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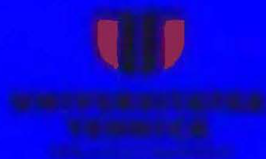

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CONTENT

Karoline Aparecida da Cruz Arruda, Gilson Gustavo Lucinda Machado, Rejiane Avelar Bastos; Gilma Auxiliadora Santos Gonçalves; *DEVELOPMENT AND EVALUATION OF JELLY MADE WITH PEACHES OUTSIDE THE IN NATURA CONSUMPTION STANDARD, SWEETENED WITH CANE BROTH* **5-13**

Meltem Serdaroğlu, Hülya Serpil Kavuşan, Elnaz Sharefi-Abadi, Burcu Sari, Hilal Can; *OXIDATIVE STABILITY OF CHICKEN MEAT EMULSION SYSTEMS: THE EFFECTS OF GELLED EMULSION AND USE OF ASCORBIC ACID AND ROSEMARY EXTRACT IN DIFFERENT PHASES* **14-28**

K.P.V.R. Karunanayake, D.W.M.M.M. Kumari and P.N. Yapa; *EVALUATION OF ANTIBACTERIAL POTENTIAL OF SELECTED CULINARY HERBS AGAINST SOME FOODBORNE PATHOGENIC BACTERIA* **29-41**

Meranda A. Tawfek, Marwa T. Hussein and Ayat E. Rizk; *LOW-FAT ICE MILK MANUFACTURED WITH FRUITS OF NABQ (ZIZIPHUSSPINA-CHRISTI L.)* **42-56**

Samaneh Hassani, Babak Pakbin, Samaneh Allahyari, Razzagh Mahmoudi, Shaghayegh Mousavi and Peyman Ghajarbeygi; *PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF FOODBORNE BACTERIAL PATHOGENS ISOLATED FROM BAGHLAVA AN IRANIAN EXPORTING PASTRY SWEET* **57-69**

Nidhi Dangi, Baljeet, S. Yadav and Ritika, B. Yadav; *RHEOLOGICAL, PASTING, THERMAL AND GEL PERMEATION CHROMATOGRAPHIC CHARACTERISTICS OF GUAR GUM ACID HYDROLYSATES* **70-85**

Olajide Adedeji, Josephine Samuel, Benjamin Ayoola, *CHEMICAL COMPOSITION, PHYSICAL AND SENSORY PROPERTIES OF DEHYDROFROZEN YAM CHIPS AS INFLUENCED BY PRE-DRYING CONDITIONS* **86-93**

Díaz G.V., Coniglio R.O., Bordaquievich M.F., Zapata P.D., Villalba, Fonseca M.; ***FUNGAL HOME-MADE ENZYMATIC COCKTAILS FOR APPLE JUICE CLARIFICATION*** **94-101**

Yueyang Symus Say, Mark Wong Kei-Fong, Eddie Ng Yin-Kwee; ***ADVERSARIAL AUTOENCODERS FOR AGRICULTURE YIELD FORECASTING*** **102-115**

Alexander G. Kruchinin, Ekaterina I. Bolshakova, Alana V. Bigaeva; ***KINETICS OF CHANGES IN THE GRANULOMETRIC COMPOSITION OF THERMODENATURED WHEY PROTEINS*** **116-127**

Mira-Elena Ionica, Violeta Nour and Felicia Tutulescu; ***INFLUENCE OF UV TREATMENT ON SOME PROPERTIES AND BIOACTIVE COMPOUNDS IN ONIONS (ALLIUM CEPA L.) DURRING STORAGE*** **128-137**

Chinyere Nkemakonam Onyekwelu and Eunice Osinachi Uzodinma; ***EFFECT FERMENTATION ON VOLATILE COMPOUNDS OF PACKAGED CASTOR OIL - MORINGA SEEDS CONDIMENT (OGIRI)*** **138-149**

Adegbola Oladele Dauda; ***QUALITY ASSESSMENT OF AFRAMOMUM DANIELLI SPICED FRUIT LEATHER FROM AFRICAN STAR APPLE FRUIT*** **150-158**

Ji Ahn Han, Ki Han Kwon; DEVELOPMENT POTENTIAL OF ERAGROSTIS TEF AS A FLOUR ALTERNTIVE **159-170**

Santhiya K., Abinaya S., Aswini R.and Nitheshlee M.; ***ASSESSMENT OF BUTTON AND OYSTER MUSHROOM NUTRITIONAL QUALITY USING VARIOUS DRYING METHODS*** **171-181**

Belkhodja Hamza, Kiari Fatima, Meddah Boumediene, Douhi Nadjet, Belarbi Maria; ***PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING ACTIVITY OF THE AQUEOUS EXTRACT OF JUGLANS REGIA (WALNUTS)*** **182-189**

Iva Paula F. dos Santos; Luísa Ozorio; Tatiana L. Azevedo; Lourdes M. C. Cabral; Caroline Mellinger-Silva; ***EVALUATION OF BOVINE MILK PROCESSING ON THE DIGESTIBILITY AND ALLERGENICITY OF MILK PROTEINS*** **190-196**

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DEVELOPMENT AND EVALUATION OF JELLY MADE WITH PEACHES OUTSIDE THE *IN NATURA* CONSUMPTION STANDARD, SWEETENED WITH CANE BROTH

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ABSTRACT
The preparation of jellies from peaches outside the standard is a way of using the fruit. Thus, the present work aimed to develop and evaluate peach jellies sweetened with sugarcane juice, made with fruits of two peach cultivars (Maciel and Top Bilt) that were out of the standard for fresh consumption. Two formulations were developed, which were elaborated with 45% peach pulp, 33% apple pulp, 20% sugarcane juice, and 2% lemon juice. In the characterization of the jellies, the following were evaluated: color (L*, a* and b*), firmness, pH, titratable acidity, soluble solids, moisture, total phenolic compounds, carotenoids, vitamin C, anthocyanins, and mold and yeast counts. After approval by the research ethics committee, the acceptance test was applied with 100 untrained tasters of both sexes. The results of the analyzes were compared by means of analysis of variance and Tukey's test at the 5% level. It was found that the jellies made with the peach cultivars are statistically different only regarding the parameters of color, a* and b* values and pH, and statistically equal regarding L*, firmness, titratable acidity, soluble solids, vitamin C, carotenoids and phenolic compounds. It is concluded that, even without the addition of commercial sucrose, the jellies of both cultivars have the potential as objects of new research about their manufacture to be made available in the market, as they present good purchase intentions.

1. Introduction

China is the world's largest producer of peaches, with an estimated production of 14.5 billion tons in 2020. Brazil is in sixth position with a production of 220 million tons (Analysis, 2020), cultivated in an area of approximately 15,995 hectares, with Rio Grande do Sul being the state with the highest production (IBGE, 2019).

The peach is a fleshy fruit belonging to the Rosaceae family which is cultivated in temperate and subtropical climates. It is highly perishable during post-harvest storage, due to its high water content, which culminates in a short shelf life under environmental conditions (Mir et al., 2018; Zhang et al., 2020). It is classified as a climacteric fruit, as it presents an increase in ethylene emission and respiration at the beginning of ripening, accompanied by a change in color, texture, and aroma (Minas et al., 2018).

According to FAO (2021), a loss of up to 50% of fruits and vegetables is estimated in developing countries. This represents, not only the loss and waste of food, but also natural resources and investments made by farmers and the country. Like other fruits, peaches can present high losses, mainly due to rapid ripening, low marketing prices, various difficulties in the production flow, or even because they do not fit the profile desired by the consumer (size, or physiological defects), even with appreciable amounts of nutrients. In this way, it can be used as a raw material for the elaboration of other fruit products (Brasil,

2005). These products include jellies, as they come from processing by cooking whole edible fruit or vegetable parts and/or pieces in the most diverse forms, pulp/juice or aqueous extracts thereof, homogenized with other ingredients that help to obtain the desired technological characteristics, such as sugar, pectin, acids and water, among other ingredients and additives allowed by current legislation, which must be concentrated until they have a gelatinous consistency, having the shape of the container in which it is packaged after processing (Oliveira et al., 2018).

The replacement of commercial sugar in jelly formulations is a challenge, as it directly influences the sweetness, viscosity, texture development and reduction of water activity (Sandrou & Arvanitoyannis, 2000; Pereira et al., 2013). Therefore, it is necessary to seek alternatives and ingredients to replace commercial sugar in the formulation.

However, the preparation of jelly added with sugarcane juice is a means of diversifying and meeting the demands of the consumer market, which is increasingly looking for quality and differentiated products that have high nutritional value, whether for aesthetic, physiological or health reasons (Reissig et al., 2016).

In light of the above, this study aimed to develop and evaluate peach jelly sweetened with sugarcane juice, made with fruits of two peach cultivars (Maciel and Top Bilt) that were out of the standard for fresh consumption.

2. Materials and methods

2.1. Materials

The experiment was carried out at the Federal Institute of Education, Science and Technology, Barbacena campus – MG, industrialization and processing of vegetables sector, physical-chemical analysis laboratory, sensory analysis and microbiological analysis of food laboratory.

The two peach cultivars used in the preparation of the jellies (Maciel, yellow pulp and Top Bilt, white pulp) were donated by the Rural Community Association of Mantiqueira do Palmital of Barbacena -MG. Fruits that do not fit *in natura* commercialization standards were used, that is, those already ripe and slightly soft, with small mechanical injuries, irregular size and shape, but without visible microbial contamination.

