	8	1:9

2.3.3. Fermentation

After cooling,liquid yeast was transferred (6%, 8% and 10%) in Laminar flow chamber and placed in dark place for fermentation for a period of 14 days. After fermentation, fermented liquor was centrifuged at 5000 rpm for 15-20 min in order to remove all yeast cells.

Supernatant was collected and stored in refrigerator at 4°C for further analysis.

2.4. Analytical Procedure

2.4.1. pH

The pH values of all samples were measured by digital pH meter of TOSCHON.

2.4.2.Titratable Acidity

Titratable acidity of fermented beverages was determined by the method of (Rangana, 2010), by using N/10 NaOH and expressed in term of malic acid.

2.4.3.Colour

Colour was estimated calorimetrically according to (Daniels, 1995).Degased sample was taken in 10 mm cuvette and absorbance was taken at 430 nm. Colour was calculated by the formula given below.

Calculations:

Colour = A × f ×25

Where:

A is absorbance at 430 nm in a 10 mm cuvette

f is dilution factor

2.4.4. Bitterness

Bitterness was estimated by the international method using iso octane extraction and bitterness was given in Bitterness Units (BU). http://dx.doi.org/10.1094/ASBCMOA-Beer-23.Briefly, in 10.0 ml Transfer 10.0 ml chilled sample a minute amount of octyl alcohol , 1 ml 3*N* HCl (reagent b) and 20 ml 2,2,4-trimethylpentane was added and centrifuge for 15 min. As soon as possible, transfer sufficient clear, upper (isooctane) layer to cuvet of spectrophotometer and absorbance was taken at 275 nm with 2, 2, 4-trimethylpentane-octyl alcohol as blank

Calculations:

Calculate bitterness units of beer by the formula,

BU = absorbance$_{275}$ × 50.

2.4.5 Ethanol content

Ethanol content in fermented liquor was estimated by the spectrophotometric method of (Caputi*et al.,* 1968).In brief, 1 ml of alcoholic sample was added directly to 30 ml with distilled water and then distilled at 70±2˚C. 20 ml of distillate was collected in a 50 ml volumetric flask containing 25 ml of potassium dichromate solution. The contents in the volumetric flask were heated at 60˚C in a water bath for 20 minutes and final volume was made to 50 ml with distilled water. After mixing and cooling the contents of the flask, the absorbance was recorded at 600 nm. The amount of ethanol in each sample was determined by using the standard curve of ethanol [0 – 20 % ethanol (v/v)].

2.5 Development of second order model

A complete second order mathematical model (Equation (1)) was fitted to the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination) and fisher's F-test. The models were then used to interpret the effect of various parameters on the response. Optimization of process parameters was carried out to predict the optimized values of selected independent variables.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_i X_j + \sum_{i=1}^{3}\beta_{ii}X_i^2 \quad (1)$$

Where,

β_0, β_{ii}, β_{ij} are constants

X_i, X_j are coded variables

The experimental data were then analyzed by multiple regression techniques to develop response functions and variable parameters optimized for best outputs. The regression coefficients of complete second order model and their significance are reported in Table 4. Thevalue of p represented the probability of significance.A high p-value indicated that the model had a significant lack of fit and therefore, considered to be inadequate. The lower the values of p, the better the model would be. The models having p-value lower than 0.1 (indicating the lack of fit is insignificant at 90% confidence level) were accepted.

3.Results and discussions

3.1. Process Optimization

3.1.1. Numerical optimization of process parameters for beer production

Numerical optimization of independent variables (blend ratio, yeast concentration and slurry ratio) was carried out by using software Design expert 10.0.1. The goal was fixed for all independent variables as per the objectives of the study. The responses namely pH, titrable acidity, colour, bitterness and alcohol content were considered for optimization. The goal setup for optimization is given in the Table 5

The optimization was carried out as per the criteria mentioned in table 5. During optimization, total 40 solutions were obtained out of which the one that suited the criteria most (as per the desirability/ objectives) was selected. Choice of the solutions was automatically retrieved by the software. The optimized values are given in table 6

3.2. Response Surface Analysis of process parameters for beer production

The effect of differentindependent variables levels treatment on pH, titrable acidity, color, bitterness and alcohol content is given in Table 3. A series of three-dimensional response surfaces were drawn using Design Expert Software 10.0.1 for the visualization of variation in responses (pH, titrable acidity, color, bitterness and alcohol content) with respect to processing variables (finger millet to apple juice ratio (X_1), yeast concentration(X_2) and malt to water ratio(X_3))**.** Since the present study involved three variables, it was necessary to fix the value of one variable in order to see the effect of two variables on the response.

Table 3. Experimental Data for beer production from combination of finger millet and apple juice

Variables				Responses				
Exp. No.	**Blend ratio of Finger millet: Apple Juice**	**Yeast concentration**	**Slurry ratio of Malted Grain: Water**	**pH**	**Titrable Acidity**	**Colour**	**Bitterness**	**Alcohol content**
1	90:10	6	1:9	5.5	0.35	34.7	17.9	10.5
2	80:20	6	1:9	5.8	0.21	36.7	17	9.4
3	90:10	10	1:9	5.2	0.12*	34.7	18.01	11.2**
4	80:20	10	1:9	5.5	0.13	37.8**	17.7	9.9
5	90:10	8	1:8	5.6	0.26	21.2	17.4	8.9
6	80:20	8	1:8	5.4	0.54	22.8	17.3	8.1
7	90:10	8	1:10	5.1	0.61	19.8	18.4	6.9
8	80:20	8	1:10	4.9	0.45	15.2*	17.5	5.5*
9	85:15	6	1:8	5.4	0.56	22.6	16.5*	8.9
10	85:15	10	1:8	5.1	0.65	15.7	18.5	8.6
11	85:15	6	1:10	5	0.63	16.2	19.8**	7.4
12	85:15	10	1:10	4.6*	0.7	17.2	16.5*	7.1
13	85:15	8	1:9	5.9**	0.78**	33.4	17.5	11.1
14	85:15	8	1:9	5.7	0.69	31.5	17.6	10.2
15	85:15	8	1:9	5.5	0.65	32.5	16.5*	10.1
16	85:15	8	1:9	5.6	0.73	33.6	16.5*	9.6
17	85:15	8	1:9	5.7	0.76	33.6	16.6	9.5
***Minimum value**				****Maximum value**				

Table 4. Results of Regression Analysis of Quality Parameters of Beer

	pH		**Titrable Acidity**		**Colour**		**Bitterness**		**Alcohol**	
	Coeff.	**P value**	**Coeff.**	**P value**	**Coeff.**	**P value**	**Coeff.**	**P value**	**Coeff.**	**P value**
Cons	5.680	0.0119^{**}	0.7260	0.0020^{***}	32.920	$< 0.0001^{***}$	16.940	0.0275^{**}	10.10	0.0016^{***}
X_1	0.025	0.6990	-1.250×10^{-3}	0.9681	0.26250	0.6720	- 0.27625	0.1688	- 0.5750	0.0263^{**}
X_2	-0.1625	0.0344^{**}	- 0.018750	0.5533	- 0.60	0.3463	-0.061250	0.7437	0.0750	0.7251
X_3	-0.23750	0.0065^{***}	0.04750	0.1588	- 1.73750	0.0222^{**}	0.31250	0.1262	- 0.950	0.0024^{***}
X_1X_2	-1.279×10^{-16}	1.0	0.03750	0.4078	0.2750	0.7531	0.14750	0.5805	- 0.050	0.8679
X_1X_3	1.4229×10^{-18}	1.0	- 0.110	0.0363^{**}	-1.550	0.1077	- 0.20	0.4579	- 0.150	0.6206
X_2X_3	-0.0250	0.7839	-5.0×10^{-3}	0.9098	1.9750	0.0511^{*}	- 1.3250	0.0012^{***}	-1.96262×10^{-16}	1.0
X_1^2	0.02250	0.80	- 0.34675	$< 0.0001^{***}$	2.440	0.0205^{**}	0.26875	0.3147	- 0.250	0.4053
X_2^2	-0.20250	0.0497^{**}	- 0.17675	0.0038^{***}	0.6150	0.4772	0.44375	0.1169	0.40	0.1995
X_3^2	-0.45250	0.0011^{***}	0.085750	0.0777^{*}	- 15.610	$<0.0001^{***}$	0.44125	0.1186	-2.50	$<0.0001^{***}$
R^2 (%)	**89.03**		**93.70**		**98.22**		**85.68**		**94.06**	
F LOF	**Not Significant**		**Not Significant**		**Not Significant**		**Not Significant**		**Not Significant**	

***, **, * Significant at 1, 5 and 10 % level of significant respectively
Cons = Constant and Coeff.= Coefficient

Table 5. Goals for optimization for independent variables/ dependent variables

Name of Independent/ Dependent variables	Goal	Lower limit	Upper limit
Blend Ratio	In range	-1	+1
Yeast Concentration	In range	-1	+1
Slurry Ratio	In range	-1	+1
pH	In range	4.6	5.9
Titrable Acidity	In range	0.12	0.78
Color	Minimize	15.2	37.8
Bitterness	Maximize	16.5	19.8
Alcohol Content	Maximize	5.5	11.2

Table 6. Optimum values of variables

Value	Blend ratio (X_1)	Yeast concentration (X_2)	Slurry ratio (X_3)
Coded	-0.952	1	-1
Actual	80.24	10	8

3.2.1. Effect of yeast concentration and malt to water ratio on pH of beer

The effect of malt to water ratio and yeast concentration on pH is depicted in figure 1.Response surface indicate that pH decreases as the yeast concentration increases. This may be because yeast concentration significantly affects the rate of fermentation. From table 3 it was also observed thatyeast concentration (X_2) and malt to water ratio (X_3) affects the pH at 1% ($P \leq 0.01$) and 5 % ($P \leq 0.05$) level of significance at both linear and quadratic term. The similar finding was observed by (Khandelwal *et al.,* 2012) who observed that pH decreases as yeast concentration increases during the preparation of blended low alcoholic beverages from under-utilized millets with zero waste processing methods.It was also observed that the pH was significantly affected by slurry ratio. The maximum pH was observed at the center value (1:9) of slurry ratio.

3.2.2. Effect of finger millet to apple juice and yeast concentration on titrable acidity of beer

The effect of finger millet to apple juice ratio and yeast concentrations on titrable acidity as shown in figure 2 it was observed that titrable acidity was significantly affected by the blend ratio. As the volume of apple juice increases and finger millet decreases the titrable acidity was found to be increased significantly.From table 3 it was also observed thatfinger millet to apple juice ratio (X_1) and yeast concentration (X_2) affects the titrable acidity at 1% ($P \leq 0.01$) level of significance at quadratic term. Our finding favor the findings of (Khandelwal *et al.,* 2012) who observed that the titrable acidity increases as the volume of apple juice increases during the preparation of blended low alcoholic beverages from under-utilized millets with zero waste processing methods.

3.2.3. Effect of malt to water ratioand finger millet to apple juice ratio on colorof beer

Figure 3 shows theeffect of malt to water ratio and finger millet to apple juice on color of beer. From figure 3 it was observed that the color was maximum at the center value (1:9) and decreased as the malt to water ratio increased.From table 3 it was also observed thatfinger millet to apple juice ratio (X_1) and malt to water ratio (X_3) affects the color of beer at 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$) level of significance at quadratic term.

3.2.4. Effect offinger millet to apple juice ratio and malt to water ratioonbitterness of beer

Effect of finger millet to apple juice and malt to water ratio on bitterness of beer depicted in figure 4.Response surface shows that bitterness decreases with increase in malt to water ratio and is not significantly affected by the finger millet to apple juice ratio. The increase bitterness is due to the addition of hops. From table 3 it was also observed thatfinger millet to apple juice ratio (X_1), yeast concentration (X_2) and malt to water ratio (X_3) not affects the bitterness of beer at any level of significance but finger millet to apple juice ratio (X_1) and malt to water ratio (X_3) affects bitterness of beer at 1% ($P \leq 0.01$) level of significance at interactive term. The similar finding was observed by (Kumar *et al.*, 2015) who observed that bitterness increases with the increase in slurry ratio and kilning temperature.

3.2.5. Effect ofyeast concentration and finger millet to apple juice ratio on alcohol content of beer

Figure 5 shows the Effect of yeast concentration and finger millet to apple juice ratio on alcohol content of beer. From figure 5it was found that alcohol content increases as yeast concentration increase while finger millet to apple juice ratio not affected significantly. From table 3 it was also observed thatfinger millet to apple juice ratio (X_1) affects the alcohol content at 5% ($P \leq 0.05$) level of significance at liner term, while malt to water ratio (X_3) affects the alcohol content of beer at 1% ($P \leq 0.01$) level of significance at linear and quadratic term. The similar finding was observed by (Amadi and Ifeanacho, 2016)who obsereved that fermentation is affected by subrate volume, mass of yeast and fermentation time.

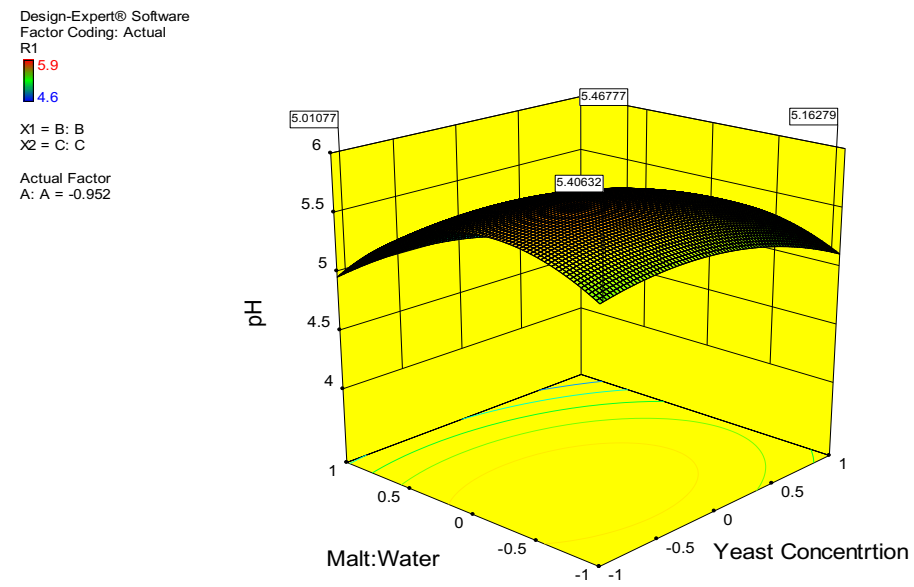

Figure1. 3D Response Surface showing the effect of malt to water ratio and yeast concentration on pH at the optimum value of finger millet to apple juice ratio

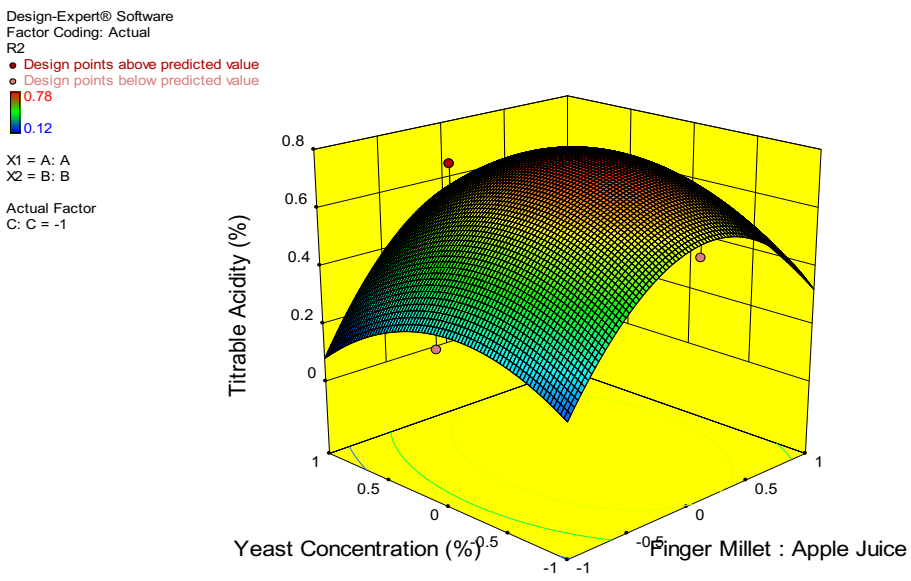

Figure 2. 3D Response Surface showing the effect of finger millet to apple juice ratio and yeast concentrations on titrable acidity at the optimum value of malt to water ratio

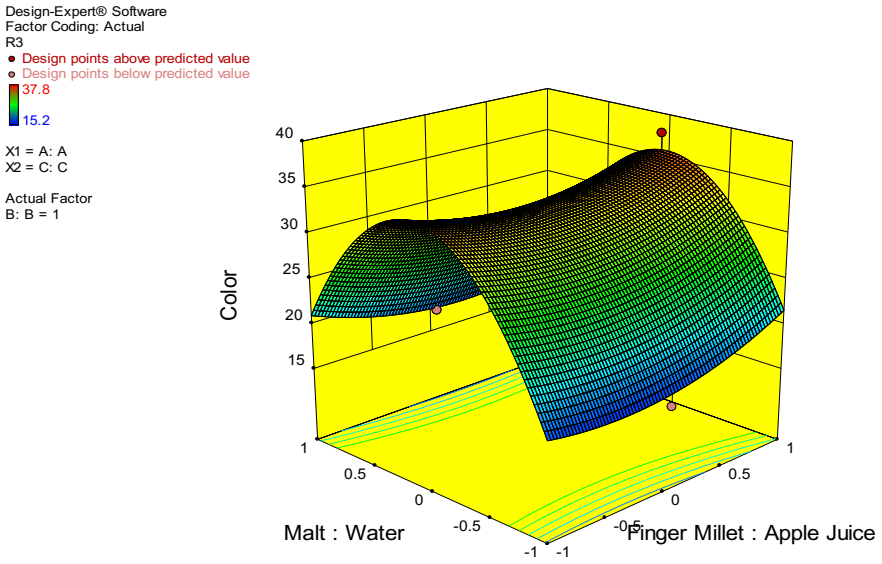

Figure 3.3DResponse Surface showing the malt to water ratio and finger millet to apple juice ratio on color of beer at the optimum value of yeast concentration