Cultivars Top Bilt and Maciel were selected for the preparation of the jellies because they present white and yellow pulp, respectively, and because they are those with the highest production and highest loss rates in the rural community of Mantiqueira do Palmital in Barbacena. The selected fruits were: pre-washed, sanitized with chlorinated water (100 mg L^{-1} 10 min^{-1}). Afterwards, they were blanched in boiling water for 5 minutes, cooled and crushed in an industrial blender, Visa LQL – 25 – stainless steel.

Pre-tests were performed to define the jelly formulation, taking into account the gel formation, and sensory attributes (color, flavor and aroma), the formulation with 45% peach, 33% apple, 20% sugarcane juice and 2% lemon juice had better sensory and gel formation characteristics. After defining the formulation, the jellies were prepared following the steps of cooking and concentration of ingredients until reaching the final point, determined by an Atago brand manual refractometer (28 – 32% soluble solids - SS), standardized at 30% SS. The hot jelly was placed in glass jars (capacity of 230 g), previously sterilized by boiling for 30 minutes and with metal lids boiled for 10 minutes. The closed packages underwent an inversion term (3 minutes), to sterilize the free space and the lid; cooled in running water until reaching an internal temperature of approximately 40 °C (temperature measured with a Benetech Infrared thermometer). The packages were labeled to identify the treatments and finally stored refrigerated until analysis time. The duration was, on average 10 days, to help better conservation until the result of the microbiological analysis was obtained, in order to carry out the sensory analysis. The jellies were evaluated for color, firmness, pH, titratable acidity (AT %), soluble solids (SS %), moisture, total phenolic compounds, total carotenoids,

vitamin C, microbiological analysis, and sensory analysis.

2.2 Analyzes

2.2.1.Coloring

Determined with a Konica Minolta CR400 colorimeter, using the L, a* and b* color scale system (CIELAB), previously calibrated, with the reading being carried out in the pot itself.

2.2.2.Firmness

Determined using a cylindrical aluminum probe with a diameter of one inch, test speed 2.0 mm/s, distance 10.0 mm and force 0.10 N. For compression analysis, 50 g of jelly were used for each measure (Pons & Fiszman, 1996).

2.2.3.pH

Using pH-meter TEKNA T-1000 according to the methodology proposed by Instituto Adolfo Lutz (IAL, 2008). The pH-meter was previously calibrated using buffer solutions (pH 4.0 and 7.0).

2.2.4. Titratable acidity (TA)

Determined by titration with a standardized 0.1 N sodium hydroxide solution, using phenolphthalein as an indicator. According to the methodology proposed by the Analytical Norms of Instituto Adolfo Lutz (IAL, 2008).

2.2.5.Soluble solids (SS g/100 g).

Determined by the refractometry method, using an Instrutherm RTD-45 refractometer (with a reading range between 28-32% soluble solids) (IAL, 2008).

2.2.6.Humidity

Determined by gravimetry in a Deleo A4SE drying and sterilization oven at 105 °C until constant dry mass, according to Instituto Adolfo Lutz (IAL, 2008).

2.2.7.Total phenolic compounds

Extracts were obtained as described by Brand Williams; Cuvelier; Berset (1995) and adapted by Rufino et. al (2007). Gallic acid was used as a reference standard and the results expressed in milligram equivalents of the acid itself (mg GAE 100 g^{-1} fresh matter).

2.2.8.Total carotenoids

The extraction and determination was carried out according to the technique described by Rodriguez-Amaya (1999).

2.2.9.Vitamin C

Determined by the Balentine method, which is based on the oxidation of ascorbic acid by potassium iodate as proposed by Tavares (1999).

2.2.10.Anthocyanins

Quantified following the differential pH method, proposed by Giusti; Wrolstad (2001), which follows the equation:

$$A = (A510nm - A700nm)\ pH = 1.0 - (A510nm - A700nm)\ pH = 4.5 \quad (1)$$

The content of monomeric anthocyanins (AM) was calculated as cyanidin-3-glycoside (PM = 449.2) using the equation:

$$AM\ (mg.100\ mL-1) = A \times PM \times dilution\ factor\ \varepsilon\ (22900) \times 1 \quad (2)$$

Where: A= Absorbance and ε = Molar Absorbance

2.2.11.Microbiological analysis

Mold and yeast counts were determined by the method of the American Public Health Association (APHA) (Silva et al., 2010). The result was compared with the limit established by Resolution - RDC no 12 of January 2, 2001 (Brasil, 2019) which establishes microbiological standards for food.

2.2.9.Acceptance test

This work was submitted to the Human Research Ethics Committee of the IF Southeast MG and approved in accordance with CAAE: 01681718.1.0000.5588. The acceptance test was applied according to the methodology described by Minim (2006) with 100 untrained tasters of both sexes in the sensory analysis laboratory of the IF Sudeste MG Barbacena campus. Potential jelly consumers were selected among staff, students and professors from the Barbacena campus, over 18 years old. The jelly samples, coded with three-digit numbers, were served accompanied by toast and a 100 mL glass of water to clean the palate between sample evaluations. The attributes color, odor, flavor, texture and global acceptance were evaluated through a structured nine-point hedonic scale, with extremes at 1 - "I disliked extremely it" and 9 - "I liked it extremely". The purchase intent test was also applied using a 5-point scale ranging from 1 - Certainly would not buy to 5 - Certainly would buy.

2.2.12.Statistical analysis

A completely randomized design was used, with the jellies made with two peach cultivars (Top Bilt and Maciel) with three replications, and analyzed in triplicate. The data obtained were subjected to analysis of variance (ANOVA) and means were compared by Tukey test at 5% significance in Sisvar 5.3 program (Ferreira, 2010).

The Acceptance Test was analyzed using the Internal Preference Map (MDPREF) methodology (Macfie; Thomson, 1994), which allowed the generation of the multidimensional affective sensory space, formed by the 100 tasters, the 2 studied samples and the 5 evaluated attributes. The data was analyzed statistically as mentioned above.

3. Results and discussions

The results of the analysis of color, firmness, pH, titratable acidity, soluble solids, moisture, phenolic compounds, carotenoids, vitamin C and anthocyanins are shown in Table 1.

Table 1. Physical and physicochemical characterization of the jellies.

Analysis	**Cv. *Maciel***	**Cv. *Top Bilt***
Color – L*	43.40 **	
a*	9.38 a*	4.30 b
b*	21.18 a	15.07 b
Firmness (N)	1.65**	
pH	3.87 b	4.21 a
Titratable acidity (%)	2.64**	
Soluble solids (%)	30**	
Moisture (%)	29.98**	
Total Phenolic Compounds (mg GAE 100 g^{-1})	2.10**	
Carotenoids (mg100 g^{-1})	0.050**	
Vitamin C (mg100 g^{-1})	0.0015**	
Anthocyanins (mg100 g^{-1})	ND	

*Equal letters, on the line, do not present significant difference. **No significant difference. ND: not detected.

The L* parameter, which indicates white (closer to 100 = light) and black (further than 100 = dark), representing luminosity, had an average of 43.40, indicating that the jellies of the two cultivars are slightly dark. Lower L* values (24.85) are reported by Santos et al (2021) in abiu diet jam with chia, indicating a darker color than the peach jams in the present study. This is probably due to the raw materials (abiu and chia) used.

There was a significant difference between the samples, regarding the a* parameter. The jelly made with peach of the Maciel cultivar had a higher a* value (9.38) when compared to that made with Cv. Top Bilt, a* (4.30), a fact possibly justified by the color of the peach cultivar Maciel pulp, which is yellow. However, both indicated color tending towards red because positive a* values indicate a tendency towards red (+a*) and negative values for green color (-a*). Lower value for the same parameter is reported by Santos et al. (2021) in diet abiu jelly with chia (0.72), indicating that the peach jellies have a color that tends more towards red than the diet abiu jelly with chia.

Positive b* values indicate yellow coloring and negative values, blue. In this variable, the samples differed from each other, and the jelly made with Cv. Maciel peach showed a higher b* value (21.18), which was already expected due to the color of the cultivar's pulp, in relation to Top Bilt (15.07). A lower value for the same parameter is reported by Santos et al. (2021), in abiu diet jelly with chia (6.69), indicating that

the peach jellies have more yellowish visual characteristics than the abiu diet jelly with chia.

The average firmness of the jellies was 1.65 N. Alower value for the same parameter was found by Oliveira et al. (2014), in the characterization of Umbu-cajá diet jellies, finding values between 0.23 and 0.43 N. A factor that can be explained by the difference in fruit species, despite having other characteristics that are very similar.

The pH is directly related to the conservation of jellies, as the lower the pH, the higher the difficulty for pathogenic bacteria growth. In addition, it is important for the texture of the jellies, as it is related to the acidity index, where values below 3.5 cause a break in the gels during processing, while values above 4.7 do not allow gel formation (Food Ingredients Brazil, 2013). There was a significant difference between the samples evaluated in terms of pH, with the highest being that of jelly from the Top Bilt cultivar (4.24). Other researchers have also found similar values, obtaining results from 4.44 to 3.68 for analyzes in mountain guava jellies, both being within the ideal established by the literature (Santos et al. 2017). Lower values for the same parameter were reported by Ben Rejeb et al. (2020), when analyzing jellies with reduced sugar content formulated from citrus fruits (ranging from 2.74 to 3.56 for sour orange and Sangue orange jellies, respectively), and by Santos et al. (2021), in abiu jelly with chia diet, where they report a pH of 3.22 for the respective product. The difference in pH values found is probably due to the raw material used in the preparation of the respective jellies, however a low pH contributes to the increase of the shelf life of the product, and a pH below 3.0 favors syneresis in jellies (Teles et al., 2017).