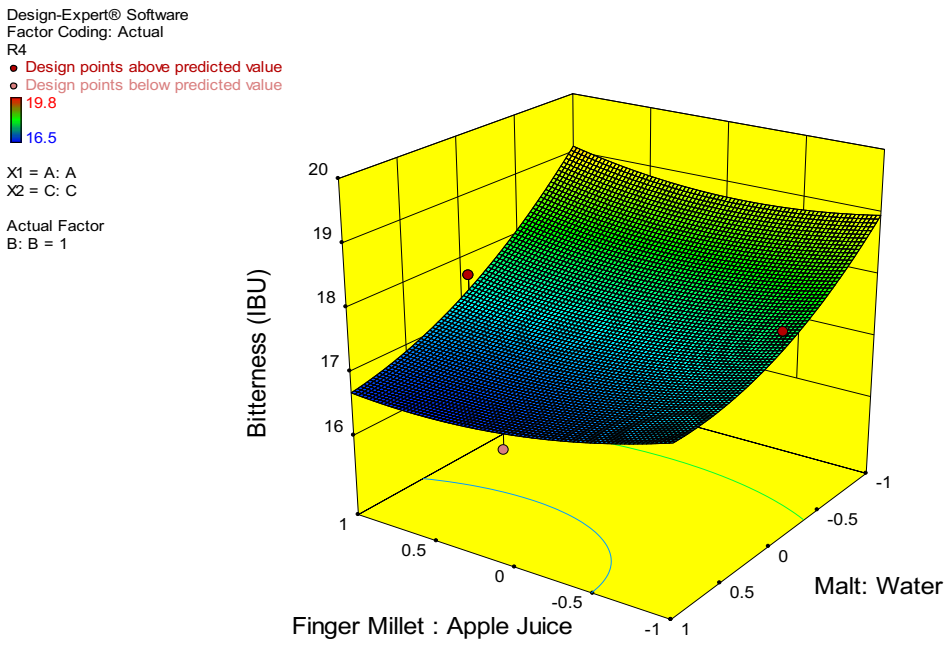

Figure 4. 3D Response Surface showing Effect of finger millet to apple juice and malt to water ratio on bitterness of beer at the optimum value of yeast concentration

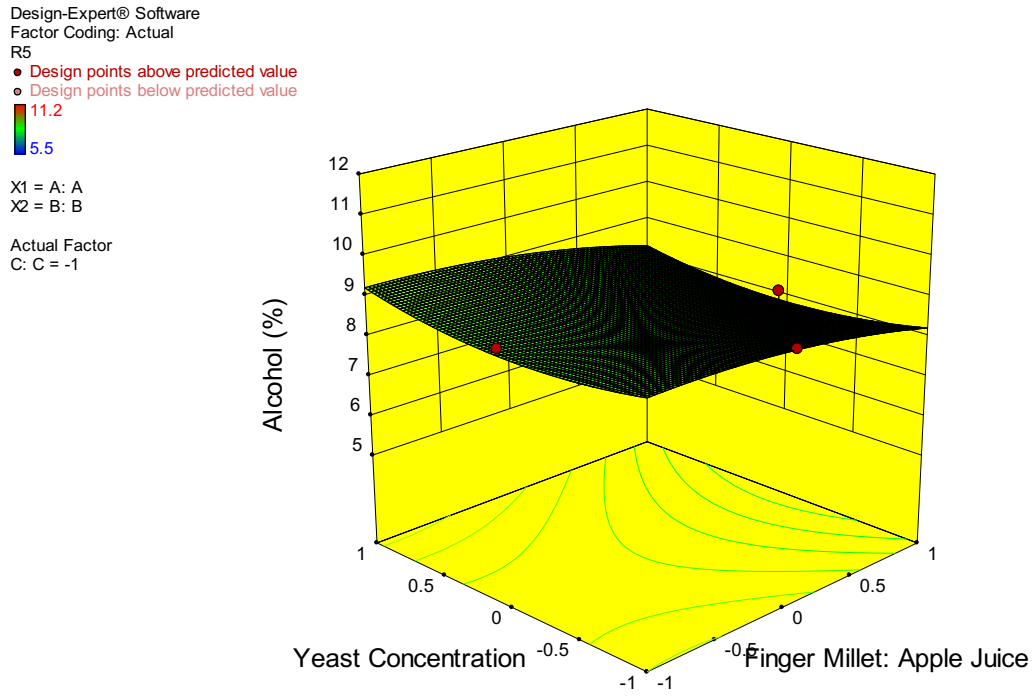

Figure 5.3D Response Surface showing effects of yeast concentration and finger millet to apple juice ratio on alcohol content of beer at the optimum value of malt to water ratio.

4. Conclusions

It could be concluded that the beer could be produced using finger millet and apple juice combination under natural fermenting conditions using 10 % yeast concentration strains as the alcohol % for finger millet to apple juice ratio 90:10 was found to be 11.2%. Optimized values of parameters for beer production were found to be 80.24:19.76 finger millet to apple juice ratio, 10% yeast concentration and 1:8 slurry ratios. The values for pH, titrable acidity, colour, bitterness and alcohol content at optimum conditions were found to be 5.12, 0.12, 17.312, 18.95 and 9.25 respectively.

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STANDARDIZATION, CHARACTERIZATION AND STORAGE STABILITY OF CURRY LEAF CHUTNEY

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ABSTRACT

Curry leaf (*Murraya koenigii*) or 'Kadipatta' is an important leaf-spice used in curries, pickles and chutneys as a natural flavoring. Curry leaf is a rich source of fibers, minerals and vitamins such as calcium, iron, phosphorus and carotene, niacin, vitamin B_2 and vitamin C. The present study aimed to standardize the Curry leaf chutney, assess its nutritional value and study the storage stability of the product in a suitable packaging material under ambient temperature (15-35ºC) conditions for a period of 90 days. Preparation of Curry leaf chutney was standardized using roasted Bengal gram (40%), Black gram (20%), dried curry leaves (10%) roasted in 10% oil and spice mix (30%) . The product had a low moisture content of 2.57%, was rich in protein (20.01%) and carbohydrates (56.99%). Sorption studies indicated that the critical moisture content for the product was found to be 9.79%, which corresponded to 68% RH indicating non- hygroscopic nature. Storage studies conducted in PET/Metallized polyester/Polythene pouches under ambient temperature (15-35ºC) conditions for a period of 90 days indicted that the overall acceptability scores for product ranged from 8.7 (excellent) to 8.2 (very good) , the tintometer colour units of Red varied from 3.3 to 3.8 units while units for yellow and blue constant. TBA values for the product also remained low indicting no rancidity development. Microbial analysis showed that an increase in the rate of growth of ACC was observed after 75 days of storage in PET/Metpoly /PE samples.

1. Introduction

The role of Indian spices in preventive and therapeutic medicine has been reported in ancient literature (Tapsell, 2006). Curry leaf (*Murraya koenigii*) or 'Kadipatta' is an important leaf-spice used in curries, pickles and chutneys as a natural flavoring (Rao *et al*.,2004; Khedkar,2015). The leaves are slightly bitter, cooling and weakly acidic in taste. The presence of volatile oils give it the distinct aroma. The leaves retain their colour and flavor even after drying, and so are utilized in fresh and dried form (Ramalakshmi *et al*., 2000). The tree is found in tropical and sub- tropical regions from Sri Lanka, India, China, Malaysia, Australia, and in the Pacific from the Mariana Islands to Vanatau and New Caledonia (Smith, 1985). The tree is native to India, Bangladesh, Sri Lanka & Andaman Islands which later spread to other parts of the world with Indian immigrants. It is widely grown almost all over India up to an altitude of 1650m. It is cultivated for its aromatic leaves and is commonly found in home gardens of South India (Joseph and Peter, 1985).

Since thousands of years, the classical Indian, Greek and Latin literature has reported the health benefits of curry leaf (Mathias, 1994). Curry leaves are known worldwide for their excellent antibacterial, antifungal, antioxidant properties, pesticidal and nutraceutical potential (Deshmukh *et al*.,1986; Pathak *et al*.,1997; Khedkar, 2015). Curry leaves contain bioactive

compounds having functional properties e.g. oxalic acid, vitamin A, koenigin, bicyclomahanimbicine, cyclomahanimbine, murrayastine, coumarine, koenidine and pypayafolinecarbazole impart nutraceutical potential to the leaves. Curry leaf is a rich source of fibers, minerals and vitamins such as calcium, iron, phosphorus and carotene, niacin, vitamin B_2 and vitamin C (Ganesan *et al*., 2013).

The leaves are a good source of vitamin A and calcium but due to the presence of high concentrations of oxalic acid, its nutritional availability is affected (The Wealth of India,2001).The TDF (Total dietary fiber), IDF (Insoluble dietary fiber) and SDF (Soluble dietary fiber) of curry leaves has been reported to be 16.3%, 13.4% and 2.9% (Punna and Rao,2004).

Traditional food adjuncts such as chutneys, pickles, papad are widely consumed and are an integral of Indian *thali* (lunch/dinner) since ages. Many studies have been conducted on the standardization and characterization of such food adjuncts. Khedkar *et al*., (2016) studied the standardization and shelf stability of a pulse based chutney powder, *Metkut*. Satyanarayan *et al*., (2013) prepared and studied the storage of flaxseed chutney. Rao et al., (2008) standardized raw mango chutney powder. Although consumed in small amount, these food adjuncts provide excellent source of nutrition along with enhancement in salivation and aiding the digestion process (Shastri, 2006). Traditionally prepared in the households, these dry and wet chutneys have a short shelf life and are available only during the season. Increasing urbanization and women work force and rising purchasing power in cities has led to a need to supply these adjuncts round the year (Khedkar *et al*., 2016).

This study was undertaken to standardize the formulation as well as process parameters ,evaluation of nutritive quality and storage stability of the Curry leaf chutney.

2. Materials and Methods

2.1. Materials

Major ingredients used in the preparation of curry leaf chutney were fresh curry leaves (*Murraya koenigii*), dehulled - split bengal gram (*Cicer arietinum*), dehulled - split black gram (*Phaseolus mungo*), whereas the minor ingredients were sesame seeds (*Sesamum indicum*), sugar, salt, dry mango powder (*Mangifera indica*), cumin seeds (*Cuminum cyminum*) and red chili powder (*Capsicum annum*). All ingredients were procured from the local market at Noida, India and were cleaned of any dirt or physical impurities.

2.2. Standardization of formulation of Curry leaf chutney

Fresh curry leaves were cleaned, washed and dried in a tray dryer at 45±2^{o}C till constant weight. Dehulled -split Bengal gram, dehulled - split black gram, sesame seeds and cumin seeds were measured, roasted in an open pan till brown and the roasted aroma developed. The dried curry leaves were measured and roasted in 10% (of the total quantity of chutney) of refined sunflower oil till crisp. The roasting temperature was kept constant at 150^{o}C. The temperature was regulated with infrared thermometer (Mextech, India).

2.2.1 Standardization of curry leaves and spice mix in Curry leaf chutney

A spice mix was prepared by weighing the sugar, salt, roasted sesame seeds, roasted cumin seeds, red chili powder and dry mango powder, grinding in a mixer grinder (Inalsa, India) and sieving it through BS30 (500µ) mesh sieve to obtain a uniform size powder. The spice mix was standardized through various trials conducted using the different levels of sugar (5-10%), Salt (2-5%), roasted sesame seeds (8-12%), roasted cumin seeds (2-5%), red chili powder (1-4%) and dry mango powder (2-5%).

For the standardization of levels of dried curry leaves and the spice mix in the formulation of curry leaves chutney, three sample were prepared using dried curry leaves and spice mix as CLC1(5:35) , CLC 2 (10:30) and CLC3 (15:25) as shown in Table 2. The levels of bengal gram and black gram were kept constant at 50 % and 10% respectively. The samples were subjected to sensory evaluation by preference

ranking test (Meilgaard *et al*.,1999) by a semi-trained panel consisting of 10 judges to evaluate the overall quality of the products by panelists who were earlier familiarized with the quality attributes of the product. The most preferred sample was ranked 1, next ranked 2 and so on.

Table 1. Composition of spice mix in Curry leaf chutney

Ingredients	Composition (% by weight)
Sesame seeds	40
Sugar	20
Cumin seeds	13.33
Dry mango powder	10
Salt	13.33
Red chili powder	3.34

Table 2. Standardization of dried curry leaves and spice mix in Curry leaf chutney

Ingredients (%)	CLC1	CLC 2	CLC3
Dried curry leaves	5	10	15
Spice mix	35	30	25
Bengal gram	50	50	50
Black gram	10	10	10

2.2.2.Standardization of major ingredients; Bengal gram and Black gram

Composition of Bengal gram and black gram, the major ingredients in the curry leaf chutney recipe was optimized by preparing 3 different samples of curry leaf chutney A, B and C using the levels of Bengal gram at 50%, 40% and 30% and Black gram at 10%, 20% and 30%. The dried curry leaves and the standardized spice mix was maintained at 10% and 30% respectively (Table 3).

Table 3. Standardization of levels of Bengal gram and black gram in Curry leaf chutney

Ingredients (%)	Sample A	Sample B	Sample C
Bengal gram	50	40	30
Black gram	10	20	30
Dried curry leaves	10	10	10
Spice mix	30	30	30

The formulation of curry leaf chutney was standardized after conducting sensory evaluation on product from the trials. Sensory evaluation was conducted by a semi-trained panel consisting of 10 judges using 9- point hedonic scale where 1= dislike extremely and 9= like extremely (Amerine *et al*., 1965). The panelists were earlier made to acquaint with the quality parameters using commercial product.

2.3. Proximate evaluation of Curry leaf chutney

The standardized samples of Curry leaf chutney were analyzed in triplicate for proximate composition. Moisture, crude fat, total protein, crude fiber and ash contents were estimated using standard methods (Ranganna, 2001). Carbohydrates were estimated by the difference method. Energy values were calculated by the standard method of summing

up the values of Total carbohydrates, crude protein and crude fat obtained and multiplying the quantity of carbohydrate and protein per 100g by 4kcal and that of fat per 100g by 9kcal respectively (Atwater and Benedict, 1902).

2.4. Sorption studies

Moisture sorption studies were conducted on the standardized samples of Curry leaf chutney by keeping 5g of each of the sample in a separate desiccators maintained at different relative humidities e.g.10%, 30%, 40%, 50%, 60%, 70% and 90%, using varying normality sulphuric acid solutions (Landrock and Proctor,1951) at 25°C.Sample weights were noted at regular intervals of one day till there was no further loss or gain in weight. Adverse changes like softness, sogginess, discoloration and mold growth were also noted from time to time. Sorption isotherm was plotted on graph. Critical moisture content and equilibrium moisture content were determined from the sorption isotherm.

2.5. Storage stability studies

The standardized samples of curry leaf chutney were packaged in PET/Metallized polyester/ PE (12µ/ 12µ/ 70µ) laminated pouches of size 10cm x 10cm, heat sealed with heat sealing machine for conducting the storage/shelf stability studies. The packaged samples were subjected to storage studies under ambient (15-35°C) storage conditions for 90 days. The storage studies included sensory evaluation using hedonic testing (Amerine *et al*.,1965) , rancidity development using TBA test, colour units measurement using Lovibond Tintometer model E (Ranganna, 2001) and microbiological evaluation (BAM, 2001).

2.6. Statistical analysis

The data were expressed as mean± S.D. Statistical analysis was carried out with SPSS version 21.0 using one-way ANOVA followed by Tukey's post hoc test for significance ($p \leq 0.05$).

3. Results & Discussion

3.1. Standardization of formultion of Curry leaf chutney

3.1.1. Standardization of curry leaves and spice mix in Curry leaf chutney

The composition of the spice mix is given in Table 1.The composition of dried curry leaves & spice mix in the preparation of curry leaf chutney was standardized based on the results of sensory evaluation by preference test shown in Table 4. The overall quality of sample CLC2 with 10% dried curry leaves, 30% spice mix, 50% Bengal gram and 10% black gram was most preferred with best score of 1.4, followed by CLC3 (2.1) and CLC1(2.5) respectively. It was noted that the addition of 15 % dried curry leaves resulted in darkening of colour as well as a bitter after taste of curry leaves. The bitterness may be due to the presence of flavor compounds pinene, sabinene, caryophyllene, cardinol and cadinene in curry leaves (Singh *et al*., 2014). A balanced proportion of curry leaves, spice mix and pulses gave an acceptable taste, body, flavour and colour.

3.1.2. Standardization of levels of pulses in the recipe of Curry leaf chutney

The sensory scores for the selection of composition of pulses, bengal gram and black gram in the formulation of curry leaf chutney has been shown in Fig.1 and Table 5. In the standardization of composition of pulses, the major ingredients, out of the three combinations, sample B scored over sample A & C in the sensory evaluation. Sample B with bengal gram (40%), black gram (20%), dried curry leaves (10%), spice mix (30%) had the characteristic colour, flavour and texture. Addition of 60% bengal gram and 10% black gram resulted in the change of colour from green to yellow with a gritty texture, whereas at 30% level of bengal gram and black gram, the colour of curry leaf chutney was pale green but with a fine texture. The composition of standardized curry leaf chutney has been given in Table 6

Table 4. Preference ranking scores for standardization of dried curry leaves and spice mix in Curry leaf chutney (n=10)

Rank/ Sample	**CLC1**	**CLC2**	**CLC3**
Mean rank ± s.d.	2.5±0.85	1.4±0.7	2.1±0.57
Remarks	More spicy, less flavour of curry leaves, dull brown colour	Good flavour of curry leaves and spices, bright green colour	Strong aroma and bitter aftertaste of curry leaves, dark green colour

Values are average ±S.D.

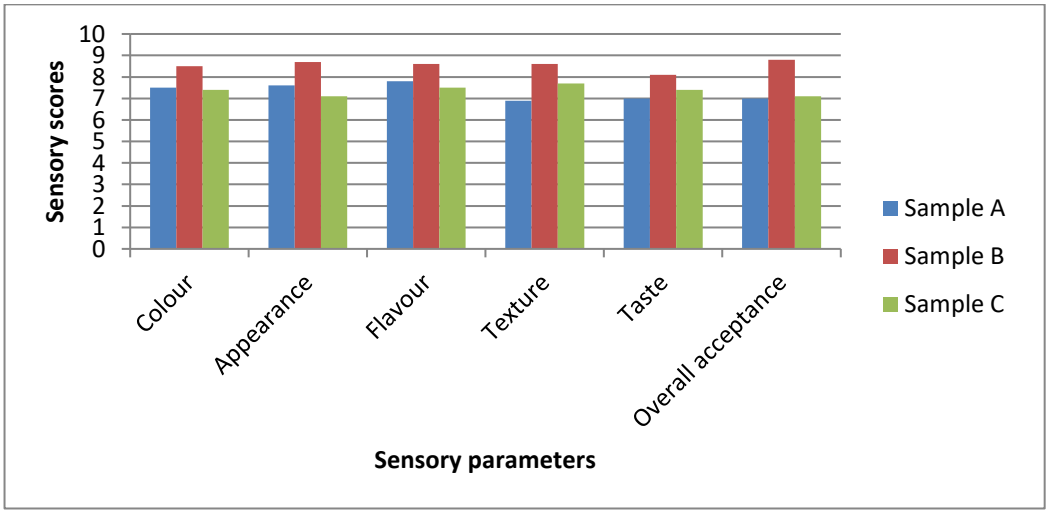


Figure 1. Sensory scores for standardization of levels of pulses in Curry leaf chutney

Table 5. Sensory scores for selection of levels of Bengal gram and black gram in Curry leaf chutney (n=10)

Parameter/sample	**A**	**B**	**C**
Colour	7.5±0.71	8.5±0.71	7.4±0.97
Appearance	7.6±0.70	8.7±0.48	7.1±0.88
Flavour	7.8±1.03	8.6±0.7	7.5±0.71
Texture	6.9±0.74	8.6±0.7	7.7±0.82
Taste	7±0.82	8.1±0.99	7.4±0.7
Overall acceptance	7.4±0.70	8.8±0.42	7.1±0.88
Remarks	Colour yellowish green, gritty texture, good aroma	Colour bright green, fine texture , fresh aroma	Colour pale green, fine texture, less aroma

Values are average ±S.D.

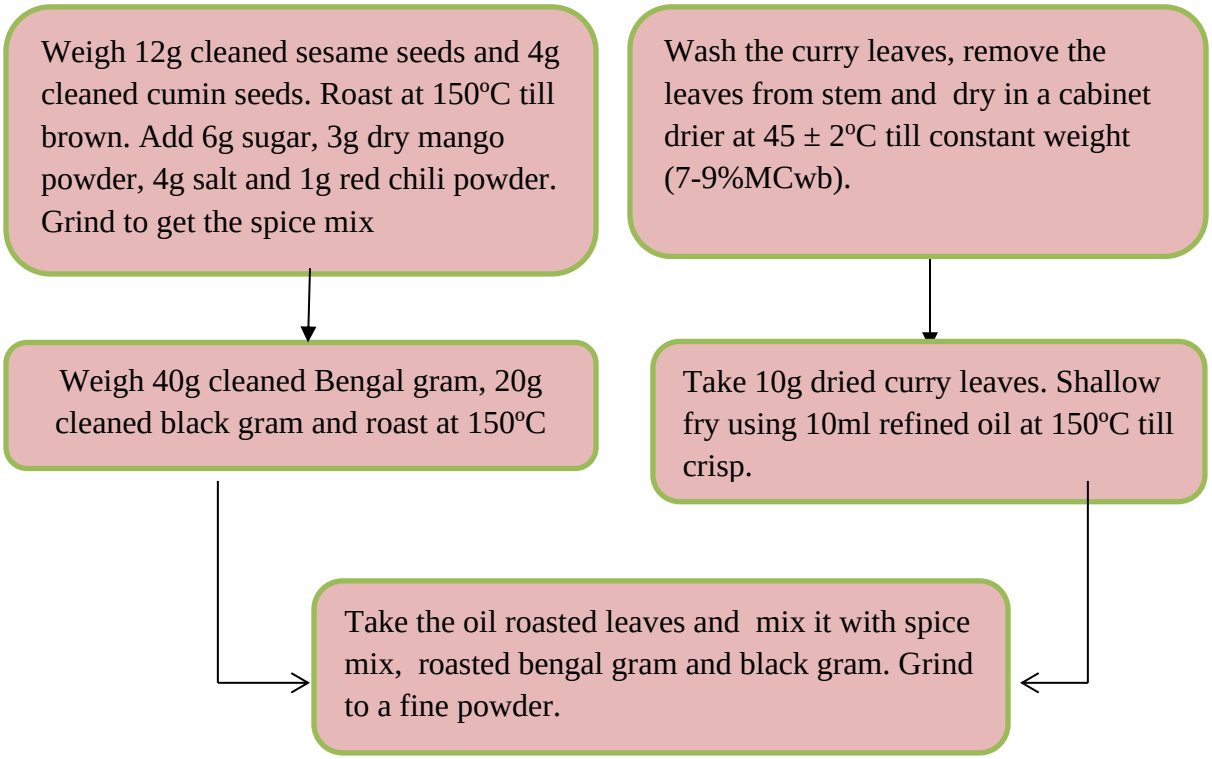
Table 6. Composition of standardized Curry leaf chutney

Ingredients	Composition (% by weight)
Dried curry leaves	10
Bengal gram	40
Black gram	20
Spice mix	30

Table 7. Proximate composition of Curry leaf chutney (per 100g) (n=3)

S.No.	Parameter (g)	Curry leaf chutney
1	Moisture	2.57±0.18
2	Protein (%N x 6.25)	20.01±0.07
3	Crude fat	14.81±0.15
4	Crude fiber	1.99±0.19
5	Total Ash	5.61±0.21
6	Carbohydrates	56.99±0.23
7	Energy value (kcal)	441.29

Values are average ±S.D. and are expressed on sample basis



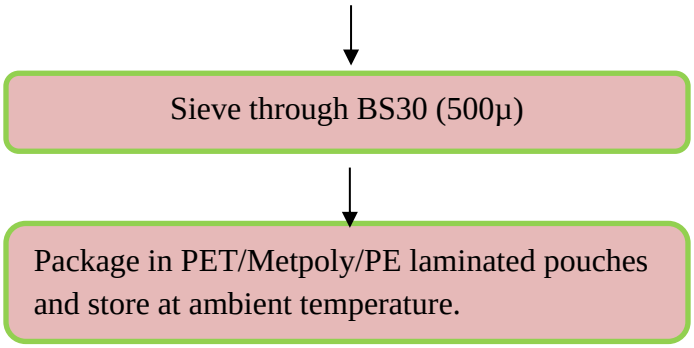


Figure 2. Standardized process for preparation of Curry leaf chutney

3.2. Proximate analysis of Curry leaf chutney

As seen from Table 7, the product had a low moisture content of 2.57%, was rich in protein (20.01%) and carbohydrates (56.99%). The crude fat content was found to be 14.81%, crude fiber 1.99%, total ash 5.61%, and energy value 441.29kcal. Gopalan *et al.* (2014) reported that bengal gram, black gram, sesame seeds and cumin seeds are a good source of protein (22.5%, 24%, 18.3% and 18.7% respectively). Sesame seeds are also a good source of fat (43.3%). The high protein and fat content can be attributed to the presence of these ingredients in the Curry leaf chutney. Rao *et al*. (2004) reported the moisture content of 5%, crude protein 16.4%, Crude fat 14.2%, total ash 4.0%, crude fiber 7.0%, carbohydrates 57%, in curry leaf chutney powder prepared from fresh curry leaves fried in 10% oil, bengal gram, black gram, green gram, coriander seeds, cumin seeds, red chili powder and tamarind pulp.

3.3. Sorption studies on Curry leaf chutney

Moisture sorption isotherm was plotted for curry leaf chutney as represented in Fig.3. The product had an initial moisture content of 2.57%, which equilibrated at48% RH. The critical moisture content was found to be 9.79%, which corresponded to 68% RH. It gained moisture quickly at RH above 72%. The product was found to be non- hygroscopic in nature. The crude fat content of the Curry leaf chutney is 14.81% indicated suitable packaging material with good light and gas barrier properties . Rao *et al*. (2004) also examined the moisture sorption isotherm of curry leaf chutney powder. The isotherm obtained was also reported to be sigmoidal, with a S- type curve, which is common for carbohydrate rich foods. Similar type of curves were obtained for instant chutneys prepared from *Pudina* (mint) and *gongura* (*Hibiscus* sp.) as reported by Satyanarayana *et al*.(2001).

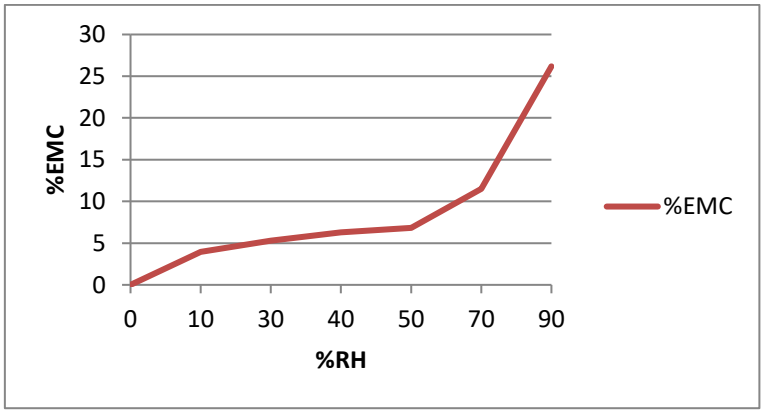


Figure 3. Moisture sorption isotherm for Curry leaf chutney using H_2SO_4 solutions of various RH

The moisture absorption isotherms for curry leaf chutney at 25ºC showed three regions, I, II & III. Region I corresponding to %RH 0-10 represent monolayer moisture content strongly bound to polar components of the product. Region II, corresponding to %RH 10-70 is also called as multi molecular phase. In this phase, the slope of the isotherm is small, indicating the changing of multi molecular water to free water. Region III for % RH above 70 where there is a steep rise in the isotherm, represents the water in the free state and available to chemical and microbial reactions (Kinsella and Fox, 1987).

3.4. Storage studies on Curry leaf chutney under ambient temperature conditions

3.4.1 Sensory evaluation of Curry leaf chutney stored under ambient temperature (15-35ºC) conditions

The sensory scores of Curry leaf chutney packed in PET/Metpoly/PE for the storage under ambient temperature conditions are presented in Table 8. The scores for product packed in PET/Metpoly/PE ranged from 8.7 (excellent) to 8.2 (very good) for overall acceptance, 8.8 to 8.1 for flavour, 8.7 to 8.2 for taste, 8.8 to 8.3 for colour respectively. Statistical analysis of the sensory scores for Curry leaf chutney packaged in PET/ Metpoly/PE and stored under ambient temperature conditions for 90 days ,was carried out using Tukey's post hoc test . The products packed in PET/Metpoly/PE did not show any significant difference in the sensory scores during the storage period.

3.4.2. Colour measurement of Curry leaf chutney stored at ambient temperature (15-35ºC) conditions

The colour changes in curry leaf chutney packaged in PET/Metpoly/PE, during the storage period of 90 days under ambient temperature conditions were measured using Lovibond Tintometer model E and are presented in Table 9. Tintometer colour units of Yellow (30.0) and Blue (7.0) remained constant during the storage period of 90 days for the product packed in PET/Metpoly/PE. The units of Red varied from 3.3 to 3.8 units indicating a colour change from fresh green to pale green.

Table 8. Sensory scores of Curry leaf chutney during storage under ambient temperature (15-35ºC) conditions for 90 days (n=10)

Parameters/ storage days	**PET/Metpoly/PE**						
	0 day	**15 day**	**30 day**	**45 day**	**60 day**	**75 day**	**90 day**
Colour	8.8±0.42 [a]	8.8±0.42 [a]	8.7±0.48 [a]	8.6±0.52 [a]	8.5±0.53 [a]	8.4±0.52 [a]	8.2±0.42 [a]
Appearance	8.7±0.48 [a]	8.7±0.48 [a]	8.5±0.71 [a]	8.4±0.70 [a]	8.3±0.67[a]	8.2±0.63 [a]	8.1±0.57 [a]
Flavour	8.8±0.42 [a]	8.8±0.42 [a]	8.6±0.52 [a]	8.4±0.70 [a]	8.3±0.82 [a]	8.2±0.79 [a]	8.1±0.74 [a]
Texture	8.5±0.53 [a]	8.4±0.52 [a]	8.2±0.42 [a]	8.1±0.32 [a]	8.1±0.32 [a]	8.0±0.47 [a]	8.0±0.47 [a]
Taste	8.7±0.48 [a]	8.5±0.53 [a]	8.4±0.52 [a]	8.3±0.48 [a]	8.2±0.42 [a]	8.1±0.32 [a]	8.1±0.32 [a]
Overall appearance	8.7±0.48 [a]	8.6±0.52 [a]	8.5±0.53 [a]	8.4±0.70 [a]	8.2±0.63 [a]	8.1±0.57 [a]	8.0±0.47 [a]
Remarks	Bright green colour, good flavour and taste	Bright green colour, good flavour and taste	Bright green colour, good flavour and taste	Bright green colour, good flavour and taste	Bright green colour, good flavour and taste	Bright green colour, good flavour and taste	Bright green colour, good flavour and taste

Similar superscripts indicate non-significant difference at p>0.05; Values are average± S.D.

Table 9 . Colour measurement of Curry leaf chutney using Lovibond Tintometer during ambient temperature(15-35ºC) storage for 90 days (n=3)

Packaging material/storage days	R/Y/B	0 day	15 day	30 day	45 day	60 day	75 day	90 day
PET/Metpoly/PE	R	3.3	3.3	3.3	3.4	3.6	3.7	3.8
	Y	30	30	30	30	30	30	30
	B	7.0	7.0	7.0	7.0	7.0	7.0	7.0

3.4.3 Estimation of oxidative rancidity in Curry leaf chutney stored under ambient temperature (15-35ºC) conditions

Curry leaf chutney had a crude fat content of 14.81%. The raw materials such as sesame seeds are rich in unsaturated fatty acids (Toma and Tabekhia, 1979). Due to the unsaturated fat content, the product was exposed to the risk of development of oxidative rancidity. The TBA test was carried out on the samples of curry leaves chutney to estimate the extent of oxidative spoilage during the storage at ambient temperature conditions for 90 days. The absorbance of the TBA reactive substances (TBARS) in the sample was measured at 530nm. The changes in absorbance for the curry leaf chutney packed in PET/Metpoly/PE and stored at ambient temperature conditions for 90 days have been shown in Table 10. The product showed non-significant, little change in absorbance from 1.01 to 1.06. Also as seen from Table 8, there was no off flavor developed during the storage period of 90 days, indicating very small/no development of rancidity in the sample. The PET/Metpoly/PE packaging material had good oxygen barrier properties, preventing the oxidative spoilage for more than the storage period of 90 days. Capriles *et al*., (2009) studied the use of rapeseed oil as a replacement for partially hydrogenated vegetable oil in snack flavoring and observed that low TBARS values and absence of off-flavours indicate that lipid oxidation did not progress significantly during the storage period.

Table 10. Changes in absorbance at 530nm in Curry leaf chutney stored under ambient temperature (15-35ºC) conditions for 90days (n=3)

Packaging material/storage days	0day	15 day	30 day	45 day	60 day	75 day	90 day
PET/Metpoly/PE	1.01	1.01	1.02	1.03	1.05	1.06	1.06

Values are average of triplicates

3.4.4 Microbiological analysis of Curry leaf chutney stored under ambient temperature (15-35ºC) conditions

Microbiological analysis of curry leaf chutney packaged in PET/Metpoly/PE carried out at 15 day intervals for 90 days of storage period in triplicates is presented in Table 11. No microbial growth was found on 0 day in samples packed in both the packaging materials. However, at the end of storage period, i.e. 90 day, samples showed growth of aerobic microorganisms as well as yeast & mold. The samples packed in PET/Metpoly/PE laminated pouches had ACC- 2.5 x 10^3 cfu/ml &YMC- 2.3 x 10^3 cfu/ml . An increase in the rate of growth of ACC was observed after 75 days of storage in PET/Metpoly /PE samples.