As for titratable acidity, there was no significant difference between samples (2.64%). This result is inferior to that of Santos et al. (2017), who found 1.70%, which is probably due to the raw material used in each experiment. Obtaining low titratable acidity values, together with high soluble solids levels and the pH, ensures certain microbiological stability of the product, since such conditions make microbial multiplication difficult.

Although the jellies did not receive added commercial sucrose, an average content of 30% SS was obtained, which is justified by the addition of sugarcane juice, naturally rich in sucrose. Similar values were found by Bernert et al. (2015) in tamarillo diet jelly (37% SS). And lower values were reported by Sousa et al. (2020) in buriti diet jelly (25.20% SS), Santos et al. (2021) in abiu diet jelly with chia (18.75% SS), and Bem Rejeb et al. (2020) in jellies with reduced sugar content formulated from citrus fruits (ranging from 26.93 to 28.42%). The soluble solids content can be associated with the moisture content, as the higher soluble solids content will indicate the lower moisture content of the jelly, which is also related to higher product conservation.

The average humidity of the jellies was 29.98%, within that required by legislation, which is a maximum of 35% (Brasil, 1978). A higher moisture values are reported by Santos et al. (2021) in abiu diet jelly with chia (61.06%), and by Sousa et al. (2020), in buriti diet jelly (64.18), expected results for diet products without the addition of sucrose, which normally requires the addition of preservatives to ensure the durability of these jellies.

With regard to phenolic compounds, there was no statistical difference between the samples, with an average of 2.10 mg GAE100 g^{-1}. The levels of phenolic compounds identified in this study are similar to those found for jabuticaba jelly evaluated by Rezende (2011), who found values ranging from 2.12 to 2.21 g L^{-1}.

Anthocyanin analysis was performed in the jellies, but there was no identification of this compound, which can be attributed to the high instability of these compounds to thermal processing, or even the small concentration of the compound in view of its high variability among peach genotypes. In general, those presenting red coloration tend to have a higher anthocyanin content (Cantín et al. 2009).

As for the carotenoids values, 0.050 mg/100 was obtained, with no significant difference

between the samples. However, this parameter is highly variable among peach cultivars, and is more concentrated in the skin (Brown et al. 2014), which may indicate that the fruits used in the preparation of jams were already poor in such compounds.

There was no significant difference between samples for vitamin C, with a mean of 0.0015 mg 100 g^{-1}. Low vitamin C values are usually identified in processed foods, especially when cooked, as these compounds are highly unstable at high temperatures and can be degraded during the preparation of the jelly. In studies carried out by Neiva et al. (2016), it was also observed that Vitamin C can be degraded very quickly in aqueous solutions under both anaerobic and aerobic conditions. However, a study indicates that the content of vitamin C in peaches can vary according to the genotype, climate, conditions and cultural practices (Cantín et al. 2009). Data obtained by Geçer (2020), corroborate the aforementioned, with levels ranging from 7.79 to 9.84 mg 100 g^{-1} of Vitamin C for different peach cultivars. This may indicate that the raw material used in the preparation of jams was already poor in vitamin C.

3.1.Microbiological characterization

The results of the analyzes of molds and yeasts indicated that there was no development of these microorganisms in the jellies. As such they are suitable for consumption and comply with the legislation (Brasil, 2019). This result indicates that the application of good manufacturing practices was efficient in obtaining the microbiological safety of the jellies.

3.2.Acceptance Test

In the sensory acceptance test, the results indicated that the jelly made with the Top Bilt cultivar obtained, in general, higher acceptance, which can be observed in the internal preference map (Figure 1), due to the higher number of vectors and their approximation to the appearance and odor attributes, located closer to the center of the graph. This, indicates that they obtained higher averages. The jelly from the Maciel cultivar was farther from the center, for the attributes of appearance (A), odor (O) and flavor (S).

The higher acceptance of the jelly from this cultivar indicates that it should be preferred for the manufacture of jellies, because although the formulation was the same for the two cultivars, the jelly from the Top Bilt cultivar proved to be more accepted by consumers, reinforcing the importance of specific studies with different fruit cultivars to define that which to be used in the preparation of a given product.

Rejeb et al. (2020), when sensorially evaluating jellies formulated from four varieties of citrus fruits and with reduced sugar content, found that there were significant differences between the acceptability, in terms of flavor, odor, color, and sweetness of the jellies, however there was no variation on acceptability for texture. The jelly made from blood orange juice obtained greater acceptance.

Curi et al. (2019), when evaluating the processing of jellies from different fig cultivars, also found that although the elaborated jellies presented good sensory acceptability, they differed statistically in terms of color, with this parameter being strongly influenced by the cultivars used in the jellies elaboration. According to the authors, different fig cultivars give rise to jellies with different physical, chemical and rheological characteristics.

This corroborates the result of the present work indicating that fruits of different varieties (cultivars) result in products with distinct sensory characteristics. This contributes to the need to carry out more research on the formulation of jellies with different peach cultivars, considering that cultivar varieties provide a wide variety of products.

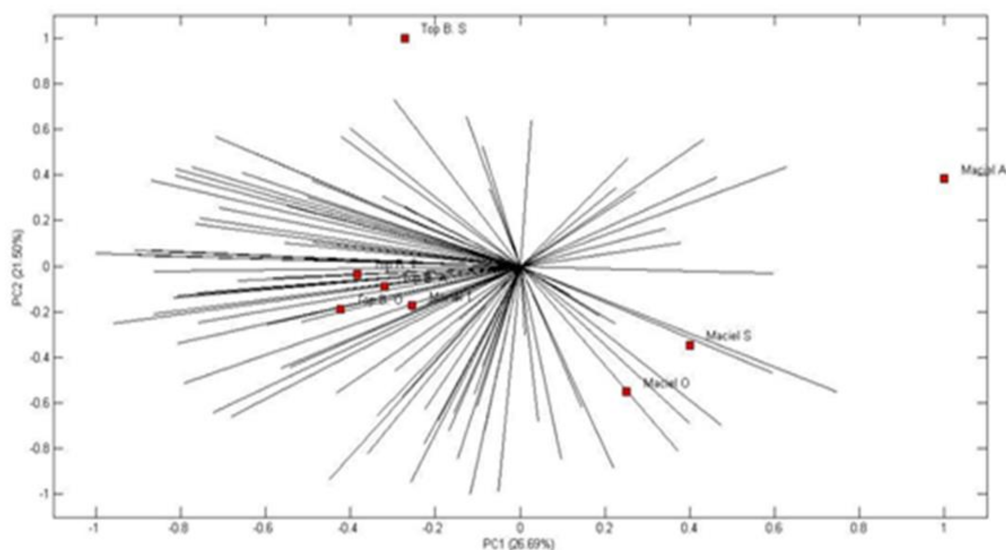

Figure 1. Internal preference map of the peach jam acceptance test.

Top B: Jelly from the Top Bilt cultivar; Maciel: Jelly from the cultivar Maciel; The appearance; S: taste; O: odor; T: texture and I: Global impression.

As for the purchase intention, there was no statistical difference between the jams of the two cultivars and the average was 3.96, being closer to the score 4 (possibly buy). This result suggests that, although sensorially, the Top Bilt jelly has greater acceptance among consumers, both can be produced and can be well marketed.

It can be said that jelly sweetened only with sugarcane juice, although not suitable for consumption by diabetics, serves the objective of offering natural food, without adding sugars or sweeteners. The products are healthier and probably of lower caloric value since the soluble solids content reached values lower than those recommended in the literature for jellies. Furthermore the jelly meets the sensory characteristics expected by the consumer, as identified in the acceptance test.

4. Conclusions

The jellies made with the peach cultivars Top Bilt and Maciel are statistically different only in terms of color, a* and b* and pH parameters they are, statistically equal in terms of L*, firmness, titratable acidity, soluble solids, vitamin C, carotenoids and phenolic compounds. It is concluded that, even without sugar and sweeteners added, jellies of both cultivars have the potential to be available in the market, as they present good acceptance and purchase intention.

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OXIDATIVE STABILITY OF CHICKEN MEAT EMULSION SYSTEMS: THE EFFECTS OF GELLED EMULSION AND USE OF ASCORBIC ACID AND ROSEMARY EXTRACT IN DIFFERENT PHASES

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ABSTRACT
In order to examine the effects of using ascorbic acid, rosemary extract, or ascorbic acid-rosemary extract combination in different phases of flaxseed oil gelled emulsion (GE) formulation on oxidative stability, model chicken meat emulsions (CMEs) formulated with as follows; beef fat-no antioxidant (C), gelled emulsion-no antioxidant (GE-No), GE containing 100 ppm ascorbic acid in the water phase (GE-A), GE containing 100 ppm rosemary extract in the oil phase (GE-R) and GE containing 100 ppm ascorbic acid in water phase and 100 ppm rosemary extract in oil phase (GE-A/R). Protein content of samples increased from 12.99% to 14.58% with the addition of GE ($P<0.05$). Water holding capacity of reformulated CMEs increased up to 66.01%. At the end of the storage using ascorbic acid and rosemary extract individually or combined in GE formulation was effective to delay the primary lipid oxidation of samples, while ascorbic acid and ascorbic acid+ rosemary extracts retarded the formation of malonaldehyde. Initial free fatty acid values ranged between 0.34%- 1.07% and the initial trend was proportional to TBARS values. Reformulated samples were lighter than the control group. a* value of control was higher while b* values were lower than reformulated CMEs throughout the storage.