Table 11. Microbiological analysis of Curry leaf chutney stored under ambient temperature (15-35ºC) conditions (n=3)

Packaging material/storage days	Parameter	0 day	15 day	30 day	45 day	60 day	75 day	90 day
PET/Metpoly/ PE	Aerobic colony count (ACC) (cfu/ml)	No growth	4.5x 10^1	1.4 x 10^2	3.2 x 10^2	5.6 x 10^2	9.1 x 10^2	2.5 x 10^3
	Yeast & Mold count (YMC)(cfu/ml)	No growth	1.0 x 10^1	4.1 x 10^1	1.2 x 10^2	4.6 x 10^2	7.4.x 10^2	2.3 x 10^3

4. Conclusions

Curry leaf chutney is a popular food adjunct in Indian cuisine. The product was standardized with dried and roasted curry leaves (10%), Bengal gram (40%), Black gram (20%) and spice mix (30%). It was rich in nutrients and can be used as a functional food adjunct. The storage studies conducted in PET/Metpoly/PE pouches under ambient temperature (15-35ºC) conditions revealed that the standardized Curry leaf chutney when packaged in suitable packaging material was shelf stable for more than 90 days. Curry leaf has medicinal, nutritional properties and are also used as flavouring agent. Use of it and other functional ingredients in food adjuncts can help in transferring the benefits to the diverse population.

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GAMMA RADIATION EFFECTS ON PHYSICOCHEMICAL, MICROBIOLOGICAL AND ANTIOXIDANT PROPERTIES OF BLACK RICE (*Oryza Sativa* L.) FLOUR DURING STORAGE

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ABSTRACT

Black rice has been categorised as a functional food because it contains high amounts of bioactive compounds. The effects of gamma radiation (at 0, 1, 2 and 3 kGy doses) on the free and bound total phenolics, antioxidant activity, and physicochemical and microbiological properties of black rice flour samples during storage were evaluated. The chemometric approach made it possible to observe the effects of irradiation and storage time on the samples. Regarding bioactive compounds, with the exception of the bound phenolic fractions the 3 kGy dose showed the highest values at time 0. At 120 days there was a decrease in these levels for all the samples, although the irradiated samples were most stable at the end of storage. Regarding the attribute of colour, the irradiation slightly modified all the parameters; in terms of microbiological analysis there was no growth of microorganisms at the end of storage.

1.Introduction

Rice (*Oryza sativa* L.) is one the most important staple foods worldwide; it is considered to be an important source of energy for populations from both developed and developing countries. In the last few years global rice production has been higher than consumption, with an annual production of almost 500 million metric tonnes (milled rice basis) and a harvested area of above 160 million hectares (USDA, 2018).

Even though white rice (non-pigmented) is the most cultivated variety, coloured (pigmented) rice has received increasing attention from researchers due to its antioxidant properties and bioactive compounds in the outer layer of the caryopsis (Finocchiaro et al., 2010; Ryu and Koh, 2017). Rice contains higher levels of phenolic acids (ferulic, caffeic and coumaric acid), than other cereal grains (Ti et al., 2014). Pigmented rice is a source of proanthocyanidins, anthocyanins and flavonoids, as well as tocopherols and vitamins (Hao et al., 2015; Rodríguez-Pérez et al., 2015).

In particular, black rice contains anthocyanins and phenolic acids, which is categorized as free phenolic acids or bound phenolic acids (Ito and Lacerda, 2019). Free

phenolic acids are extractable mainly by using an 80% methanol solution. As the bound phenolic acids are present in insoluble form, a strong alkali is added to the residue obtained after the extraction of free phenolics (Alves et al., 2016). These bioactive compounds, which are present in different amounts in rice varieties, are associated with reducing the risks of developing several chronic diseases such as cancer, diabetes, cardiovascular disease (Hao et al., 2015).

It has known that irradiation is a powerful processing technology to inactivate microorganisms and insects to ensure hygienic quality, as well as extending the shelf life of foods. Gamma radiation is considered to be a physical and environmentally friendly technology that is widely acknowledged for its various applications in the food industry (Kumar et al, 2017).

Understanding the effects of radiation by using chemometrics could provide significant information concerning the bioactive compounds that are present in pigmented rice. This is fundamental to develop new food products and to improve human health. Therefore, the purpose of this research was to investigate the effects of gamma radiation on the free and bound total phenolics, antioxidant activity and physicochemical and microbiological properties of black rice flour during storage by using chemometrics.

2. Materials and methods

2.1. Sample preparation and radiation treatment

All reagents were of the highest grade commercially available. The biodynamic black rice used in the experiments was cultivated according to Demeter biodynamic standards (Demeter International, 2012) and purchased in a local supermarket in the city of Curitiba, Paraná, Brazil. The black rice flour was obtained according to the methodology used by Ito et al. (2018).

All samples were irradiated at doses of 0, 1, 2 and 3 kGy at a 0.221 kGy h^{-1} dose rate in a ^{60}Co gamma irradiator (Gammacell Excell 220 - MDS Nordion, Ottawa, Canada). The irradiation treatments were performed in the Centre for Nuclear Energy in Agriculture at the University of São Paulo, Brazil (CENA/USP).

2.2. Physicochemical analysis

The colour attributes lightness (L^*), redness (a^*), and yellowness (b^*) of the black rice flour samples were determined using a HunterLab MiniScan EZ colourimeter (Reston, VA, USA.), as described by Ito et al., 2016.

The moisture was determined gravimetrically in an air oven at 105 °C (AOAC 935.29). The water activity (A_w) was measured with a digital A_w meter (Aqualab®, USA).

2.3. Extraction and phenolic composition

2.3.1. Extraction of free and bound phenolics

The free phenolics were extracted using the method reported by Sumczynski et al. (2016) with minor modifications. Briefly, the sample (0.5 g) of black rice flour was treated twice with 8 mL of 80% aqueous methanol using an ultrasound device (47 kHz, 130 W, Ultrasonic Cleaners, Vernon Hills, USA) at 35 °C for 1 h. The supernatants were combined after centrifugation at 5000 *g* (HIMAC CR-GII, Hitachi, Ibaraki, Japan) for 30 min at room temperature and their pH was adjusted to 4.5−5.5.

To extract the bound phenolics the residues of black rice flour obtained above were re-washed using 20 mL of water. After removing the water the samples were blended twice with 20 mL of 4 M NaOH for 2 h in an ultrasonic device. The mixture was then adjusted. After centrifugation, the supernatant was used as the bound phenolic extract.

2.3.2. Phenolic composition

The free and bound phenolic fractions were determined by colorimetric analysis using Folin-Ciocalteu reagent, as described by Singleton, and Rossi (1965), with modifications. The absorbance was recorded at a wavelength of 720 nm after one hour of reaction and the measurements were performed using a

microplate reader (Epoch microplate spectrophotometer, Synergy-BioTek, Winooski, VT, USA). The total phenolic content was calculated as the sum of the free and bound phenols. The results were expressed as mg of gallic acid equivalents (GAE) per gram of black rice flour (mg GAE g^{-1}).

The total anthocyanins content (TAC) was determined according to the pH differential spectrophotometric method adapted for microplate (Giusti & Wrolstad, 2001). Firstly, two solutions were prepared: one buffer at pH 1.0 (0.025 mol L^{-1} KCl water buffer, acidified with HCl) and another buffer at pH 4.5 (0.4 mol L^{-1} sodium acetate water buffer, acidified with HCl). Subsequently, using the method reported by Shao et al. (2014), aliquots of the extract were transferred to a 96-well microplate and 290 µL of corresponding buffer (pH 1.0 and 4.5) and allowed to equilibrate for 30 min. The absorbance was measured at 520 and 700 nm. The TAC was expressed as mg cyanidin-3-glucoside equivalent (C3G) per g of black rice flour.

The anthocyanin extracts were also analysed using HPLC, as previously described by Pedro et al. (2016), with minor changes. The analysis was performed in an Alliance 2695 separation module (Waters, Milford, MA, USA) coupled with photodiode detector (model PDA 2998, Waters, Milford, MA, USA), a quaternary pump and an auto sampler. Firstly, the extracts were filtered then 10 µL of sample was injected into the HPLC system. The separation was then performed using a XTerra® MS C18 column with dimensions of 4.6 × 250 mm, 5 µm (Waters, Milford, MA, USA) kept at 20 °C with a flow of 1.0 mL min^{-1}. The black rice flour anthocyanins were identified and quantified at 515 nm using a DAD detector by comparing the retention time with the standard of cyanidin-3-glucoside in the concentration range from 0.01 to 0.25 mg L^{-1} ($y = 25482x - 20152$; $R^2 = 0.999$).

The total flavonoids (TF) were quantified by UV–Vis spectrophotometry (Shimadzu UV-1800) at 374 nm as described by Pedro et al, (2016). The total flavonoid content was expressed as mg quercetin equivalents (QE) per g of black rice flour.

2.4. In *vitro* antioxidant activity

The total antioxidant potential of the black rice flour extracts was determined by assessing the ABTS scavenging activity of the extracts using the method described by Re et al. (1999), with modifications. The absorbance was recorded at a wavelength of 734 nm after the solution had been allowed to stand in the dark for 30 min. The results were compared with a standard curve (trolox 100–1000 µmol L^{-1}) and expressed in µmoL trolox equivalent per g of black rice flour (µmol TE g^{-1}).

The DPPH radical scavenging activity of the extracts was determined according to the method of Brand Williams et al. (1995), with minor modifications. The absorbance was recorded at a wavelength of 517 nm after the solution had been allowed to stand in the dark for 30 min. A standard curve (DPPH = 0.001 × absorbance; $R^2 = 0.995$; $p < 0.001$) was plotted using different concentrations of trolox (0.1 – 300 µmol L^{-1}). The results were expressed in µmol trolox equivalents per gram of sample (µmol TE g^{-1}).

2.5.Microbiological analysis

In order to evaluate the microbiological quality of the black rice flour, the following analyses were performed: *Bacillus cereus*, thermotolerant coliforms and *Salmonella* sp, according to the National Health Surveillance Agency, Collegiate Board Resolution No. 12 (ANVISA, 2001). In this study, the samples were included in the food group "flour, pasta and bakery products (industrialised and packaged) and similar" with a maximum tolerance for an indicative sample being $3x10^3$ NMP g^{-1} or CFU g^{-1} of *Bacillus cereus*; 10^2 NMP g^{-1} or CFU g^{-1} of thermotolerant coliform; and the absence of *Salmonella* sp in 25 grams. The analyses were performed in triplicate during the first and last stages of the research.

2.6. Statistical analysis

The experimental data were presented as the mean ± standard deviation. The analyses were performed using STATISTICA v.13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). One-way analysis of variance (ANOVA) was used to study the effects of gamma radiation on bioactive compounds, antioxidant activity and colour parameters. Duncan's tests were conducted to determine differences between the means at 95% confidence level ($p < 0.05$). Pearson's correlation analysis (r) was applied between the different response variables to assess the strength of the correlation between the responses.

Principal component analysis (PCA) was used to analyse the interrelationships between the parameters. The hierarchical cluster analysis (HCA) was based on Euclidean distance and Ward's method. In order to compare the results between the groups proposed by HCA, homogeneity of variance (Levene's test) and analysis of variance (one-way ANOVA or Kruskal-Wallis ANOVA) were applied (Ito et al., 2016).

3. Results and discussions

3.1. Effects of gamma radiation on bioactive compounds and antioxidant activity during storage

The results obtained for phenolic compounds (PC) and antioxidant activity by the ABTS and DPPH assays regarding the free, bound and total phenolic fractions of the black rice flour are summarised in Table 1. Analysing the values for PC and antioxidant activity (ABTS and DPPH) of the free phenolic fraction of the black rice flour, it was observed that the sample with a 3 kGy dose showed the highest values compared with the samples treated with doses of 0, 1 and 2 kGy. At 120 days there was a decrease in the levels for all the samples ($p<0.05$).

Table 1..Effects of gamma radiation on the levels of phenolic compounds (PC) and antioxidant activity of free, bound and total phenolic fractions of black rice flour at the beginning (T_0 – zero days) and end of storage (T_f – 120 days).

Analysis	Doses (kGy)	T_0(0 days)	T_f (120 days)
Free PC (mgGA g)	0	5.28^{Da}±0.03	3.25^{Cb}±0.04
	1	5.79^{Ba}±0.03	3.58^{ABb}±0.03
	2	5.67^{Ca}±0.01	3.54^{Bb}±0.03
	3	5.89^{Aa}±0.03	3.64^{Ab}±0.03
Bound PC (mgGA g)	0	1.84^{Aa}±0.02	0.95^{Db}±0.03
	1	1.67^{Ba}±0.02	0.99^{BCb}±0.04
	2	1.61^{Ca}±0.02	1.09^{Ab}±0.02
	3	1.59^{Ca}±0.01	1.01^{Bb}±0.02
Total PC (mgGA g)	0	7.12^{Ca}±0.02	4.19^{Bb}±0.06
	1	7.46^{Aa}±0.01	4.57^{Ab}±0.06
	2	7.28^{Ba}±0.03	4.63^{Ab}±0.01
	3	7.47^{Aa}±0.03	4.65^{Ab}±0.05
Free ABTS (mgGA g)	0	6.37^{Da}±0.03	5.19^{Db}±0.03
	1	7.35^{Ca}±0.03	6.05^{Bb}±0.02
	2	7.59^{Ba}±0.01	6.16^{Cb}±0.02
	3	8.13^{Aa}±0.02	6.77^{Ab}±0.03
Bound ABTS (mgGA g)	0	2.52^{Aa}±0.03	1.60^{Cb}±0.03
	1	2.48^{Ba}±0.03	1.61^{Bb}±0.03
	2	2.36^{Ca}±0.01	1.63^{Ab}±0.02

	3	2.35^{Da}±0.03	1.63^{Ab}±0.02
Total ABTS (mgGA g)	0	8.89^{Da}±0.01	6.80^{Db}±0.01
	1	9.83^{Ca}±0.06	7.66^{Cb}±0.04
	2	9.96^{Ba}±0.02	7.79^{Bb}±0.01
	3	10.48^{Aa}±0.01	8.40^{Ab}±0.05
Free DPPH (mgGA g)	0	6.19^{Da}±0.03	4.95^{Db}±0.03
	1	6.37^{Ca}±0.03	5.01^{Bb}±0.03
	2	6.60^{Ba}±0.02	5.13^{Bb}±0.02
	3	6.68^{Aa}±0.02	5.14^{Ab}±0.03
Bound DPPH (mgGA g)	0	2.14^{Aa}±0.03	1.31^{Cb}±0.02
	1	2.09^{Ba}±0.03	1.31^{Cb}±0.01
	2	2.05^{Ca}±0.01	1.33^{Bb}±0.03
	3	2.03^{Da}±0.02	1.34^{Ab}±0.02
Total DPPH (mgGA g)	0	8.33^{Da}±0.06	6.26^{Db}±0.01
	1	8.47^{Ca}±0.01	6.32^{Cb}±0.03
	2	8.65^{Ba}±0.03	6.46^{Bb}±0.05
	3	8.72^{Aa}±0.04	6.48^{Ab}±0.01

Note - Results are expressed as mean ± standard deviation; Different capital letters in the same column indicate significant difference between the doses; Different small letters in the same line indicate significant differences during the time of storage. The significant differences at a level of 5% were performed by Duncan's test.

This increase in PC and antioxidant activity of the free phenolic fraction of gamma irradiated samples can be ascribed to the development of new double bonds due to of radiation degradation, which reduced the reactivity of the free radicals (Kumar et al., 2017). Another explanation is that, gamma irradiation may modify/activate some enzymes in rice and change the post-harvest physiology during storage (at room temperature), resulting in an improved synthesis of phenolic acids (Zhu et al., 2010).

The total phenolic content showed behaviour that was similar to that of the free fractions, where the irradiated samples showed the highest values compared with control sample ($p<0.05$); both at the beginning (T_0) and at the end (T_f). The free phenolic fractions accounted for about 78% of the total phenolic contents and 77% of the total antioxidant activity (ABTS and DPPH assays) in the irradiated samples. The main phenolic acids found in pigmented rice are protocatechuic, synaptic, vanillic, p-coumaric and ferulic acid (Zhang et al., 2015).

The values for PC and antioxidant activity of the bound phenolic fraction showed behaviour that was distinct from the free phenolic fraction of the black rice flour. The levels of the control sample were highest at time 0; however, the irradiated samples were most stable at the end of storage ($p<0.05$). The concentration of these phenolic fractions may be dependent on the radiation dosage, the time of storage, technological processes and also the specific nature of the product. Zhang et al., (2015) also found the TPC in soluble fractions were higher than insoluble bound fractions in the black rice; it could be due to some genes that control the linkage between phenolics and lignins which assorted resulting in different offsprings of rice.

Total flavonoids comprise a hydrophilic group of phenolic compounds, to which anthocyanins (highly coloured substances that are recognised for their antioxidant activity and are responsible for the red-purple colour of most vegetables) belong. Cyanidin-3-glucoside (88% of total anthocyanins) is a major anthocyanin in black rice (Abdel-Aal et al., 2006).

Evaluating the total anthocyanin content (TAC), cyanidin-3-glucoside (C3G) and total flavonoids (TF) at 120 days, the results were significantly different ($p < 0.05$) for all samples. The TAC values ranged from 1.84 - 1.52

mgC3G.g-^{1}; cyanidin-3-glucoside ranged from 1.43 – 1.26 mg.g^{-1} and TF ranged from 0.65 – 0.49 mgCE.g^{-1}. It is noteworthy that the sample with a 3 kGy dose at time 0 showed the highest values, and the control sample (0 kGy) at 120 days showed the lowest values. Sultan et al. (2018) also found a significant increase in TAC in pigmented brown rice flour using an irradiation dose of 2.5 and 5 kGy, as well as Zhu et al. (2010), who evaluated brown rice at an irradiation dose of 6 kGy.

3.2. Effects of gamma radiation on colour attributes during storage

With regard to the effects of gamma radiation on the colour attributes of the black rice flour during storage, the irradiation slightly modified all the parameters ($p < 0.05$), as shown in Figure 1.

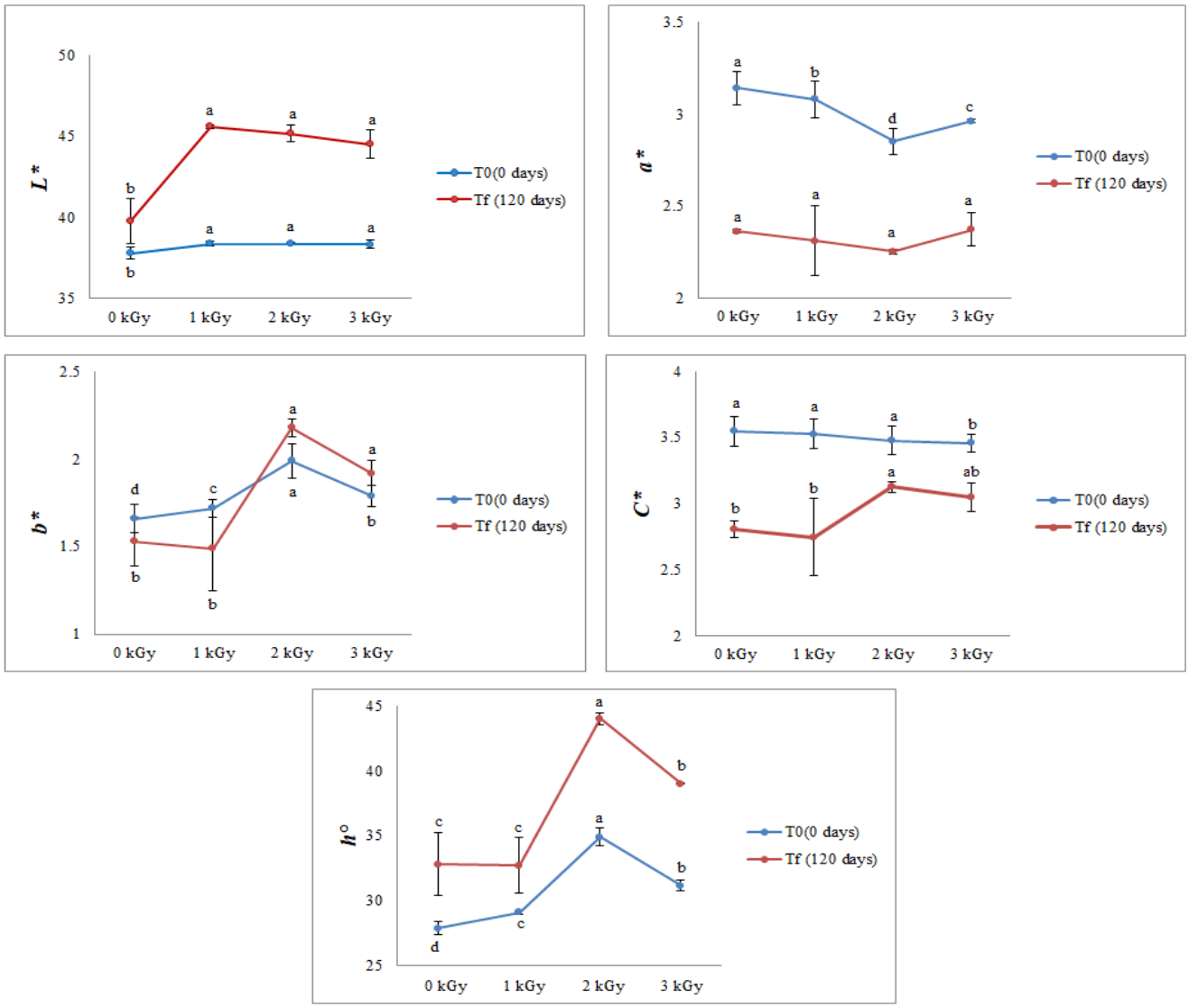

Figure 1. Effects of gamma radiation on the colour attributes (*L*, a*, b*, C** and *h*°) of black rice flour at the beginning (T_0 – zero days) and end (T_f) of storage.

In the irradiated samples, the values for Lightness (*L**) and hue angle (*h*°) were higher than the control sample. Moreover, these parameters increased for all the samples during storage, indicating a loss of colour intensity. *L** is negatively correlated to colour intensity

(Lago-Vanzela et al., 2014) and this behaviour may be associated with a decrease in phenolic content (Figueiredo-González et al., 2013). The *a** (red-green) values were lower in the irradiated samples and during storage this parameter decreased for all the samples.

The irradiated sample with a 2 kGy dose showed higher values for the *b** (yellow-blue) parameter and during storage the best stability for the chroma (*C**) parameter. The effects of gamma radiation on the colour parameters have been reported by other authors. For example, slight changes were found in hazelnuts and almond kernels treated with low doses (Mexis and Kontominas, 2009; Mexis et al., 2009). Therefore, these effects differed due to the individual characteristics of each product.

3.3. Multivariate analysis

The principal component analysis shown in Figure 2 relates to a two-dimensional graphical representation of the black rice flour samples. Principal Components 1 (eigenvalue 13.62) and 2 (eigenvalue 2.28) explained 93.5% of the variance of the data. The formation of two clusters based on the time of storage can be observed.

The left area of Principal Component 1 contained the samples with higher levels of free, bound and total PC, free bound and total DPPH, bound and total ABTS, TAC, TF and *C**. The right area of Principal Component 1 contained the samples with higher values for the *a** parameter. The upper area of Principal Component 2 contained a concentration of samples with higher levels of cianidin-3-glucoside, free ABTS, *L**, *b** and *h*°.

The samples analysed at day 0, were located in the second and third quadrants (left side) and the samples analysed at 120 days were located in the first and fourth quadrants. PCA was an appropriate approach to verify the differences between the gamma radiation doses and storage time in the samples of black rice flour.

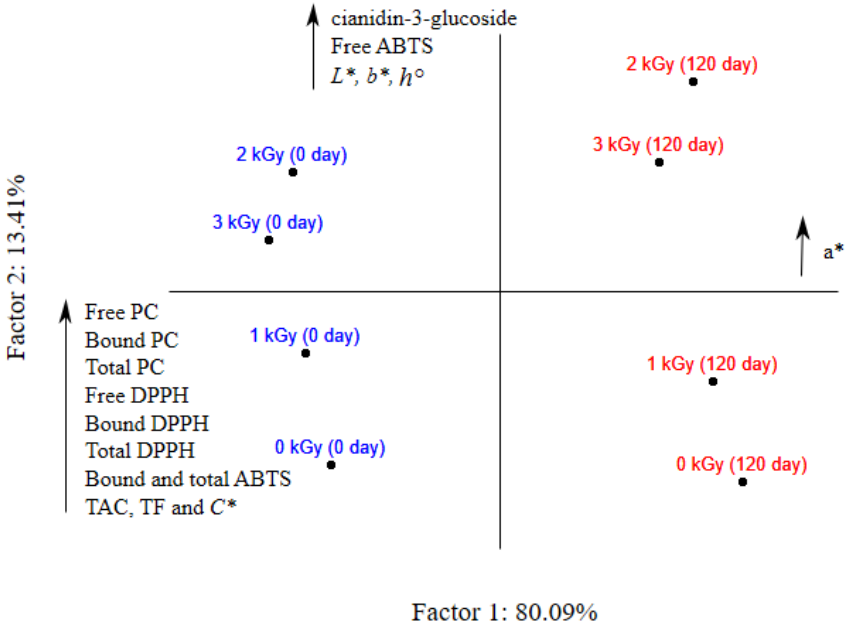

Figure 2. A scatter plot (Principal Component 1 × Principal Component 2) in relation to free, bound and total phenolic compounds, antioxidant activity and colour attributes in irradiated black rice flour during 120 days of storage.

The dendrogram in Figure 3 shows the association between the studied variables. The TAC showed a strong association and positive correlation with the free and total PC ($r = 0.96$; $p < 0.001$) and free and total antioxidant activity by DPPH ($r = 0.97$; $p < 0.001$). The TAC was

also positively correlated with cianidin-3-glucoside (r = 0.76; p < 0.05), TF (r = 0.96; p < 0.001), free and total ABTS (r = 0.94; p < 0.05) and the *C** parameter (r = 0.93; p < 0.05). The bound PC, bound ABTS and bound DPPH also showed a strong association and positive correlation (r = 0.99; p < 0.001), as well as the cianidin-3-glucoside and free ABTS (r = 0.96; p < 0.05). Several studies have also demonstrated that phenolic content has a highly positive correlation with antioxidant activities (Shen et al., 2009; Shao et al., 2013).

The *L** and *a** colour parameters showed a strong association and positive correlation (r = 0.83; p < 0.05); in relation to the *h*° attribute there was an association with *b** (r = 0.74; p < 0.05).

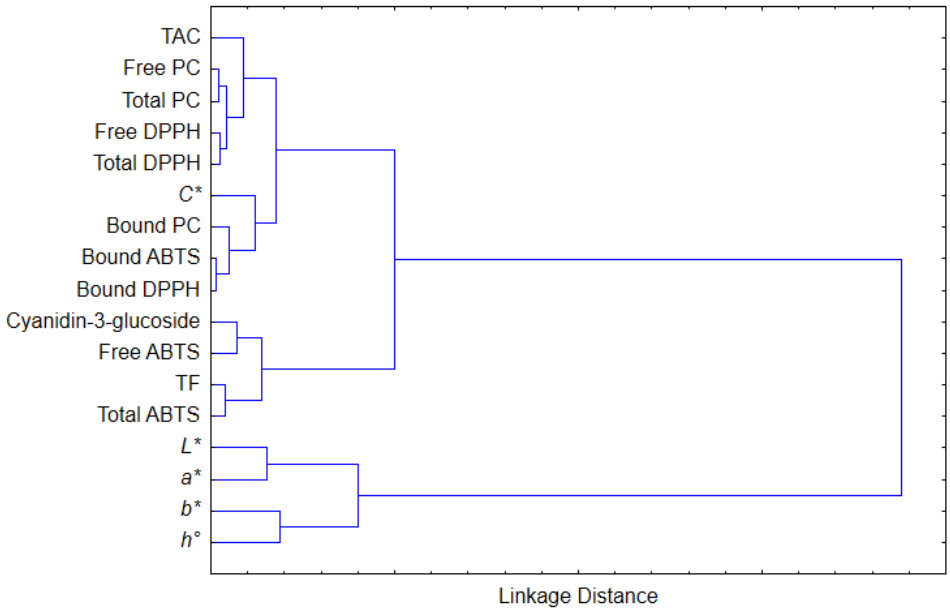

Figure 3. Dendrogram obtained from hierarchical cluster analysis for irradiated black rice flour during 120 days of storage applied to the variables in relation to free, bound and total phenolic compounds, antioxidant activity and colour attributes.

The similarity of the samples was evaluated by using hierarchical cluster analysis, where two clusters were suggested (Figure 4). Cluster 1 was characterised by samples analysed at 0 days and Cluster 2 was characterised by samples that were analysed at 120 days Table 2 shows that the means for each dependent variable were compared, and the ANOVA results for the clusters obtained by hierarchical cluster analysis were calculated.

Cluster 1 (0 days) had higher values for all the parameters, except (p > 0.05) for cianidin-3-glucoside, *b** and *h*°. Cluster 2 (120 days) showed lower values, which could have been due to the period of storage.

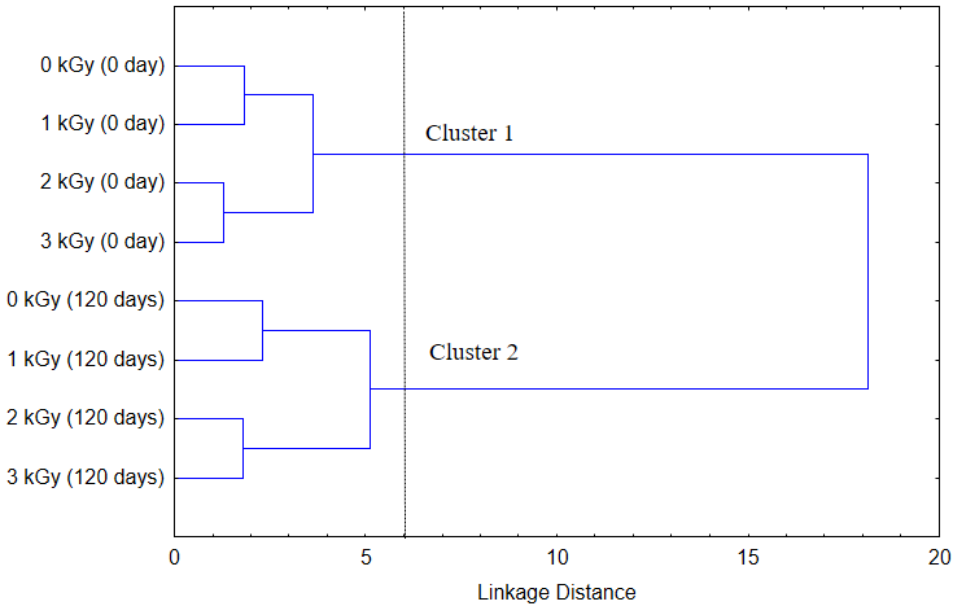

Figure 4. Dendrogram obtained from hierarchical cluster analysis for irradiated black rice flour during 120 days of storage applied to the samples in relation to free, bound and total phenolic compounds, antioxidant activity and colour attributes.

Table 2.Phenolic content – PC (mg GA g); antioxidant activity – ABTS and DPPH (µmol TE g), total anthocyanin content – TAC (mg C3G g^{-1}), cianidin-3-glucoside (mg g^{-1}), total flavonoids - TF (mg QE g^{-1}) and colour attributes of irradiated black rice flour using hierarchical cluster analysis (HCA).

Variables	Cluster 1 n= 4	Cluster 2 n=4	PSD	p-value*	p-value**
Free PC	5.66 [a]	3.50 [b]	1.53	0.52	<0.05*
Free ABTS	7.36 [a]	6.04 [b]	0.93	0.81	<0.05*
Free DPPH	6.46 [a]	5.06 [b]	0.99	0.05	<0.05*
Bound PC	1.68 [a]	1.01 [b]	0.47	0.30	<0.05*
Bound ABTS	2.43 [a]	1.61 [b]	0.58	<0.001	<0.05*
Bound DPPH	2.08 [a]	1.32 [b]	0.54	<0.05	<0.05*
TAC	1.81 [a]	1.58 [b]	0.16	0.67	<0.05*
Cianidin-3-glucoside	1.38	1.31	0.05	0.25	0.14
TF	0.63 [a]	0.53 [b]	0.07	0.93	<0.05*
L*	43.7 [a]	38.17 [b]	3.91	<0.05	<0.05*
a*	2.32 [a]	1.38 [b]	0.66	0.98	<0.05*
b*	1.79	1.78	0.01	<0.05	0.77
C*	3.5 [a]	2.93 [b]	0.40	<0.05	<0.05*
***h*°**	37.15	30.77	4.51	0.16	0.08

Note: Results expressed as mean ± pooled standard deviation. PSD: pooled standard deviation; *Probability values obtained by Levene's test for homogeneity of variances; **Probability values obtained by one-way ANOVA or Kruskal–Wallis test. Different letters in the same line represent statistically different results ($p < 0.05$).

3.4. Effects of gamma radiation on physicochemical and microbiological properties during storage

There was an increase in all the samples during 120 days of storage regarding moisture and water activity (A_w). The moisture content ranged from 10.54 (3 kGy - 0 days) to 11.60 g.100g-1 (0 kGy – 120 days) and A_w values ranged from 0.41 (2 and 3 kGy - 0 days) to 0.48 (0 kGy – 120 days). The control sample (0 kGy) had the highest increase, 9.02% for moisture and 14.94% for A_w. This increase can be associated with the hygroscopic of flours and their tendency to respond to changes in ambient relative humidity, as well as the transfer properties of water vapour from the packaging.

Similar results were found by Silva et al. (2010), who evaluated the oxidative stability in irradiated wheat flour and cornmeal, and who observed a significant increase in moisture in the samples from the first 30 days onwards. Marathe et al. (2002) found that moisture levels in irradiated and non-irradiated wheat flour increased at 120 days of storage.

Regarding the microbiological analyses, the results were below those required by legislation (ANVISA, 2001). In the control sample (0 kGy), the colony-forming units per gram CFU g^{-1} were $<10^2$ for *Bacillus cereus*; the most probable number per gram NMP g^{-1} was < 3.0 for thermotolerant coliforms; and there was an absence of *Salmonella* sp in 25 grams. There was no growth of microorganisms during the storage period of 120 days. In the irradiated samples (1, 2 and 3 kGy) there were non-detectable values for these bacteria. Feliciano et al., (2017) demonstrated the effectiveness of low-dose gamma irradiation for the microbial decontamination of brown rice for a prolonged period of storage in ambient conditions.

4. Conclusions

Our study concluded that the effects of gamma - radioisotope ^{60}Co on black rice flour resulted in an increase in phenolic compounds and antioxidant activity of the free and total phenolic fraction, as well as total anthocyanins content, cyanidin-3-glucoside and total flavonoids. The dose of 3 kGy showed the highest values; at 120 days there was a decrease in these levels for all samples. However, the irradiated samples were most stable at the end of storage. With regard to the colour attributes, the irradiation slightly modified all the parameters. For the microbiological analysis, there was no growth of microorganisms in the samples during the storage period of 120 days.

The results demonstrated that the chemometric approach proved to be effective in facilitating the visualisation of the positive effects of irradiation, which may improve the shelf life of black rice flour. This pigmented rice can be a valuable ingredient in gluten-free, cereal products with higher nutritional value, as well as helping to partially reduce negative impacts on the environment.