1.Introduction

Increasing trends in consumer's demand for healthy foods have led to meat industry to focus on research and development studies to improve healthier meat product formulations. In the application of producing healthier meat products, using vegetable and seed oils in liquid form yields technological problems, undesired organoleptic properties, and increase oxidative changes. To prevent these undesirable changes in quality, liquid oils can be modified by using various methods such as structured emulsions which involve gel and double emulsions (Alejandre *et al.,* 2016; Öztürk *et al.,* 2017), interesterification (Kılıç and Özer, 2017), and organogelation (Barbut *et al.,* 2016) have been researched previously. Gelled emulsions (GE) are considered to be one of the effective ways to solidify liquid oils by entrapping the liquid oil in gel network which is created by polysaccharides and/or enzymes (Serdaroğlu *et al.,* 2016; Serdaroğlu *et al.,* 2017).

One of the main problems when improving the fatty acid composition of meat products by the addition of oils that contains high amounts of unsaturated fatty acids is their oxidative susceptibility which results in deterioration of the nutritional and sensorial perspective of meat products (Kumar *et al.,* 2015). Therefore, these oils should be protected to make them more stable against oxidative changes during processing and storage (Carneiro *et al.,* 2013). In this respect gel networks which are created by polysaccharides, enzymes, and heat treatment

provide an opportunity to protect unsaturated fatty acids against oxidation (Wang *et al.,*2018; Kavuşan *et al.,*2020). In some cases, using highly oxidative oils in gelled emulsion formulations remains incapable in terms of inhibition of oxidative changes due to high perishability, therefore antioxidant incorporation to gelled emulsion formulation may retard the oxidation reactions better than gelled emulsion itself (Alejandre *et al.,* 2019). For this purpose, *Murraya koenigii* berries extract (Kumar and Kumar, 2020) and olive leaves extract (Robert *et al.,* 2019), rosemary extract (Erdmann, *et al.,* 2017) loaded O/W emulsions or double emulsions were used successfully in meat products. Also, lyophilized *Melissa* extract (Poyato *et al.,* 2013) and curcumin, quercetin, rutin hydrate, and ascorbic acid (Noon *et al.,* 2020) were used in gelled emulsion formulations for the prevention of sunflower, olive, and linseed oils. Up to now, there is only one research regarding the use of green tea extract in the formulation of fish oil gelled emulsion (Pourashouri *et al.,* 2020). The findings of this research presented green tea extract loaded gelled emulsion did not improve oxidative stability. Instead of using antioxidant in the water phase, Mosca *et al.* (2013) stated that the best strategy to preserve the emulsions is the combined use of antioxidants both in water and oil phases, to promote a synergistic effect and the regeneration of antioxidants mediated by the interfacial layer.

Lipid oxidation could be retarded by sequestration of free radicals from the medium, chelation of metallic ions, inhibition of free radical producing enzymes, activation of endogenous antioxidant enzymes, and prevention of lipid peroxidation (Carocho *et al.,* 2018). Using synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) has been related health problems and their application in the food industry has limited (Rojas and Brewer, 2008). Thus, the use of natural antioxidants such as fruit or vegetable extracts and essential oils in meat product formulations have been subjected to researches in recent years (Oswell *et al.,*2018; Pateiro *et al.,* 2018).

Water-insoluble rosemary (*Rosmarinus officinalis* L.) extracts have strong antioxidant effects depending on their phenolic compounds such as rosmanol, rosmariquinone, rosmaridiphenol, carnosic acid, and carnosol. The antioxidant mechanism of rosemary extract is based on its breaking free radical chain as a result of hydrogen donation provided by phenolic compounds (Haile, 2015). Ascorbic acid is water-soluble organic compound that one of the most important properties of ascorbic acid is its ability to remove environmental oxygen (Varvara *et al.,* 2016).

“Polar paradox” is explained by Porter (1993) stated that polar antioxidants are more effective than apolar antioxidants in bulk oils while apolar antioxidants were more efficient in O/W emulsions due to the localization of antioxidants in different phases where oxidation occurs (Shahidi and Zhong, 2011; Sørensen *et al.,* 2011). Although this phenomenon has been predominantly approved, new pieces of evidence from elaborative studies have found that not all antioxidant compounds in O/W emulsions behave according to this phenomenon (Shahidi and Zhong, 2011; Poyato *et al.,* 2013).

To the best of our knowledge, there is limited search focused on the combined use of different antioxidants in gelled emulsion formulations (Noon *et al.,* 2020) or their use in meat products. Therefore, the objective of this study was to examine the effects of the combined use of ascorbic acid and rosemary extract in different phases of GE formulation on oxidative stability of model chicken meat emulsion where beef fat is replaced by GE prepared with flaxseed oil.

2. Materials and methods (TNR 12 Bold, No ident)

2.1. Materials

Chicken breast meat (74.72% moisture, 2.43% fat, 20.77% protein, 1.67% ash), skinless and boneless was kindly donated by a local processor (Abalıoğlu, Manisa, Turkey). Flaxseed oil (17% oleic acid, 15% linoleic acid,

and 59% linolenic acid) was taken from Ege University Agriculture Faculty (Izmir, Turkey). Polyglyserol polyricinoleate (PGPR) was obtained from Çağdaş Chemicals Co. (Turkey). Gelatin was purchased from Sigma-Aldrich (USA) and egg white powder was purchased from Dr. Gusto (Turkey). Inulin powder (Inulin: 88-92%) was obtained from BENEO-Orafti. Ascorbic acid and rosemary extracts were supplied from Kimbiotek (Turkey).

2.2.Preparation of emulsion gels formulated with different antioxidant

Gelled emulsions were prepared with flaxseed oil, egg white powder, inulin, and gelatin according to the method implemented by our research group (Kavuşan *et al.,* 2020). The formulation of gelled emulsions is given in Table 1. Four different batches of gelled emulsions are formulated as follows: (No) no antioxidant added to GE; (A) 100 ppm ascorbic acid added to the water phase of GE; (R) 100 ppm rosemary extract added to the oil phase of GE, and (A/R) 100 ppm ascorbic acid added to the water phase and 100 ppm rosemary extract added to the oil phase of GE respectively. Antioxidant addition was performed after the heating procedure when the temperature of emulsions reached room temperature. Prepared emulsions were stored at 4 ºC overnight to use in the model chicken meat emulsion formulation.

Table 1. Formulation of gelled emulsions used in model chicken meat emulsion

	100 g emulsion							
	Water phase (g)					**Oil phase (g)**		
	Egg white powder	**Inulin**	**Gelatin**	**Water**	**Ascorbic acid**	**PGPR**	**Flaxseed oil**	**Rosemary extract**
No	3	8	2	37	-	3.2	46.8	-
A	3	8	2	37	0.01	3.2	46.8	-
R	3	8	2	37	-	3.2	46.8	0.01
A/R	3	8	2	37	0.01	3.2	46.8	0.01

*Rosemary extract added into oil phase, while ascorbic acid added into water phase. Addition level of antioxidants was 100 ppm.
No= no antioxidant added to gelled emulsion formulation
A= 100 ppm ascorbic acid added to water phase of gelled emulsion formulation
R= 100 ppm rosemary extract added to oil phase of gelled emulsion formulation
A/R=100 ppm ascorbic acid added to the water phase and 100 ppm rosemary extract added to the oil phase of GE respectively

Table 2. The formulation of model chicken meat emulsion

Samples	**Meat**	**Beef fat/GE**	**Water**	**NaCl**	**STTP**	**$NaNO_2$**
C	63.41	9.75	24.39	1.95	0.49	0.01
GE	63.41	20.83	13.31	1.95	0.49	0.01
GE-R	63.41	20.83	13.31	1.95	0.49	0.01
GE-A	63.41	20.83	13.31	1.95	0.49	0.01
GE-A/R	63.41	20.83	13.31	1.95	0.49	0.01

C: only beef fat added chicken meat emulsions
GE: only GE added chicken meat emulsions
GE-R : CME prepared with GE incorporated with rosemary extract in oil phase
GE-A: CME prepared with GE incorporated with ascorbic acid in water phase
GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

2.3.Processing of model chicken meat emulsion

The preparation of the chicken meat emulsion method of Cofrades *et al.* (2008) was used with some modifications. After trimming visible fat and connective tissue, chicken breast and fat are minced through a grinder with a 3 mm plate. Minced meat was mixed for 60 s. in Thermomix (Vorwerk, Wuppertal, Germany), fat or GE, fifty percent of the ice, and curing ingredients (NaCl, STTP, $NaNO_2$) were added and homogenized at 2500 rpm for 60 s. more. Control sample was formulated with 9.75% beef fat, while 20.83 % gelled emulsion was added to all other CME samples to reach the same fat content as the control samples. The formulation of the model chicken meat emulsion is given in Table 2. The final temperature of emulsions was lower than 12 ºC for all batches. Twenty-five g of samples were filled in 50 ml tubes, then tubes were centrifuged at 2500 rpm for 60 s. Samples were then heated for 30 min. in a 70 ºC water bath, after the heating process, all heat-treated samples were cooled (approximately 25 ºC) and afterward stored for 7 days at 4 ºC.