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PERFORMANCE EDIBLE COATING CONTAINING OLEORESIN FROM GINGER EMPRIT (ZINGIBER OFFIVINALE VAR. AMARUM) AND ITS EFFECT ON CONSUMER PREFERENCE PROPERTIES

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ABSTRACT

Ginger oleoresin (GO) as natural compounds becoming widely used to edible film, and coating for extending shelf life product. The objective of this study was to investigate the performance oleoresin and accept of consumer sensory properties from ginger emprit. Preliminary UHPLC-MS assay analysis showed that most components of oleoresin (24.7%) geranial and then gingerdione (10.2%). The methods were active concentration 1%, 1.5% and 2% (wt) GO applied onto edible coating toward beef meatballs. The resulting minimum inhibitory showed GO 1.5% was the optimum concentration against Gram-positive bacteria (S. aureus) 4.3 mm and Gram-negative bacteria (Salmonella) 0.5 mm. During the storage period, minimum meatballs quality was determined based on microbiological (Total Plate Count/TPC) and pH. Meatballs with edible containing GO 1.5% preserve the best quality with resulted in 1.83 log reduction of TPC and average pH value at 5.7. On sensory properties, the attribute was colour, taste, flavour intensity, juiciness, and hardness. Only 2% GO concentration has a negative effect on colour.

1. Introduction

In Indonesia, meatball or known as 'bakso' is popular street food. The consumption of meatball has increased by 18.5% over the last decades due to the relatively convenient purchase and availability (Kurniati et al, 2014; Purnomo & Rahardiyan, 2008). Currently, in Indonesia food adulteration is a major issue in the meatball household-firm and is causing concerns among consumers and local food authority (Rahmania et al, 2015). The presence of prohibited chemical material in food products to gain economic benefits are serious matters in view of consumer health and protection. One of the major authenticity issues of household-firm meatball product using the prohibited material (e.g formalin, borax) for shelf life extension (Ministry of Health, 2017).

Meatball is contained high protein which prompt transforms the meatball to be a susceptible product for the growing of pathogenic and spoilage microorganisms (Kerry et al., 2006). The edible coating is simple method have been developed for shelf life extension to inhibit the growth of undesirable microorganisms and reduce lipid oxidation in the meat-based product. Recently, a variety of

plant resource such as oleoresin for antimicrobial compound applied in edible coatings or films have been developing for application in fresh red meat, fish products and processing food (Horita et al., 2018; Granato, Nunes, & Barba, 2017; Nikmaram et al., 2017; Lorenzo, Batlle, & Gómez, 2014). The oleoresins characteristic is dark brown oil has an unstable mixture of essential oils and resin, non-volatile components that are hydrophobic, and lipophilic in the form of viscous liquids lifting specific scent plant or herbs obtained by extraction (Yasni, 2017).

There are numerous studies on isolation and activities of ginger oleoresins deals with chemistry reactions (Singh et al., 2008; Babu et al.,2018), and anti-bacterial (Auta et al., 2011; Park et al., 2008; Shahidi and Hossain, 2018); however, indigenous ginger from Indonesia emprit (*Zingiber Offivinale var. Amarum*) are not studied so vastly. The purpose of the present study was to investigate ginger emprit oleoresin as alternative antimicrobial agents, against Gram-negative bacteria (Salmonella) and Gram-positive bacteria (S. aureus) and evaluate the effects of using oleoresin when incorporated in beef meatball based coating toward consumer sensory properties. Our goal to find a simple adopting compound and method which can be done by household firms.

2. Materials and methods

2.1. Materials and Media culture

The mature and healthy of ginger emprit (*Zingiber officinale var. Amarum*) were bought from the local market in Malang City, Indonesia. Fresh beef meatball were purchased from household firms.

Glycerol, sulfuric acid (H_2SO_4), Boric acid, NaOH and Petroleum ether used were of analytical grade were purchased from Merck (Darmstadt, Germany). The inhibition zone tests using culture medium of Nutrient agar Merck (Darmstadt, Germany) and resazurin Sigma Aldrich (Missouri, USA) as a metabolic indicator. Clinical isolated pathogenic Salmonella and S.aureus were purchased from Medical Laboratory (University of Muhammadiyah Malang).

2.2. Preparation of oleoresin extraction

The simplicial form ginger was properly weighed to 200 gr put into a beaker glass then added 1L 96% ethanol. The beaker glass perfectly preserved from light and evaporation. The stirring inside the beaker glass was achieved magnetically at 200 rpm for 6 hours. After the extraction, samples were filtrated by a Buchner funnel and Whatman filter paper 42mn and the remaining solvent was removed by rotary evaporation at 50 ºC for 20 min (Fernández-Ronco et al, 2008; Singh et al., 2008). The obtained light yellow colored oil with a pleasant odor was dried over anhydrous sodium sulfate yield was then calculated as the grams and stored in sealed vials at (4 ± 2 ºC) in dark for further use and UPLC-MS analysis.

2.3. Analysis of chemical oleoresins presumptive composition

Ginger oleoresin components identification and presumptive amount present were determined by using UHPLC-MS (Angler Laboratory, Surabaya, Indonesia). Operated in negative ion mode in a capillary temperature of 100°C, gas atomizer with a flow rate of 25 L/hour, the source of voltage +2.9 kV in full scan mode (range 100 −700 m/z) at 30°C temperature, an Acquity UPLC BEH C18 (2.1 mm × 50 mm, 1.7 m; Waters, USA).

Tabel 1. Presumptive identification of the components in ginger oleoresin

t_R (min)	Molecular Formula	Compounds	Fragments (m/z)	Concentration (Peak %)
1.26	$C_6H_{14}NO_5$	Glucosamine	180.0866	0.3
3.51	$C_{10}H_{16}O$	Geranial	224.0716	24.7
4.43	$C_{10}H_{12}O_2$	*Eugenol*	237.1119	36.8
5.1	$C_{17}H_{26}O_4$	6-Gingerol	293.1757	8.9
5.7	$C_{17}H_{24}O_4$	Gingerdione	291.1599	10.2
6.32	$C_{17}H_{24}O_3$	6-Shogaol	275.1651	4.7
6.42	$C_{19}H_{30}O_5$	5-Acetoxy-6-gingerdiol	337.2008	Trace
7.40	$C_{23}H_{34}O_4$	Dehydro-12-gingerdione	373.2375	7.8
9.25	$C_{19}H_{32}ON_5$	2,2-Dimethoxyethyl	368.2431	Trace
Total				93.7%

A ternary gradient elution consisting of 0.1% (v/v) formic acid in water (system A) and 0.1% (v/v) formic acid in acetonitrile (system B), at a flow rate of 0.2mL. min-1 with injection volume of 5 µL. Mass data were processed by the MassLynx V4.1 software.

2.4. Disc diffusion

Both pathogenic strains were cultured in nutrient broth for activation at 37 ºC for 24 h. Afterwards, inoculation into nutrient agar discs which aseptically prepared with the spread plate method. Then, put over sterilized Whatman filter paper no.1 with 5 mm diameter has been dipped into 20 ml of coating solutions containing GO with concentration 1% (1µL/100 µL), 1.5% (1.5 µL/100 µL), and 2% (2 µL/100 µL). The plates were incubated in an upright position at 37 ºC, each plate is examined every next three days (3, 6, 9 and 12). The diameters of inhibition zones (in mm) were measured (Seol et al., 2009; Noori et al. 2018).

2.5. Preparation of coating solutions

Crude ginger weighed 5g dissolved in 100ml distilled water and stirred at a controlled temperature of 80ºC and 1100 rpm for 45 min. 1.2 g (30% wt of crude) glycerol was added as a plasticizer. Then, oleoresin was added with constant stirring to reach a final concentration of oleoresin 1 to 2% wt of crude ginger. After, 10 min stirring the solutions were applied for meatball dipped in a coating solution for 2 minutes and 1h for dried.

2.6. Meatball quality testing

2.6.1. Total Plate Colony (TPC)

Samples 20 g were prepared aseptically and put into sterile plastic 180 ml containing a sterile water mashed up by stomacher® 400C (10^{-1}) for 2 min. 1 mL of solution 10^{-1} dilution was taken by used micropipette and 2 min homogenized in 9 ml sterile water (10^{-2}) using vortex mixer, this step is repeated until reached (10^{-6}) (Wasteson and Hornes, 2009). Serial dilutions 10^{-5} and 10^{-6} were taken 1 mL using the pour plate method on nutrient agar Merck (Darmstadt, Germany). All plates were incubated at 37°C for 48 h, colonies data were transformed into logarithms of the number of colony forming units (Log CFU/g).

2.6.2. pH analysis

Meatballs were measured using digital pH meter (SI analytics LAB 875) instrument after samples (20 g) have been homogenized in distilled water (10 ml).

2.6.3. Sensory evaluation

Semi-trained 20 member panellists (13 females and 7 males) were selected from undergraduate students the department of food science and technology of the University of Muhammadiyah Malang evaluated the total acceptance of samples. Panelists were provided

with an assessment form, plastic bowl, napkin, toothpick, a cup of water, and palate cleansers (plain crackers) to use between samples. All panelists had a background in beef meatball evaluation and were selected based on their sensitivity and limit detection of flavor intensity and taste concentrations of oleoresin. The attributes considered in the sensory evaluation were colour, taste, flavor intensity, juiciness, hardness and overall acceptability using 5 points descriptive scale, where 5 = extremely desirable, 1 = extremely undesirable and score of 3 was taken as the lower limit of acceptability.

2.7. Statistical Analysis

All experiments were performed in twice with analysis of variance (ANOVA) (SPSS Inc, Version 20) performed with a completely randomized design. Duncan's test ($p < 0.05$) was used to detect differences among mean values of meatballs properties in all test intervals.

3.Results and Discussions

3.1. Identification of the components in ginger oleoresin

The Careful identification of GO emprit was carried out HPLC assay result which interpretation contains a specific large number of compounds in Table 1. From Table 1 it is evident that in GO existing four major components of 9 components constituting 93.7% of the total weight. Most of the findings have similarity with other variety of ginger (*var. rubrum and var. officinale*) which has been reported in previous study (Agrawal et al. 2001, Kamaliroosta et al. 2012 and Gurdip Singh et al 2008). The differences only on the amount, Singh et al. (2008) identified Eugenol (49.8) and geranial (25.9%) in our study indicated amount *Eugenol* (36.8%), geranial (24.7%) and glucosamine trace (0.3%) was rarely found in rhizome plants. While specific emperit comparison with Arijanti et al. (2019) identified 6-Gingerol have the main components of GO on Gingerol 1.8%, Shogaol 0.10% and Zingerone 0.86%. There are many factors could be drive differences in the chemical composition of GO such as genetic, production conditions, environmental, weather conditions, distillation conditions and other factors (Rehman et al., 2016; Blair et al., 2001).

3.2. Antimicrobial activity testing

The performance results from antimicrobial activity testing of GO against S. aureus (Gram-positive) and Salmonella (Gram-negative) has different effective concentration is shown in Fig 1. GO concentration starting at 1% (1µL/100µL), 1.5% (1.5 µL/100µL), and 2% (2µL/100µL) was found to be more susceptible to against Salmonella gram-negative.

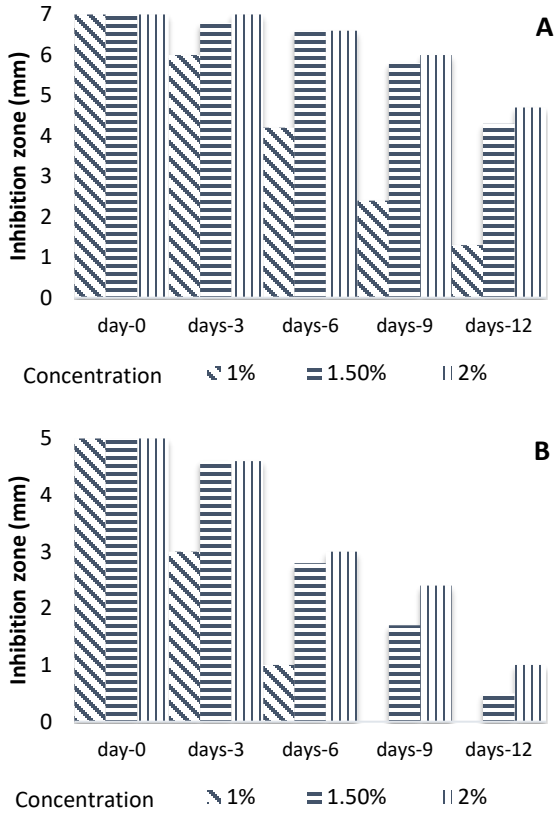

Figure 1. Anti-bacterial activity of meatball edible coatings containing ginger oleoresin against S. aureus (A) and Salmonella (B). Data shown are the means ± standard deviation ($P < 0.05$).

The difference GO was comparatively more effective against S. aureus gram-positive. This discrepancy of the higher resistance of gram-

negative due to the cell wall structural have an outer membrane containing a thin peptidoglycan layer and lipopolysaccharide. It acts permeability barrier lead to reducing bioactive compounds activity by absorption of reactive oxygen species (ROS) thereby affecting the performance of GO (Russell, 2003). The intense inhibiting activity of GO against gram-positive could be due to interactions between principal component properties of phenolic compounds (Eugenol, geranial, gingerdione, 6-Gingerol and 6-shogaol) with the positive charged cell wall (Calo et al., 2015).

In the recently, GO study has been used to inhibit the activity of pathogens which the performance ability dependent on the concentration used to. Our finding, first treatment GO 1% (1µL/100µL) for 72 h storage (3 days) inhibiting activity by more than 90% for S. aureus and 65% for Salmonella. Although the GO (1µL/100µL) be able to inhibit S. aureus for 288 h storage (12 days) but did not with Salmonella which is rotted in early 150 h storage for (6 days 4 hours). In contrast, increasing the GO concentrations to 1.5% (1.5 µL/100µL), and 2% (2µL/100µL) reveal significant performance could inhibit up to 288 h (12 days) these two bacteria with a clear zone for S. aureus (4.3 and 4.7 mm) and Salmonella (0.7 and 1.1 mm).

Interestingly, even though sing et al (2008) study reported that GO is less effective against S. aureus in disc diffusion method. Furthermore, Mesomo et al (2013) result demonstrated that GO has slight inhibition activity against salmonella. However, from our data indicated GO from Emprit variant has high-performance and effect is comparable to chloramphenicol against these bacteria. This suggests that different variant ginger have different strength and characteristics of phenolic compounds which might responsible for inhibition performance.

3.3. Beef meatballs quality testing

3.3.1. Total plate count (TPC)

Total plate count (TPC) indicating the level of microorganism in it during storage study from 0 to 12 days. The control sample has increased significantly ($p<0.05$) 6.12 Log CFU/g at 6 days, because it was completely putrefied was not recorded up to end of a storage.

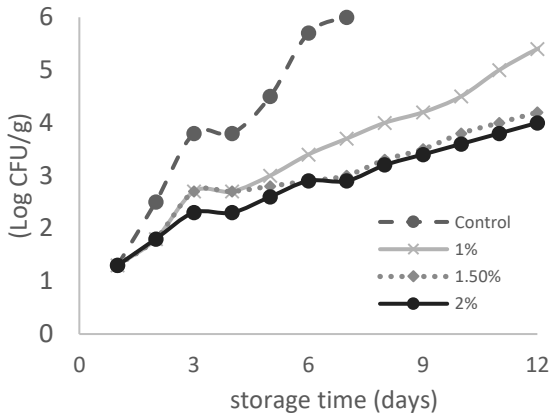

Figure 2. Effect of the edible coatings containing ginger oleoresin on the microbial growth (Log CFU/g) of total plate count (TPC) values in meatballs during storage at $4^0C\pm 1^0C$. Data shown are the means ± standard deviation ($p < 0.05$).

Addition of 1% (1µL/100 µL) coated oleoresin into meatballs significantly decreased value the TPC ±1.2 log CFU/g up to 9 days. Higher addition of oleoresin into meatballs 1.5% (1.5µL/100 µL) to 2% (2µL/100 µL) was significantly control the microbial growth of meatballs under 4.4 log CFU/g at the end of storage time (12 days). Within this result, the meatball can be consumed according Indonesia National Standards which is TPC does not exceed log $1x10^5$ CFU/g (SNI.3818:2014). Similar case on GO Noori et al (2018) result demonstrated the concentration treatment have significant effects inhibit the population of moulds and yeast on chicken breast fillets during storage.

More specific Widayat et al (2017) using liquid smoke containing Eugenol of GO could inhibit inactivates intracellular enzymes for forming process that causes the lysis on the cell wall of microbes.

Table 2. Sensory properties of meatballs edible coating containing ginger oleoresin (*n*=20 panelists)

Storage time (Day)	(%)	Colour	Taste	Flavour Intensity	Juiciness	Hardness	Overall
0	1	6.4±0.04	6.0±0.63	6.4±1.24	6.2±2.07	6.4±0.17	6.0±0.77
	1.5	6.0±0.23	5.9±0.08	6.3±0.32	6.0±1.13	6.1±0.63	6.0±0.33
	2	6.3±0.48	5.9±0.15	6.2±0.67	6.0±0.01	6.0±0.01	6.0±0.61
3	1	5.1±0.44	5.9±0.27	6.0±0.44	6.2±1.20	6.0±0.31	6.0±0.11
	1.5	5.7±0.05	5.5±0.85	6.0±0.25	6.0±0.12	6.1±0.12	6.0±0.22
	2	6.0±0.48	5.7±0.16	6.0±0.05	6.0±0.31	6.0±0.01	6.0±0.90
6	1	4.7±0.57	4.0±0.21	3.7±0.30	6.0±0.53	5.9±0.05	5.8±0.05
	1.5	5.5±0.35	5.5±0.17	5.7±0.05	5.9±0.17	6.1±0.61	6.0±0.12
	2	5.3±0.05	5.1±0.25	4.8±0.23	5.9±0.90	6.0±0.45	6.0±0.91
9	1						
	1.5	4.6±0.23	3.7±0.63	3.9±0.50	5.7±0.54	4.4±0.40	5.2±0.84
	2	4.0±0.12	3.4±0.05	3.5±0.46	5.5±0.12	4.5±0.81	5.1±0.13
12	1						
	1.5	3.4±0.52	3.6±0.22	3.6±0.51	4.9±0.13	3.7±0.82	4.3±0.10
	2	3.0±0.02	2.0±0.47	1.3±0.86	4.8±0.22	3.6±0.21	4.0±0.92
Control		6.6±0.01	6.2±0.20	6.3±0.08	6.2±0.25	6.7±0.25	6.3±0.19

3.3.2. pH Analysis

The control samples had higher pH value ranged from 5.8 to 6.34, while oleoresin treatment samples had the pH value ranged from 5.8 to 6.0 which is differed significantly ($p < .05$). In line with these result, Sallam et al. (2004) study also findings storage time factor had a significant ($p < .05$) influence on increasing of pH values. An increase may be attributed to protein degradation of metabolites by bacterial action in a beef meatball (Karabagias et al. 2011) in this situation spoilage often produce highly malodorous volatile substances (Alfreider et al., 2002; Xiao et al. 2013).