2.4.Experimental design

Five different batches of chicken meat emulsions (CMEs) were produced. Control (C) samples formulated with beef fat and no antioxidant, four other CMEs were prepared with gelled emulsions without antioxidant (GE-No), gelled emulsion added rosemary extract to oil phase (GE-R), gelled emulsion added ascorbic acid to water phase (GE-A) and gelled emulsion added rosemary extract to the oil phase and ascorbic acid to the water phase (GE-A/R).

2.5.Chemical composition

Moisture and ash contents of samples were determined following AOAC (2012) procedures. Fat content was analyzed according to Flynn and Bramblet (1975). Protein content of samples was determined by using DUMAS method with LECO nitrogen analyzer (FP-528, USA).

2.6.Water holding capacity (WHC)

The ability of CMEs to keep water determined as described by Hughes *et al.* (1997). 10 g CME samples heated in a water bath (90 ºC, 10 min) and centrifuged for 10 min. at 4000 rpm. Final weight of samples recorded and WHC was found as the percentage of retained water related to moisture content.

2.7. pH

pH values were measured with a pH-meter (WTW pH 330i/SET, Germany) on different points of CME samples for every individual group.

2.8.Oxidation analysis

Peroxide and TBARS analyses were performed to observe oxidative changes in CME samples on the 0, $3^{rd,}$ and 7th days of storage. In the CME samples, the peroxide value was determined after the fat/oil in the sample was extracted (chloroform). The potassium iodide was oxidized with the peroxide oxygen in the fat/oil afterward the iodine was released, and this free iodine was titrated with thiosulfate (Koniecko, 1979).

TBARS analysis was based on the measurement of the intensity of the formed pink color (malonaldehyde) as a result of oxidation at a wavelength of 532 nm. The obtained absorbance value was multiplied by 5.2 and the malonaldehyde concentration of the product was determined as mg MA/ kg (Witte *et al.,* 1970).

2.9.Free fatty acid (FFA)

Free fatty acid value was analyzed according to AOAC (2012) procedure.

2.10 Color

Color parameters of chicken meat emulsions were measured quadruple for each group during refrigerated storage by using a portable colorimeter (Chromameter CR400, Minolta, Japan) and parameters were phrased as lightness (L*), redness (a*), and yellowness (b*).

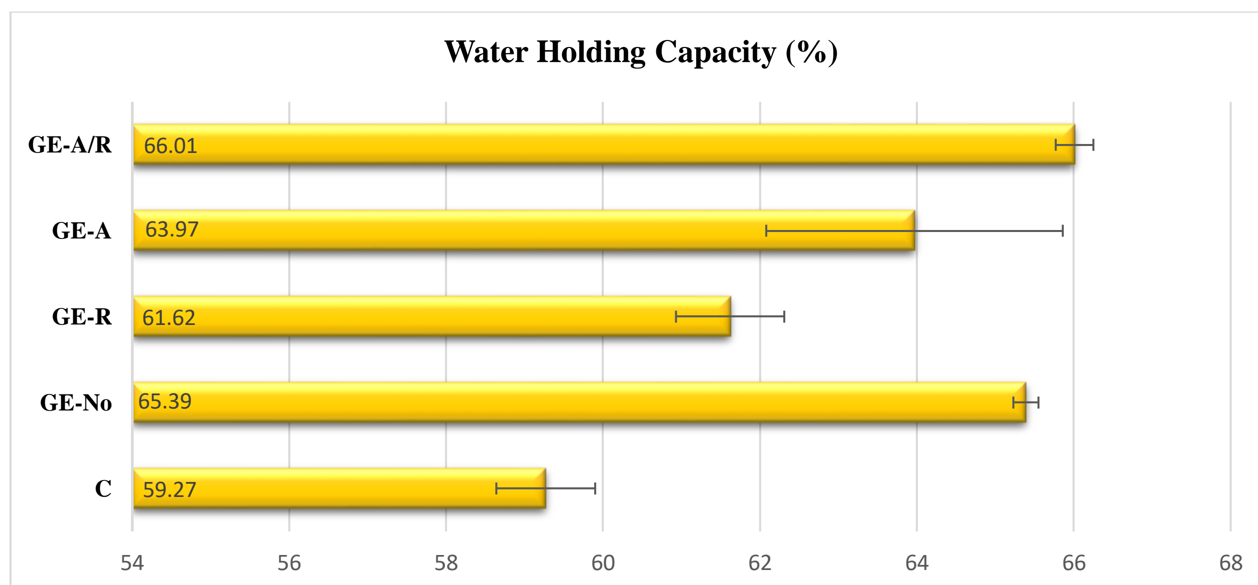
2.11.Statistical analysis

Statistical analyses were carried out using SPSS program (IBM, version 21.0, USA). A one-way ANOVA was applied to examine the effects of using gelled emulsions loaded with antioxidants to different phases on the chemical composition and water holding capacity. A model that included a two-way analysis of variance (ANOVA) was carried out to analyze the effect of treatment (C, GE-No, GE-R, GE-A, GE-A/R) and storage day (days 0, 3, and 7) on the pH, color, peroxide and TBARS value analyses. For these analyses, treatments and storage days were considered as fixed effects, while trial was included in the model as a random effect. Significant differences that affect analysis are examined by Duncan multiple tests at a 95% confidence level. The results of this study were reported as the mean values and standard deviation of the mean.

3.Results and discussions

3.1. Chemical composition and water holding capacity

Table 3 presents the chemical composition of heat-treated chicken meat emulsions. All chemical composition parameters except fat content are affected by the fat source (P<0.05). Moisture, protein, fat, and ash contents of samples varied between 69.46-71.71%, 12.99-14.58%, 12.36-12.76%, and 2.94-3.14% respectively. It is observed that samples incorporated with GE had lower moisture content compared to control sample prepared with beef fat, however, the adverse trend has been observed in protein and ash contents (P<0.05). These findings could be associated with egg white powder added to GE formulation. Lower moisture content of samples could be the result of the calculated equal fat contents of CME samples. In model meat emulsions when fat is completely replaced with gelled emulsion, lower moisture content was reported by Serdaroğlu *et al.* (2016). In a study where pork back fat in frankfurter formulation replaced by unripe banana by-products and pre-emulsified sunflower oil, the addition of pre-emulsion had a moisture increasing effect was reported (Pereira *et al.,* 2020).



ab: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.
C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

Figure 1. Water holding capacity of chicken meat emulsions

Table 3. Chemical composition and of chicken meat emulsions

Sample	Moisture (%)	Protein(%)	Fat(%)	Ash(%)
C	71.71±0.72[a]	12.99±0.64[b]	12.36±0.05	2.94±0.12[b]
GE	69.46±0.67[b]	14.19±0.09[a]	12.98±0.42	3.08±0.01[a]
GE-R	70.14±0.28[b]	14.13±0.32[a]	12.67±0.04	3.06±0.00[a]
GE-A	69.81±0.14[b]	14.58±0.29[a]	12.57±0.47	3.04±0.04[ab]
GE-A/R	69.88±0.67[b]	14.22±0.41[a]	12.76±0.32	3.14±0.06[a]

ab: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

WHC values of CME samples are shown in Figure 1. All GE added samples were more successful in terms of keeping water compared to C treatment (P<0.05). The increment in WHC could be explained by the water absorption ability of inulin in GE formulation. Inulin also reduced the water release in CME probably due to the strong electrostatic interactions and hydrogen bonds between meat proteins. The same phenomena are explained by covalent bonds of gelatin which is included in the aqueous phase of gel emulsion, which may also increase the water-holding of the system (Serdaroğlu *et al.,*2016). Negative effects on the liquid release have been reported in a study where gelled emulsion containing olive oil, chia mucilage + carboxymethylcellulose, or sodium alginate was added to model meat emulsions (Câmara *et al.,*2020). GE-No samples had good water retention ability than GE-R sample (P<0.05), however, they had similar WHC values with GE-A and GE-A/R. The lowest WHC values (61.62%) were found in samples incorporated with GE loaded with rosemary extract (P<0.05). WHC of samples formulated with GE is in line with pH results, samples with higher pH had high WHC (Figure 1). Additives might affect the space between the filaments and net charges of protein molecules thus help to keep moisture in the structure (Yogesh *et al.,* 2012). Another reason for alterations in WHC could be the possible effect of protein oxidation that yielded conformational changes in proteins. Also, similarly, Serdaroğlu *et al.* (2017) reported an increment in WHC in samples prepared with a gelled emulsion containing pumpkin seed oil in model meat emulsions.

3.2. pH

Table 4 shows the pH values of CME samples throughout the 7 days of storage at 4 °C. Initial pH values ranged from 6.11-6.16. Antioxidant addition to GE affected the pH values (P<0.05). Control samples prepared with beef fat, GE-No, and GE-A/R samples had similar pH values which were higher than GE-R and GE-A (P<0.05). Also, meat emulsions formulated with a gelled emulsion containing olive oil, chia mucilage + whey resulted in lower pH values (Câmara *et al.,* 2020). Pourashouri *et al.,* (2020) reported that fish sausages formulated with fish oil gelled emulsion had higher pH values than control treatments, nevertheless, samples that contain green tea extract in gelled emulsion formulation had lower pH values than gelled emulsion added samples. At the end of the storage pH values were 6.23, 6.18, 6.22, 6.22, and 6.19 for C, GE-No, GE-R, GE-A, and GE-A/R respectively. The highest pH value belonged to C sample (P<0.05). Relatively lower pH values could be attributed to the usage of flaxseed oil or gelatin. Similarly, Pintado, Herrero, Jimenez-Colmenero and Ruiz-Capillas (2016) reported that frankfurters formulated with gelled emulsion consisting of 2 % gelatin showed the lowest pH.

Table 4. pH values of chicken meat emulsions throughout 7 days of storage

pH	0th day	3rd day	7th day
C	$6.16\pm0.02^{a,y}$	$6.22\pm0.02^{a,x}$	$6.23\pm0.01^{a,x}$
GE	$6.15\pm0.01^{a,z}$	$6.23\pm0.01^{a,x}$	$6.18\pm0.01^{c,y}$
GE-R	$6.11\pm0.01^{b,z}$	$6.20\pm0.01^{b,y}$	$6.22\pm0.01^{b,x}$
GE-A	$6.12\pm0.01^{b,y}$	$6.22\pm0.01^{a,x}$	$6.22\pm0.01^{b,x}$
GE-A/R	$6.15\pm0.00^{a,z}$	$6.22\pm0.01^{a,x}$	$6.19\pm0.00^{c,y}$

abc: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.
C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

3.3. Peroxide value

Peroxide values of samples are displayed in Table 5. Initial peroxide values were affected by fat replacement (P<0.05). All peroxide values were lower than 25 meqO2/kg which is a limit for fatty food products (Evranuz, 1993). At the beginning of the storage, even samples formulated with GE had higher PV than the control treatment, combined use of antioxidants lowered the PVs amongst the GE added treatments. Peroxide values of all samples increased (P<0.05) during the storage except GE-A, the addition of ascorbic acid maintained the oxidative stability along with the storage. Freire *et al.* (2017) reported that when animal fat in pork patties was replaced with perilla oil gelled emulsion at levels of 66 and 100%, modified samples had higher hydroperoxide concentration at the end of the storage. The highest PVs were obtained in C (7.98 meqO2/kg) and GE-No (7.96 sample meqO2/kg) groups (P<0.05). In a study conducted by Pelser *et al.* (2007) using pre-emulsified flaxseed oil resulted in higher peroxide values in fermented sausages than control groups at the end of the storage. At the end of the storage replacing beef fat by flaxseed oil had neither lowering nor increasing effect on PV, however, the use of ascorbic acid and rosemary alone or combined in GE are found to be effective to delay primary lipid oxidation by locating oil-water interphase where hydroperoxides exist. Similar to our results, incorporation of blackthorn branch extract through gelled emulsion prepared with microalgal oil in reduced-fat beef patties resulted in lower peroxide values (Alejandre *et al.,* 2019).

Table 5. Peroxide values of chicken meat emulsions throughout 7 days of storage

Peroxide (meqO2/kg)	0th day	3rd day	7th day
C	$2.95\pm1.01^{b,z}$	$5.96\pm0.05^{a,y}$	$7.98\pm0.01^{a,x}$
GE	$6.96\pm0.96^{a,x}$	$4.98\pm1.00^{a,y}$	$7.96\pm0.02^{a,x}$
GE-R	$6.91\pm1.01^{a,x}$	$4.44\pm0.51^{a,y}$	$5.63\pm0.56^{b,xy}$
GE-A	$5.95\pm0.05^{a,x}$	$4.97\pm0.98^{a,x}$	$5.99\pm0.02^{b,x}$
GE-A/R	$3.39\pm0.48^{b,z}$	$4.61\pm1.17^{a,xy}$	$6.00\pm0.00^{b,x}$

ab: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.
C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

3.4. TBARS values

Replacement of beef fat and storage period were found effective on TBARS values (P<0.05). Initially, C sample had the lowest (0.25 mg MA/kg) TBARS value. All modified treatments had higher TBARS values due to the presence of flaxseed oil in formulation, however, induvial use of rosemary (GE-R samples) or ascorbic acid (GE-A samples) resulted in lower TBARS values. The mean TBARS values of CME samples which ranged from 0.25- 1.04 mg MA/ kg are displayed in Table 6. TBARS values of all samples were lower than limiting threshold value (2 mg MA/kg) (Witte *et al.,*1970). During the storage decrements and followed by increments have been observed in TBARS values of all antioxidants added counterparts (P<0.05). Increments recorded in TBARS values during storage possibly be explained by a higher formation ratio of malonaldehydes than the disappearance, nonetheless, after a while, the disappearance ratio surpasses the formation ratio, thus, TBARS values tend to decrease (Delgado-Pando *et al.,*2011). The rate of malonaldehyde disappearance throughout the storage may have exceeded the rate of production as a consequence of intermolecular reactions between malonaldehydes and amino acids or proteins (Jamora and Rhee, 2002). Throughout the 3 days of storage, the C sample had the lowest value while GE-A/R had the highest (P<0.05). Up to 3-day TBARS values of antioxidant added samples raised, GE-A/R showed the highest value amongst all samples and storage days, however, on day 7 rosemary and ascorbic acid showed an antioxidant effect. Similar to our findings, W/O nanoemulsion formulated with orange essential oil and cactus fruit acid was used in emulsified meat products to substitute pork fat up to 5%, resulted in lower TBARS values in a dosed manner at the end of the storage (Almaráz-Buendia *et al.,* 2019). Oxidative reactions could be retarded better in salami formulated with O/W emulsion (caprylic, capric, and lauric triglyceride mixture) loaded with 100 g/kg rosemary than sample formulated with emulsion without rosemary (Erdmann *et al.,* 2017). Even so, the amount of GE is the same as beef fat added to control group, TBARS values were lower in samples formulated with gelled emulsion containing flaxseed oil after 7 days of storage. Lower TBARS values are probably linked to the preservation of easily perishable flaxseed oil against oxidation by the surrounding gel network which is created by inulin and gelatin. Also, it could be said that using GE incorporated with ascorbic acid or combined with rosemary was found as a successful strategy to control the oxidation reactions at the end of 7-days of storage. Also, some studies reported that the combined use of various antioxidants with ascorbic acid decreased the TBARS values (Kim *et al.,* 2013; Hwang *et al.,* 2017).

It is thought that well protection of highly perishable flaxseed oil by the gelled emulsion which is formed with inulin and gelatin could be the reason for lower TBARS values. Similarly, Robert *et al.* (2019) reported that in a meat system double emulsion added with olive leaves extract exhibited a successful barrier against oxidation for linseed, fish, and olive oils mixture.

In our study, we found that ascorbic acid with a hydrophilic character was found more effective than lipophilic rosemary extract in model chicken meat emulsions. This opposite 'polar paradox' behavior could be explained using egg white powder and inulin may enhance the antioxidant activity of ascorbic acid through interactions or the level of rosemary extract could be insufficient. Also, oxidation occurs in oil-water interphase, so nonpolar antioxidants above critical concentration goes into the oil phase instead of collecting at the oil-water interphase. Another probable reason for this particular

result is that pro-oxidants in the aqueous phase could be inactivated by the effect of ascorbic acid. Opposite to our results, in fish sausages, hydrophilic polar green tea extract in fish oil gelled emulsion formulation did not show an antioxidant effect compared to the samples formulated with gelled emulsion alone due to soy protein aggregates placed in the interphase (Pourashouri *et al.,* 2020). In a study reported by Noon *et al.* (2020) low concentration (0.1 and 0.4 mg) of ascorbic acid was found more effective than non-polar curcumin in terms of delaying the oxidation in sunflower oil emulsions where polysorbate 20 is used as surfactant throughout the 7 days of storage.

Table 6. TBARS values of chicken meat emulsions throughout 7 days of storage

TBARS (mgMA/kg)	0th day	3rd day	7th day
C	$0.25 \pm 0.01^{e,y}$	$0.25 \pm 0.03^{c,y}$	$0.94 \pm 0.03^{a,x}$
GE	$0.71 \pm 0.03^{b,y}$	$0.71 \pm 0.02^{b,y}$	$0.83 \pm 0.03^{b,x}$
GE-R	$0.62 \pm 0.02^{c,z}$	$0.97 \pm 0.00^{a,x}$	$0.86 \pm 0.03^{b,y}$
GE-A	$0.57 \pm 0.01^{d,y}$	$0.72 \pm 0.08^{b,x}$	$0.48 \pm 0.02^{c,y}$
GE-A/R	$0.87 \pm 0.01^{a,y}$	$1.04 \pm 0.13^{a,x}$	$0.53 \pm 0.01^{c,z}$

abcd: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

Table 7. Free fatty acid values of chicken meat emulsions throughout 7 days of storage

FFA (%oleic acid)	0th day	3rd day	7th day
C	$0.34^{c,z} \pm 0.07$	$0.68^{d,y} \pm 0.13$	$1.68^{b,x} \pm 0.07$
GE	$1.01^{a,y} \pm 0.08$	$0.83^{cd,y} \pm 0.01$	$1.45^{b,x} \pm 0.33$
GE-R	$0.36^{c,y} \pm 0.12$	$1.15^{ab,xy} \pm 0.15$	$1.69^{b,x} \pm 0.28$
GE-A	$0.64^{b,y} \pm 0.13$	$0.97^{bc,y} \pm 0.13$	$2.79^{a,x} \pm 0.55$
GE-A/R	$1.07^{a,y} \pm 0.08$	$1.23^{a,y} \pm 0.12$	$2.40^{a,x} \pm 0.14$

abc: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xyz: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.