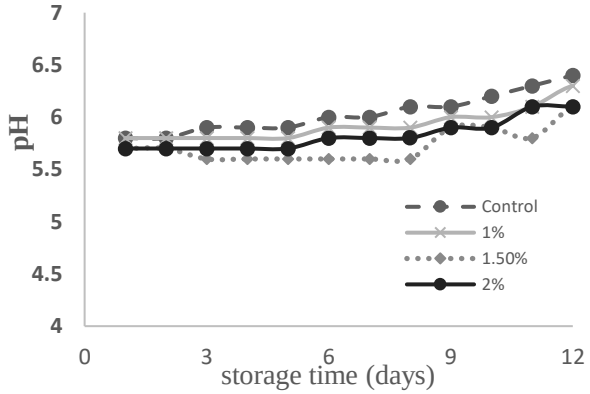

Figure 3. Effect of the edible coating containing ginger oleoresin on pH values in meatballs during storage at $4^0C\pm 1^0C$.

3.3.3. Sensory analysis

Sensory analysis beef meatballs score showed that increased percentage additional oleoresin concentration and refrigerator storage time give effect toward less acceptable in panelist sensory qualities. The sensory preferences on color, taste, flavor intensity juiciness, and hardness levels significantly decreased ($p<0.05$) gradually up to end of the storage (12 days).

Except, oleoresin concentration 1% was not recorded at a day of 9 and 12 because it was completely rotted. In the current study, the colors difference of beef meatballs caused by ginger oleoresin negatively affected the sensory color and flavor intensity evaluation results samples with 2% had higher scores. However, it was evaluated this decrement of preference in color and flavor intensity did not have a negative effect and no significant
differences ($p >.05$) were seen in taste, hardness and overall acceptance of the samples. These

results are in conformity with a study conducted by Turgut et al., (2017) that the addition of pomegranate peel extract in beef meatballs during frozen storage was not a significant difference in terms of taste. Moreover, the taste of the beef with added kaffir lime leaves oleoresin did not induce a significant taste and juiciness of kaffir lime (Utami et al., 2014)

4. Conclusions

These results demonstrated and verified that oleoresins of ginger *emprit* had a potential antibacterial into beef meatball could help to improve shelf life without ignoring consumer preference. The negative impact come from color property which is not affected in taste, flavor intensity and overall acceptance scores. From the inhibit the growth of bacteria oleoresins as natural preservatives have a simple application and low-cost.

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THE EFFECT OF THE DRYING AND EXTRACTION METHODS ON THE PECTIN YIELD AND THE OPTIMIZATION OF MICROWAVE-ASSISTED PECTIN EXTRACTION FROM KAFFIR LIME (*CITRUS HYSTRIX*) POMACE

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Box-Behnken design;
Extraction methods;
Kaffir lime pomace;
Microwave-assisted;
Pectin yield.

ABSTRACT

This research aimed to determine the effect of the drying methods (hot air oven and microwave oven) and extraction methods (water bath and microwave oven) on the pectin yield from kaffir lime (*Citrus hystrix*) pomace. The optimal conditions for pectin extraction were studied, and the equation for predicting the pectin yield was determined. The drying method did not significantly affect the pectin yield, but the extraction method did significantly affect the yield. The pectin yield from kaffir lime pomace extracted with a microwave oven (34.07%) was 1.5 times higher than that extracted with a hot air oven (22.32%). For the determination of the optimal conditions for the microwave-assisted pectin extraction from kaffir lime pomace, a Box-Behnken design was used with 3 factors at 3 levels, including the solid to liquid ratio (1 to 12, 1 to 30, and 1 to 48 g/mL), the pH (1, 1.5, and 2), and the microwave irradiation time (10, 20, and 30 min). The optimal conditions were 1 to 23 solid to liquid ratio, pH 1.6, and an 18-min irradiation time with the microwave power at 450 W, which resulted in a yield of 29.21%. The equation for the prediction of the pectin yield was obtained from fitted experimental data (R^2 = 0.93). The chemical properties of pectin extracted from the optimal conditions included the moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid content and esterification level, which were 9.57%, 2.85%, 526.87 g, 10.46%, 92.79% and 64.00%, respectively.

1. Introduction

Pectin is a heteropolysaccharide polymeric compound that confers structure on the primary plant cell wall. The primary structure results from a polymer of galacturonic acids linked by α-1,4-glycosidic bonds. Pectin is used as an ingredient in many foods. It has been used as a gelling agent in jams and jellies, a stabilizing agent in dairy products and yogurt, and a thickener in sauces, seasonings, heavy syrups, dressings, drinks, etc. (Thakur *et al*., 1997; Sila *et al*., 2009). In addition to its direct use as a food ingredient, pectin is also used as an edible fiber in the form of dietary supplements for health effects such as reducing cholesterol and blood sugar levels (Voragen *et al*., 1995; Koseki, *et al*., 1986).

In studies by Wang *et al*., 2007; Li *et al*., 2012; Maran *et al*., 2013; Maran *et al*., 2014; Thirugnanasambandham *et al*., 2014; Maran *et al*., 2015; and Maran and Prakash, 2015,

pectin was extracted from apple pomace, sugar beet pulp, orange peel, *Citrullus lanatus* fruit rind waste, dragon fruit peel, mango peel waste, and *Carcia papaya* L. peel waste by drying the raw material in a hot air oven and extracting the pectin in a microwave oven. The effects of the type of acid (i.e., hydrochloric or sulfuric acid), the pH (1-4), the solid to liquid ratio, (1 to 5 – 1 to 30 by weight to volume), the microwave power (150-640 W), and pectin extraction time in a microwave oven (1-17.4 min) on the yield were studied. The highest pectin yield of 25-29% was obtained from mango peel waste, *Citrullus lanatus* waste, fruit rind waste, and *Carcia papaya* L. peel waste. The optimum conditions for pectin extraction were between 400-477 W power in a microwave oven, a 2-20 min of extraction time, a pH adjusted with hydrochloric acid to between 1.8-2.7, and 1 g:15 mL to 1 g: 24 mL solid to liquid ratio (Maran *et al*., 2014; Maran *et al*., 2015; Maran and Prakash, 2015). Pectin was extracted from orange peel and apple pomace at a yield of 19% and 16%, respectively. The optimum extraction conditions were a microwave power of 400-500 W, a 2-3 min extraction time, a pH adjusted with hydrochloric acid to 1.5-2.7, and a 1 g:15 mL to 1 g: 20 mL solid to liquid ratio (Wang *et al*., 2007; Maran *et al*., 2013). Pectin extracted from the dragon fruit peel had the lowest yield of 7%.

No research has studied the effect of drying samples in a hot air oven or a microwave oven and the effect of pectin extraction in a water bath or a microwave oven on the pectin yield from kaffir lime pomace, nor has any research studied the optimum conditions for pectin extraction from kaffir lime pomace in terms of yield. The anticipated benefits from such research are a lower cost of kaffir lime pomace disposal, which would add value to that agricultural waste and methods that would enhance the efficiency of pectin extraction in terms of yield. Therefore, this study aimed to determine the effect of drying kaffir lime pomace in either a hot air oven or a microwave oven and investigate the effects of a pectin extraction method by a water bath or a microwave oven on the pectin yield. The drying and extraction methods for pectin from kaffir lime pomace that produced the highest yield were chosen to conduct the optimization of pectin extraction. In addition, we also determine of the optimum conditions and the equation to predict the pectin yield for pectin extraction from kaffir lime pomace, and the physical and chemical quality of the extracted pectin were compared with commercial pectin.

2. Materials and methods

2.1. Raw materials

Kaffir lime (*Citrus hystrix*) was purchased from the Nonthaburi Market, Mueang, Nonthaburi, Thailand, weighed 37±3 g, was approximately 6 months old, and had a dark green peel.

2.2. Kaffir lime pomace preparation

The kaffir limes were cut in half, and the skin was peeled to get rid of the essential oils. They were then squeezed to remove the juice, and the seeds were also removed. The kaffir lime pomace was boiled with 95% ethanol at a ratio of 1:1 (w/v) in a water bath (WNB 22, Memmert, Germany) with 15 L of water at 80 °C for 30 min to eliminate dirt and filtered through a mesh to separate the liquid. The kaffir lime pomace was then squeezed with a squeezing machine to remove the liquid until only the kaffir lime pomace remained.

2.3. Effect of the drying and extraction method on the pectin yield from kaffir lime pomace

The kaffir lime pomace was dried in a hot air oven or a microwave oven as detailed in section 2.3.1 and 2.3.2, respectively; then, it was ground with a grinding machine (SK

100, Retsch, Germany). The dried kaffir lime pomace powder was kept in a zippered polypropylene bag and stored in a desiccator at room temperature until the pectin was extracted. The moisture content of the kaffir lime pomace powder was determined according to an AOAC method (2000). The pectin was extracted in a hot air oven or a microwave oven as detailed in section 2.3.3 and 2.3.4, respectively. The suspension was filtered through a white cloth to separate the liquid from the sludge. The liquid was cooled in a beaker to 40 °C by immersion in ice water, then set aside. The sludge was extracted twice with distilled water. The pH was adjusted with hydrochloric acid to the pH in the first extraction and filtered again. The liquid from both extractions were combined and precipitated with 95% ethanol at a ratio of 1:1.5 (v/v). The mixture was quickly mixed to accumulate the precipitate, then set aside at room temperature for 1 h, after which it was filtered with a white cloth that was folded twice and placed in a colander. The pectin precipitate was washed with 95% ethanol three times and dried in a hot air oven (UF 110, Memmert, Germany) at a temperature of 55 °C for 5 h until the pectin had moisture content below 12% as determined by McCready's method (1954). The pectin was then weighed to determine the yield, after which it was ground in a grinding machine (ZM 100, Retsch, Germany).

2.3.1. Drying kaffir lime pomace in a hot air oven

The kaffir lime pomace was dried by spreading it evenly in a 18×27 cm^2 rectangular tray at 1 kg per tray, then dried in a hot air oven (UF 110, Memmert, Germany) at 55 °C for 18 h to a moisture content of approximately 12% (dry basis). The experiment was repeated three times.

2.3.2. Drying kaffir lime pomace in a microwave oven

Kaffir lime pomace was dried by spreading it evenly in a 18×27 cm^2 rectangular tray at 1 kg per tray, then dried in a microwave oven (MW 7803, SEVERIN, Germany) at 900 W for 20 min to a moisture content of approximately 12% (dry basis). The experiment was repeated three times.

2.3.3. Pectin extraction from dried kaffir lime pomace in a water bath

This pectin extraction method was modified from a study by Shaha *et al*. (2013). Kaffir lime pomace powder (10 g) dried in a hot air oven and a microwave oven as in section 2.3.1 and 2.3.2 was placed in a 1-L beaker and 400-mL of distilled water was added (1:40 weight by volume) The pH was adjusted with hydrochloric acid to pH 1.5. The mixture was then extracted in a water bath (WNB 22, Memmert, Germany) containing 15 L of water at 90 °C for one hour.

2.3.4. Pectin extraction from dried kaffir lime pomace in a microwave oven

The pectin extraction method was modified from a study by Maran *et al.* (2014) and Shaha *et al.* (2013). Dried kaffir lime pomace powder (10 g) obtained as in sections 2.3.1 and 2.3.2 was placed in a 1-L beaker and 400-mL of distilled water was added (1:40 weight by volume). The pH was adjusted with hydrochloric acid to 1.5. The mixture was extracted in a microwave oven (MW 7803, SEVERIN, Germany) by placing the beaker in the center of a rotating tray at 450 W for 20 min.

2.3.5 Statistical analysis

The effects of drying and extracting kaffir lime method was studied in an experiment that employed a 2x2 Factorial Design with three replicates per treatment. An analysis of variance (ANOVA) was performed using the Minitab version 16 software for a two-way ANOVA with two drying methods (hot air and microwave oven) and two extraction methods (water bath and microwave oven). The difference between each treatment mean was compared using Tukey's test at a 95% confidence level.

2.4. Determination of the optimum conditions and the equation to predict the pectin yield for pectin extraction from kaffir lime pomace

We found that kaffir lime pomace dried in a hot air oven (55 °C, 18 h) had a more uniform moisture content than that dried in a microwave oven (900 W, 20 min). However, the pectin yield from kaffir lime pomace extracted with a microwave oven (34.07%) was 1.5 times higher than that extracted with a hot air oven (22.32%). Therefore, we chose drying in a hot air oven and extraction in a microwave oven to conduct experiments to determine the optimum conditions for pectin extraction in terms of yield.

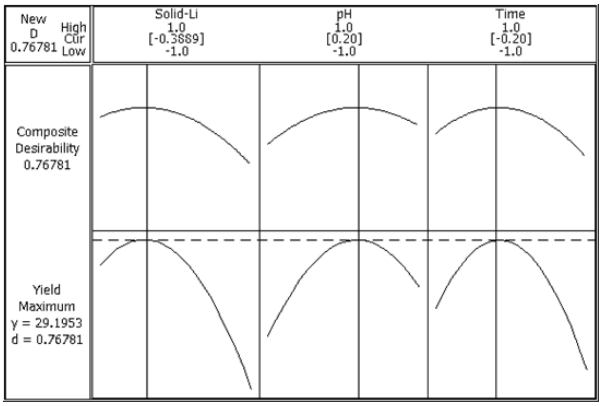

Figure 1. Determination of the optimum conditions for microwave-assisted pectin extraction from pomace using coded values

The extraction procedure was modified from studies by Maran *et al*. (2014) and Shaha *et al*. (2013). Dried kaffir lime pomace powder (10 g) was placed in a 1,000-mL beaker, and distilled water was added according to the ratios in Table 1.

The pH was adjusted with hydrochloric acid to the pH values shown in Table 1. The mixture was then extracted in a microwave oven (MW 7803, SEVERIN, Germany) by placing the beaker in the center of the rotating tray. After the specified time, the suspension was filtered through a white cloth to separate the liquid from the sludge. The liquid was cooled in beaker to 40 °C by soaking in ice water, then set aside. The separated sludge was extracted twice with distilled water. The pH was adjusted with hydrochloric acid to the pH in the first extraction and the extract was filtered again. The liquid from both extractions was combined and precipitated with 95% ethanol at a ratio of 1:1.5 (v/v). The mixture was quickly mixed to accumulate the precipitate, set aside at room temperature for 1 h, and filtered with a white cloth folded twice and placed in a colander. The pectin precipitates were washed with 95% ethanol three times, and dried in a hot air oven (UF 110, Memmert, Germany) at a temperature of 55 °C for 5 h to moisture content below 12%. The pectin was weighed to determine the yield, then ground with a grinding machine (ZM 100, Retsch, Germany). The physical and chemical properties of the pectin were then determined.

Table 1. Experimental plan for the Box-Behnken Design showing the ratio of kaffir lime pomace powder to distilled water, pH, and the microwave irradiation time for pectin extraction

Exp. No.	*Factors*			*Yield (% wet basis)*
	Ratio of kaffir lime pomace powder to distilled water (g/mL)	*pH*	*Extraction time (min)*	
1	1:48	2	20	20.31
2	1:48	1.5	10	14.36
3	1:48	1.5	30	26.16
4	1:30	1.5	20	17.06
5	1:30	2	10	26.50
6	1:30	1	30	12.62
7	1:30	1.5	20	12.38
8	1:12	1	20	19.59
9	1:48	1	20	17.69
10	1:12	2	20	15.90
11	1:30	1.5	20	16.16
12	1:30	1	10	22.39

13	1:12	1.5	10	30.46
14	1:30	2	30	26.68
15	1:12	1.5	30	28.33

2.4.1. Determination of pectin properties

The physical and chemical properties of the pectin from this study and a commercial food grade pectin (Apple Pectin AP104 HP, China) were determined.

a. The color was measured with a Hunter Laboratory Colorimeter (Color Quest 45/0 Reston, Virginia) by adding 3 g of pectin powder to a clear plastic container for color measurement, placing it at the measurement spot, closing the lid, and measuring the color at 4 spots 3 times per sample.

b. The moisture content of the kaffir lime pomace powder was determined according to an AOAC method (2000).

c. The moisture content of the pectin was determined according to McCready's method (1954).

d. The amount of ash was determined according to McCready's method (1954).

e. The equivalent weight was determined according to Ranganna's method (1995).

f. The amount of methoxyl was determined according to Ranganna's method (1995).

g. The amount of anhydruronic acid was calculated according to Mohamed and Hasan's method (1995).

h. The degree of esterification was calculated according to the method of Owens *et al.* (1952).