C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

3.5. Free fatty acid

The initial FFA values were between 0.34 and 1.07% (oleic acid) and increased to 1.45–2.79% (oleic acid) after 7 days of storage (Table 7). At each period of storage treatment formulated with beef fat had lower FFA value (0.34%). Microbial and muscle lipases attack mainly unsaturated fatty acids for hydrolysis. Since flaxseed oil contains high amounts of unsaturated fatty acids it was expected that lipolysis was more rapid in all modified treatments. It could be seen that, just after the production (day 0), FFA value of GE-No and GE-A/R showed similar trends

with TBARS values (Table 6). Samples with rosemary extract and control samples had similar FFA values at the beginning of storage. No significant differences were observed in the FFA values on the 3rd day of storage compared to day 0 except C samples. Ascorbic acid addition was ineffective to block the generation of FFA (P<0.05). FFA values of GE-No and GE-R were similar to C at the end of storage. However, on the 7th day, FFA values of all samples showed an increase, as a result of the hydrolysis of phospholipids which is in an agreement with other studies (Geçgel *et al.,* 2015; Reddy *et al.*, 2017).

3.6. Color

Table 8 shows the L* values of treatments formulated with GE and/or GE combined with antioxidants. L* values varied between 76.33-82.94, and the incorporation of GE resulted in higher L* values (P<0.05). Same findings also reported for model meat emulsions formulated with GE consisted of gelatin and inulin (Serdaroğlu *et al.,* 2016; Serdaroğlu and Öztürk, 2017).

Table 8. L*, a* and b* values of chicken meat emulsions throughout 7 days of storage

L*	**0th day**	**3rd day**	**7th day**
C	76.33±0.49c,y	78.69±0.13d,x	78.06±0.50e,x
GE	82.94±0.40a,x	82.67±0.93a,x	82.49±0.47a,x
GE-R	82.30±0.39a,x	82.33±0.16a,x	81.76±0.32b,x
GE-A	82.32±0.32a,x	81.42±0.13b,y	81.10±0.10c,y
GE-A/R	78.75±0.50b,y	80.11±0.37c,x	80.33±0.26d,x
a*	**0th day**	**3rd day**	**7th day**
C	0.65±0.16b,y	2.07±0.17a,x	1.98±0.13a,x
GE	-0.20±0.11c,z	0.77±0.15c,x	0.51±0.02d,y
GE-R	0.36±0.21b,y	0.83±0.15c,x	0.76±0.16c,x
GE-A	0.51±0.25b,y	1.31±0.13b,x	0.99±0.03b,x
GE-A/R	1.14±0.07a,x	0.83±0.07c,y	0.53±0.04d,z
b*	**0th day**	**3rd day**	**7th day**
C	8.46±0.36c,x	7.42±0.25c,y	6.81±0.16c,z
GE	14.24±0.06ab,x	12.67±0.15b,z	13.05±0.25b,y
GE-R	14.48±0.22ab,x	13.57±0.25a,y	13.73±0.59a,xy
GE-A	13.94±0.45b,x	12.86±0.55b,y	13.01±0.13b,y
GE-A/R	14.70±0.22a,x	13.84±0.21a,y	13.89±0.36a,y

abc: means with the different letter in the same column are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates. xy: means with the different letter in the same row are significantly different (*P<0.05*), all values are mean ±standard deviation of three replicates.
C: only beef fat added chicken meat emulsions, GE: only GE added chicken meat emulsions, GE-R : CME prepared with GE incorporated with rosemary extract in oil phase, GE-A: CME prepared with GE incorporated with ascorbic acid in water phase, GE-A/R: CME prepared with GE incorporated with rosemary extract in oil phase and ascorbic acid in water phase.

Gelled emulsions have abundantly smaller droplet sizes than beef fat globules therefore the reflection of light on the surface of these treatments was more than in control groups. Increased L* values were also reported by Poyato *et al.* (2014). Using rosemary or ascorbic acid separately in GE did not affect initial L* values. L* values of GE-No and GE-R groups remained stable throughout the storage, while L* values of C and GE-A/R

samples increased. The results obtained on day 7 showed that C samples had the lowest L* values (P<0.05). Similar to our results, Barros *et al.* (2020) reported that tiger nut oil emulsion increased the L* values of beef burgers.

a* values of samples are presented in Table 8. Concerning a* values (red color), there was a significant increase from day 0 to 3rd day for all treatments (P<0.05). This increment indicates the conversion of meat pigment to nitroso pigments (Sakata, 2000). On day 0, GE treatment showed the lowest a* value, while GE-A/R had the highest (P<0.05). a* values were affected by the storage time (P<0.05). An increment followed by a decrement was seen in a* values of all treatments throughout the storage, C treatment showed higher a* values compared to other counterparts. After 3rd day of storage using GE and incorporating the antioxidants reduced the * values. Green tea extract-loaded fish oil gelled emulsion also had the same reducing effect on the fish sausages (Pourashouri *et al.,* 2020).

Yellowness (b*) does not greatly impact the appearance of meat color, however, this value is negatively correlated with the oxidation processes (Luciano *et al.,* 2009) while as the lipid oxidation proceeds a* values of meat decrease (Zhang *et al.,* 2013). The lowest b*values of control samples are related to the oxidation process. As can be seen in Table 5, on the last day of storage higher TBARS values were found for C samples, therefore the color of C treatment may have been negatively influenced.

b* values were changed between 6.81-14.70 during 7 days of storage (Table 8). At all storage periods, C treatment formulated with beef fat exhibited the lowest b* values (P<0.05). Similar findings also reported by Wang *et al.* (2018) in Harbin sausages formulated with camellia oil gelled emulsion at different levels. Also, an increase in b* values reported in hamburgers with animal fat is replaced with hydrogelled emulsion formulated with linseed oil and chia (Heck *et al.,* 2019). There was a general decline in all samples throughout the storage most probably due to the lipid oxidation reactions (P<0.05).

4. Conclusions

According to the results found in this study, it was possible to produce stable gelled emulsions formulated with rosemary extract and/or ascorbic acid. Oxidation reactions of model chicken meat emulsions were affected by both GE addition and antioxidant incorporation. Lipid oxidations at the end of the storage were lower in samples formulated with a gelled emulsion containing flaxseed oil by the reason of the preservation of easily perishable flaxseed oil against oxidation by surrounding with gel network which is created by inulin and gelatin. Gelled emulsion is a good source for highly perishable oils; however, inhibition of oxidation is more pronounced when ascorbic acid or ascorbic acid+rosemary extract loaded to the gelled emulsion formulation which was used as a fat source. Polar ascorbic acid was found the most effective antioxidant unlike 'polar paradox'. However further researches should be implemented with different emulsion formulations and antioxidants or different meat products

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EVALUATION OF ANTIBACTERIAL POTENTIAL OF SELECTED CULINARY HERBS AGAINST SOME FOODBORNE PATHOGENIC BACTERIA

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ABSTRACT

Culinary herbs consist of bioactive compounds which play an important role as natural antimicrobial agents. The present study was carried out with the objective of evaluating the antibacterial activity of extracts from selected culinary herbs; *Trachyspermum involucratum*, *Laurus nobilis*, *Coriandrum sativum*, *Allium tuberosum*, *Allium schoenoprasum*, *Melissa officinalis*, *Origanum majorana*, *Origanum vulgare*, *Rosmarinus officinalis*, *Santolina chamaecyparissus,* and *Satureja hortensis*. Different extraction solvents (sterilized distilled water, hot distilled water (80°C), absolute methanol, and acetone) were used against three foodborne pathogens (*E. coli* NCTC 10418, *E. coli* ATCC 25922, and *Enterococcus faecalis*) using the agar-well diffusion method. Statistical analysis using two-factor factorial completely randomized design in SAS software revealed that all solvent extracts of *Trachyspermum involucratum* has the highest antibacterial activity ($p<0.05$) followed by *Rosmarinus officinalis*, *Santolina chamaecyparissus*, *Satureja hortensis*, *Origanum vulgare,* and *Coriandrum sativum* against all tested bacteria with variable potential. Further, hot distilled water (80°C) extract of *Trachyspermum involucratum* had significant antibacterial activity against *E. coli* NCTC 10418 (14.67 ± 1.53 mm). In particular, organic extracts of *Rosmarinus officinalis*, *Santolina chamaecyparissus* and *Satureja hortensis* had strong antibacterial activity against *E. coli* NCTC 10418 and *Enterococcus faecalis*. Overall, *Enterococcus faecalis* has highly inhibited the growth followed by *E. coli* NCTC 10418 and *E. coli* ATCC 25922 in extracts of the best anti-bacterially active herbs. The minimum inhibitory concentration of above the herb extracts was 0.2 g mL^{-1} against most of the tested pathogens. It can be concluded that culinary herbs are potentially effective as natural antimicrobials against tested foodborne pathogens.

1. Introduction

Foodborne diseases are an acute illness, a condition that occurs after ingestion of contaminated food and water. Major foodborne illnesses can be caused by foodborne pathogens like bacteria, fungi, viruses, parasites, and/or toxins through contaminated food or water (Sudershan *et al*., 2014). Amongst the various infectious pathogenic microbes *Corynebacterium diphtheria*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus, Enterococcus faecalis,* and *Pseudomonas aeruginosa* are of prime importance as common foodborne pathogens (Panpatil *et al*., 2013).

Today, food safety has become an important public health issue and researchers and

regulatory bodies in food industries are constantly bothered with an increase in the number of food poisoning and spoilage caused by pathogenic foodborne microorganisms (Pesavento *et al*., 2015). It is important to reduce the activity of foodborne pathogens in order to prevent the occurrence of foodborne diseases.