2.4.2. Statistical analysis

The Response surface methodology (RSM) using Box-Behnken Design was computed with the Minitab version 16 software for the three factors x_1, x_2, and x_3 respectively including the ratio of kaffir lime pomace powder to distilled water, the pH, and the pectin extraction time (min) at a constant of microwave power (450 W). A total of 15 experiments of pectin extraction from kaffir lime pomace using microwave irradiation were done. The experiments at the center point were conducted 3 times and had the experimental order as shown in Table 1. The values used in the experiments had three levels including -1, 0, and 1. Both the actual and coded values for the ratio of kaffir lime pomace powder to distilled water, the pH, and the extraction time that are shown in Table 2.

Table 2. Ratio of kaffir lime pomace powder to distilled water, pH, and pectin extraction time from kaffir lime pomace using microwave irradiation that are actual and coded values

Coded value	**Actual value**		
	Ratio of kaffir lime pomace powder to distilled water (g/mL)	**pH**	**Extraction time (min)**
-1	1 : 12	1	10
0	1 : 30	1.5	20
1	1 : 48	2	30

3. Results and discussions

3.1. Drying and extraction methods to yield

Two drying methods for the pomace in were studied: a hot air oven at 55 °C for 18 h and a microwave at 900 W for 20 min. The results showed that the drying method significantly ($p<0.05$) affected the final moisture content of the pomace. The moisture content of the pomace dried in a microwave oven had an mean of 10.61% (wet basis) or 11.87% (dry basis), which was significantly higher ($p<0.05$) than that dried in a hot air oven with a mean moisture content of 9.26% (wet basis) or 10.20% (dry basis). The pomace had a more even moisture content in the hot air oven than in the microwave oven because drying in hot air allowed the heat to diffuse into the pomace more slowly and evenly than drying in a

microwave oven, while in the microwave oven, the pomace absorbed the microwave energy and turned it into heat, which spread throughout the pomace from a high temperature areas to low temperature areas. The pomace at the corners or edges was exposed to more intense microwave irradiation than that in other areas because the microwave radiation accumulated in that area, causing the pomace to become dryer than in other areas (Buffler, 1993). Therefore, pomace dried in a microwave oven had an uneven moisture content.

Table 3. The pectin yield from kaffir lime pomace dried in a hot air oven or a microwave oven and extracted in a water bath or a microwave oven

Drying method[NS]	**Extraction method**	
	Microwave oven (450 W, 20 min)	**Water bath (90 °C, 1 hr)**
	% dry basis	**% dry basis**
Hot air oven (55 °C, 18 hr)	33.28 ± 1.85	21.31 ± 1.39
Microwave oven (900 W, 20 min)	34.86 ± 2.62	23.33 ± 1.26
$\bar{x}$ ± S.D.	34.07 ± 2.20 [A]	22.32 ± 1.62 [B]
Significant interaction	**Significance**	
Drying method	NS	
Extraction method	*	
Drying method × Extraction method	NS	

Values are mean ± S.D. (three replicates)

NS and * indicate not significant and significant at p = 0.05, respectively.

The values in the same row followed by different superscript (A-B) were significantly different ($p < 0.05$).

From Table 3, The interaction of the drying and extraction methods and the main factor was the drying method but it did not significantly affect the yield ($p \geq 0.05$), while the key factor was the extraction method, which did significantly affect the yield ($p<0.05$). Extraction in a microwave oven yielded 1.50-fold more pectin than extraction in a water bath because the microwave radiation was converted to heat by the vibration of the charged particles or the rotation of polar molecules, causing them to collide with nearby molecules after the object was exposed to a microwave radiation and absorbed the energy. As a result, heat was generated quickly. Therefore, the heat was distributed evenly, which caused a higher yield. Heating in a water bath was different; the heat was transferred from high temperature areas to the low temperature areas from the hot water in the bath into the samples that adhered to the wall of the beaker. The heat was subsequently transferred to the samples in the center of the beaker by thermal conduction and convection. A comparison of the yield of pectin extracted from pomace dried in a microwave oven and a water bath revealed that the yield was 23.33% (dry basis), which was less than that obtained by Shaha *et al*. (2013) who extracted pectin from kaffir lime peel and obtained a 37.00% yield. Maran *et al*. (2013) extracted pectin from orange peel and obtained a 19.24% yield. The yields differences were due to the different raw materials, extraction methods, and extraction conditions.

The pectin yield from pomace dried in a microwave oven (900 W, 20 min) and extracted in a microwave oven (450 W, 20 min) was the highest at 34.86% (dry basis), but it was not significantly different from pomace dried in a hot air oven (55 °C, 18 h)

and extracted in a microwave oven (450 W, 20 min) which had a yield of 33.28% (dry basis) (see Table 3).

3.2. Optimum conditions for the highest yield from kaffir lime pomace extracted in a microwave oven and the equation for predicting the yield

Table 4. Analysis of variance of regression model in terms of four independent variables (Actual value) for yield of pectin

	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	460.064	460.064	51.118	7.02	0.022*
Linear	3	80.663	80.663	26.888	3.69	0.097
Square	3	249.721	249.721	83.24	11.43	0.011*
Interaction	3	129.68	129.68	43.227	5.94	0.042*
Residual Error	5	36.405	36.405	7.281		
Lack-of-Fit	3	29.236	29.236	9.745	2.72	0.280
Pure Error	2	7.169	7.169	3.584		
Total	14	496.469				

Note: * Significant at $p<0.05$.

DF = The degrees of freedom of an estimate of a parameter

Seq SS = The sequential sum of squares for each term in the model

Adj SS = The adjusted sum of squares for a term in the model

Adj MS= The adjusted mean square = Adj SS/DF

Table 4 shows that the linear and quadratic effect of the three independent variables (the ratio of the pomace powder to distilled water, pH, and pectin extraction time) played a significant role in the yield of pectin ($p<0.05$), and the lack of fit was equal to 0.280, ($p\geq0.05$), indicating no significant difference, which meant that the equation did not have a significant lack of fit or that the equation fit the experimental results well. Therefore, the equation (1) was suitable to predict the yield from the microwave-assisted extraction of kaffir lime pomace.

Equation for Predicting Yield:

$$Y = 28.4887 - (2.7147x_1) + (1.6238x_2) - (0.276x_3) - (4.6388x_1^2) - (4.3759x_2^2) - (6.0787x_3^2) - (0.7844x_1x_2) + (5.2704x_1x_3) + (2.0068x_2x_3) \quad (1)$$

$R^2 = 92.67$ %

where

Y is the yield (% wet basis)

x_1 is the ratio of kaffir lime pomace powder to distilled water in coded units (value -1 to 1)

x_2 is pH in coded units (value -1 to 1)

x_3 is extraction time in coded units (value -1 to 1)

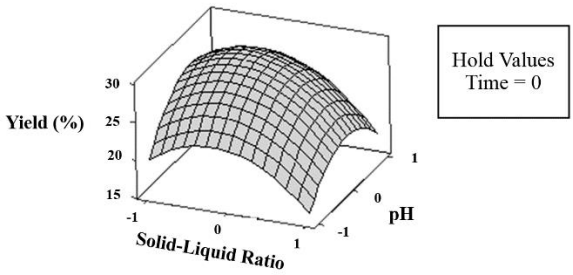

Figure 2. Three-dimensional graph of the yield from a microwave-assisted pectin extraction from pomace (Y) at various pomace to water ratios (x_1) (g/mL) and pH (x_2) by using microwave power at 450 W for 20 min ($x_3 = 0$)

Figure 2, shows the major factors that influenced the yield were the pomace to water ratio and the pH. When the pomace to water ratio was less than 1 to 16.5 (code = -0.75) and higher than 1 to 33.6 g/mL (code = -0.2) and the pH was below 1.35 (code = -0.3) and above 1.88 (code = 0.75) the yield was lower. If the pomace to water ratio (x_1) is lower, more solution is necessary to extract the pectin and thus more pectin is produced. A larger concentration gradient resulting from the higher solvent to solid ratio during the diffusion of the internal material into the solution would accelerate the mass transfer, thereby increasing the extraction efficiency. Too much or a too dilute solution would lower the yield. If too little solution is used for extraction, it will not be enough to extract

the pectin from the cells (Guo *et al*., 2001). The pH (x_2) affected the pectin extraction by facilitating the extraction of the pectin from the cells, thus increasing the extraction efficiency (El-Nawawi and Shehata, 1988). Pectin is solubilized in two steps. First, the pectin is depolymerized via a β-elimination reaction that occurs when heated at a neutral or weakly acidic pH, causing the molecules to be small enough to solubilized from the cell walls. In the second step, the pectin is degraded by a thermal process due to acid hydrolysis (pH<3) (Sila *et al*., 2009). Pectin is very stable at approximately pH 3.5, which is its pKa (Sila *et al*., 2009). At a pomace to water ratios ratio of 1:16.5 (Coded value = -0.75) to 1:33.6 g/mL (Coded value = 0.2) and pH 1.35 (Coded value = -0.3) to 1.88 (Coded value = 0.75), the yield was the highest at 29.21%.

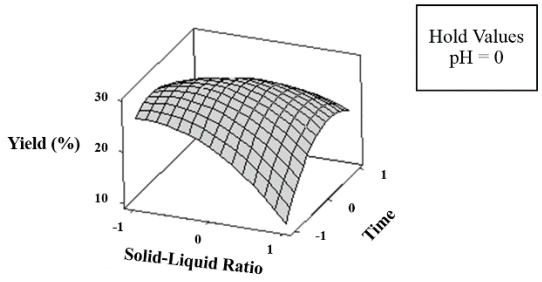

Figure 3. Three-dimensional graph of the yield from the microwave-assisted pectin extraction from pomace (*Y*) at various pomace to water ratios (x_1) (g/mL) and extraction times (x_3) at 450 W and pH 1.5 (**x_2 = 0**)

Figure 3 shows that the pomace to water ratio and the extraction time were the key factors that affected the yield. Time affects the absorption of the microwave energy in the extraction process before it is transformed into heat in the extracting solution. Thus, a longer time would yield higher pectin. However, excessive time produces excessive heat; therefore, thermal pectin hydrolysis could occur, lowering the yield (Xianzhe *et al*., 2011). At pomace to water ratios of 1:13.8 (code = -0.9) to 1:33.6 g/mL (code = 0.2) and extraction times of 13 min (code = -0.7) to 23 min (code = 0.3), the yield would be the highest at 29.21%.

At pH 1.35 (code = -0.3) to 1.88 (code = 0.75) and an extraction time of 16 min (code = -0.4) to 24.9 min (code = 0.49), the yield would be the highest at 29.21%. If the pH and extraction time are higher or lower than in the appropriate range, the yield will be lower (Figure 4).

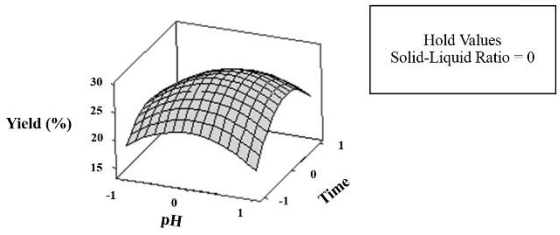

Figure 4. Three-dimensional graph of yield from microwave-assisted pectin extraction from pomace (*Y*) at various pH (x_2) and extraction times (x_3) at 450 W and a pomace to water ratio of 1:30 g/mL (x_1 = 0)

3.3 Validation of a Mathematical Model

From the optimum conditions obtained from the Minitab 16 software, the mathematical model was validated with experiments using microwave-assisted pectin extraction from pomace (the condition used were a pomace to water ratio of 1:23, pH 1.6, and an extraction time of 18 min at 450 W).

There experiments were performed in triplicate. A yield of 28.89 ±. 1.25% was obtained. When compared to the values obtained from the model of 29.20%, the difference was 1.06%, which was similar, indicating that the model can predict the percentage yield well.

3.4. Study of physical and chemical properties of extracted pectin

A pomace powder sample dried in a hot air oven to a moisture content of 10.20 ±

0.24% (dry basis) was extracted in a water bath and microwave oven to study the physical and chemical properties of the pectin obtained. The results are shown in Table 5.

Table 5. Physical and chemical properties of pectin

Physical & chemical properties	**Commercial pectin[X]**	**Pectin dried with hot air and extracted with mw[Y]**	**Pectin dried with hot air oven and extracted with water bath[Z]**
Color value			
L^*	82.64 ± 0.02^{A}	50.04 ± 0.03^{B}	43.14 ± 0.05^{C}
a^*	3.21 ± 0.02^{A}	1.36 ± 0.01^{B}	0.26 ± 0.01^{C}
b^*	14.12 ± 0.04^{A}	12.00 ± 0.03^{B}	10.78 ± 0.04^{C}
C^*	14.49 ± 0.03^{A}	12.08 ± 0.03^{B}	10.78 ± 0.04^{C}
h^*	77.18 ± 0.05^{C}	83.53 ± 0.06^{B}	88.64 ± 0.03^{A}
Moisture content by dry basis (%)	9.55 ± 0.28^{B}	9.57 ± 0.18^{B}	11.66 ± 0.40^{A}
Ash content by dry basis (%)	2.05 ± 0.04^{C}	2.85 ± 0.03^{A}	2.27 ± 0.04^{B}
Equivalent weight (g)	1066.12 ± 1.55^{A}	526.87 ± 1.61^{B}	475.89 ± 1.50^{C}
Methoxyl content (%)	13.67 ± 0.02^{A}	10.46 ± 0.02^{B}	9.78 ± 0.02^{C}
A.U.A. content (%)	94.14 ± 0.09^{A}	92.79 ± 0.21^{B}	92.52 ± 0.20^{B}
Degree of esterification (%)	82.46 ± 0.03^{A}	64.00 ± 0.03^{B}	60.03 ± 0.06^{C}

Note $^{A, B, C}$ indicate the average of horizontal data is significantly different ($p<0.05$) (n=3)

[X] commercial food grade pectin (Apple Pectin AP104 HP, China)

[Y] Pectin dried with hot air oven and extracted with microwave oven. Extraction conditions were a pomace to water ratio of 1:23 w/v, pH 1.6, an extraction time of 18 min, and 450 W (the optimum conditions obtained in this study).

[Z] Pectin dried with hot air oven and extracted with water bath. Extraction conditions were a pomace to water ratio of 1:40 w/v, pH 1.5, extraction time of 1 h, and a water bath temperature of 90 °C (Shaha *et al*., 2013).

From Table 5, it was found that the color value of the pectin varied significantly ($p<0.05$). The results indicate that L^*, a^*, b^*, and C^* values for the high methoxyl commercial pectin were higher than those of the pectin from the pomace extracted in a water bath and the pectin from the pomace extracted in a microwave oven. The color of pectin from this research was green shade.

Because of the residual color from kaffir lime peel.

The ash content indicates the pectin quality. A higher ash content indicates more contaminants in the pectin. Good quality pectin must have low ash content. Related research has also found that the ash contents of the extracted pectin were between 2.8-8.5%. Pagan and Ibarz (1999) showed an ash content of pectin extracted from peach peel of 3.0 ± 0.2% and Pagan *et al*. (2001) showed an ash content of pectin extracted from peach peel at 2.8 ± 0.3%.

The equivalent weight is the amount in grams of pure polygalacturonic acid. It depends on the degree of esterification, which is correlated with the number of free carboxylic groups in one gram mole equivalent to one gram mole of hydroxy. It can be obtained by titration with sodium hydroxide (Ranganna, 1997). The equivalent weights of the pectin from pomace extracted in a water bath and the pectin from pomace extracted in a microwave oven in this study were similar to that of Shaha *et al*. (2013), who extracted pectin from kaffir lime peel and obtained an equivalent weight of 210 - 735.4 g.

The methoxyl content is an important variable that determines the gelation time of the pectin and the sensitivity of its response to polyvalent cations. If the methoxyl content is high, it indicates that that the pectin will gel

quickly. The methoxyl content of all 3 samples was higher than 9%.

The anhydrouronic acid (A.U.A.) content indicates the purity of pectin because the main component of pectin is esterified polygalacturonic acid. Table 5 shows that the A.U.A. content of the pectin from pomace extracted in a water bath and the pectin from pomace extracted in a microwave oven were similar to values obtained by Shaha *et al.* (2013), which had an A.U.A. content of 38-98%, but a comparison with the A.U.A. content of commercial pectin with the pectin from pomace indicated that the pectin from pomace had a significantly lower A.U.A. contents than commercial pectin ($p<0.05$).

The degree of esterification of all 3 samples was higher than 50%, so they were categorized as high methoxyl pectins and had a degree of esterification according to the FDA standard of at least 50% degree of esterification. The degree of esterification of pectin extracted in a water bath and extracted in a microwave oven were similar to that obtained by Shaha *et al.* (2013), which had a degree of esterification of 58-65%.

Pectin extracted in a microwave oven had chemical properties similar to pectin extracted in a water bath, and when comparing the chemical properties of the 2 samples with those of the commercial pectin, it was found that pectin extracted in the microwave oven had chemical properties more similar to those of commercial pectin.

4. Conclusions

The best method for pectin extraction from pomace was in a microwave oven because it produced an average yield approximately 35% higher than extraction in a water bath (the average yields of pectin extracted in a water bath and in a microwave oven were 22.32 ± 1.32 and 34.07 ± 2.20, respectively.

The optimum conditions for using microwave radiation to facilitate pectin extraction from pomace to obtain the highest yield of 29.20% was a pomace to water ratio of 1:23, a pH of 1.6, and an extraction time of 18 min at 450 W.

The pectin extracted from pomace dried in a hot air oven and extracted in a microwave oven had light green color, a moisture content of 9.57 ± 0.18% (dry basis), an ash content of 2.85 ± 0.03%, an equivalent weight of 526.87 ± 1.61 g, a methoxyl content of 10.46 ± 0.02%, an A.U.A. content of 92.79 ± 0.21%, and a degree of esterification of 64.00 ± 0.03%. The pectin extracted in this study can be categorized as high methoxyl pectin.

5.References

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