In the food industry, the use of food antimicrobials has become one of the promising solutions for foodborne diseases (Sankar *et al*., 2013). However, the indiscriminate use of chemical preservatives and the misuse of commercial antibiotics over the years have been led to the emergence of antibiotic-resistant pathogens that can amplify foodborne infections has become another major health concern (Witkowska *et al*., 2013). In this regard, the use of natural agents as herbs especially, the culinary herbs provide a better alternative for the development of novel drugs for human being and play a vital role in the alterations of worse conditions produced by infectious foodborne microorganisms (Weerakkody *et al*., 2010).

Culinary herbs are herbaceous plants which primarily used for, and associated with, adding to or enhancing the flavor, aroma, pungency, and appearance of foods (Opara and Chohan, 2014). Culinary herbs have always been an important component of the human diet, which add characteristic flavor to the staple foods, as well as being used for food preservation. They are secondarily used as a natural antimicrobial agent, which contains bioactive compounds. Therefore, today culinary herbs are considered as the most important source for the development of natural antimicrobial agents for different selective foodborne pathogens (Bozin *et al*., 2007).

Therefore, this research is one of the attempts to focus on the potential of culinary herbs as natural antimicrobial agents to control the foodborne diseases caused by selective foodborne pathogenic bacteria. The main objective of this study was to evaluate the antibacterial potential of selected culinary herb extracts and determine their Minimum Inhibitory Concentration (MIC) against selected foodborne bacterial pathogens.

2. Materials and methods

2.1. Materials

2.1.1. Collection of Culinary Herbs

Asamodagam (*Trachyspermum involucratum* L.), bay leaves (*Laurus nobilis* L.), coriander (*Coriandrum sativum* L.), garlic chives (*Allium tuberosum*), onion chives (*Allium schoenoprasum*), lemon mint (*Melissa officinalis*), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.), rosemary (bush & tree type) (*Rosmarinus officinalis* L.), santolina/lavender cotton (*Santolina chamaecyparissus* L.) and savory/summer savory (*Satureja hortensis*) were collected from Agriculture Research Station – Seetha Eliya, Department of Agriculture, Sri Lanka (Table 1).

Table 1. Types of Culinary Herb

Common Name	Family Name	Scientific Name	Parts used
Asamodagam/ Ajamoda	Apiaceae	*Trachyspermum involucratum* L.	Leaves
Bay leaves	Lauraceae	*Laurus nobilis* L.	Leaves
Coriander/Cilantro	Apiaceae	*Coriandrum sativum* L.	Leaves
Garlic chives	Liliaceae	*Allium tuberosum*	Leaves
Onion chives	Liliaceae	*Allium schoenoprasum*	Leaves
Lemon mint	Lamiaceae	*Melissa officinalis*	Leaves
Marjoram	Lamiaceae	*Origanum majorana* L.	Leaves
Oregano	Lamiaceae	*Origanum vulgare* L.	Leaves

Rosemary (Bush)	Lamiaceae	*Rosmarinus officinalis* L.	Leaves
Rosemary (Tree)	Lamiaceae	*Rosmarinus officinalis* L.	Leaves
Santolina/Lavender cotton	Asteraceae	*Santolina chamaecyparissus* L.	Leaves
Savory/Summer savory	Lamiaceae	*Satureja hortensis*	Leaves

2.1.2. Pathogen Collection

Pure cultures of foodborne bacterial pathogens; *Escherichia coli* NCTC 10418, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* were obtained from the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka. The bacterial strains were grown in Nutrient agar medium at 37 ^{0}C temperature and the stock cultures were maintained in Nutrient agar medium at 4 ^{0}C and sub-cultured at regular intervals (Allard *et al.*, 2018).

2.2. Methods

2.2.1. Preparation of Culinary Herb Extracts

Culinary herb samples were washed with tap water, dried under shade, and ground separately using a motor and pestle. The ground sample was sieved through a 10 mm sieve. Then 10 g of sieved sample was dissolved in 100 mL of sterilized distilled water, hot distilled water (80 ^{0}C), absolute methanol, and absolute acetone to prepare the extracts (Hinneburg *et al.*, 2006). The mixture was swirled continuously at 120 rpm in an orbital shaker for one hour. Extracts were filtered through Whatman filter paper No: 01 (150 mm). The filtrate was concentrated by rotary evaporator under reduced pressure at 40 ^{0}C temperature and stored at 4 ^{0}C for further analysis (Wong and Kitts, 2006).

2.2.2. Preparation of Bacterial Inoculum

Bacterial inoculum was prepared by matching with 0.5 McFarland turbidity standards (10^8 CFU/mL) and UV visible spectrophotometer was used to measure the absorbance (Mostafa *et al.*, 2018).

(0.5 McFarland turbidity = 0.08 to 0.10 absorbance specification at 625 nm)

2.2.3. Antibiotic Susceptibility Testing (ABST)

Antibacterial activity of herb extracts against test pathogens was assessed by the Agar Well Diffusion method (Sharififar *et al.*, 2007). 100 µL of bacterial inoculum prepared by matching with 0.5 McFarland turbidity standards (10^8 CFU/mL) was spread on the Mueller Hinton (MH) agar in Petri plates using a sterile cotton swab. Wells of 8 mm diameter was bored in the inoculated media with the help of a sterile cork-borer (8 mm). Then, each well was filled with 50 μL of test herb extract (Reller *et al.*, 2009). 50 μL aliquots of positive control and negative control were dispensed into wells of the same plate. The plates were incubated at 37 ^{0}C for 24 - 48 h aerobically. After incubation, the diameter of the inhibition zone was measured in millimeters using a vernier caliper (Ruangpan and Tendencia, 2004).

Sterilized distilled water, hot distilled water (80 ^{0}C), absolute methanol, and absolute acetone were used as negative controls and Amoxicillin was used as a positive control for bacterial pathogens (Jeyaseelan and Jashothan, 2012).

2.2.4. Determination of Minimum Inhibitory Concentrations (MIC)

The crude extraction was done with solvents and prepared 0.2 g mL^{-1}, 0.15 g mL^{-1}, 0.1 g mL^{-1}, and 0.05 g mL^{-1} different concentrations separately and stored in -20 ^{0}C in order to determine the Minimum Inhibitory concentration (MIC) of different herb extracts using the Agar Well Diffusion method as previously described in the ABST testing (Shan *et al.,* 2007).

2.2.5. Statistical Analysis

All experiments were done in triplicate (n = 3) and the inhibition zone diameter was expressed as means ± standard deviation (SD). The results were statistically analyzed in two-

factor factorial Completely Randomized Design (CRD) using the SAS program (Version 9.0, SAS Institute Inc. USA). Means were compared using Tukey's simultaneous test set at $p < 0.05$.

3. Results and discussions

3.1. Antibacterial Activity of Herb Extracts

Twelve herb species were investigated to evaluate their antibacterial activity against some foodborne pathogenic bacteria, including two strains of *E. coli* (*E. coli* NCTC 10418 and *E. coli* ATCC 25922) and one strain of *Enterococcus faecalis* using the Agar-well diffusion method. Evaluation of the antibacterial activity of these herb extracts was recorded by the mean diameters of the inhibition zone of all herb extracts against three foodborne pathogens in Table 2. The results revealed that most of the herb extracts were potentially effective in suppressing microbial growth of foodborne pathogenic bacteria with variable potency.

According to Tukey's simultaneous test set at $p<0.05$ in the SAS program, *Trachyspermum involucratum* L. has the highest antibacterial activity against three foodborne pathogenic bacteria followed by *Rosmarinus officinalis* L. (Bush), *Santolina chamaecyparissus* L., *Satureja hortensis*, *Origanum vulgare* L. and *Coriandrum sativum* L. respectively. Other herbs have a minimum or may not have the antibacterial activity against the tested foodborne bacterial pathogens.

Table 2. Antibacterial Activity (Diameter of Inhibition Zone) of Herb Extracts

Herb	Type of Extract	Antibacterial Activity (DIZ) (mm)		
		E. coli NCTC 10418	*E. coli* ATCC 25922	*Enterococcus faecalis*
***Origanum vulgare* L.**	Distilled water	$8.33^{b} \pm 0.58$	$10.00^{ab} \pm 1.00$	$11.00^{b} \pm 1.00$
	Hot Distilled water	$8.33^{b} \pm 0.58$	$8.68^{ab} \pm 0.58$	$10.00^{b} \pm 0.00$
	Acetone	$8.33^{b} \pm 0.58$	$8.00^{b} \pm 0.00$	$15.33^{a} \pm 0.58$
	Methanol	$10.67^{a} \pm 0.58$	$10.67^{a} \pm 1.15$	$11.33^{b} \pm 0.58$
***Rosmarinus officinalis* L. (Tree)**	Distilled water	$8.00^{b} \pm 0.00$	$8.00^{c} \pm 0.00$	$8.00^{b} \pm 0.00$
	Hot Distilled water	$8.00^{b} \pm 0.00$	$8.33^{bc} \pm 0.58$	$9.67^{b} \pm 0.58$
	Acetone	$10.67^{a} \pm 0.58$	$9.67^{a} \pm 0.58$	$17.67^{a} \pm 1.53$
	Methanol	$8.33^{b} \pm 0.58$	$9.33^{ab} \pm 0.58$	$8.67^{b} \pm 0.58$
***Rosmarinus officinalis* L. (Bush)**	Distilled water	$9.33^{b} \pm 0.47$	$9.33^{b} \pm 0.58$	$8.00^{c} \pm 0.00$
	Hot Distilled water	$8.67^{b} \pm 0.58$	$9.00^{b} \pm 0.00$	$8.33^{c} \pm 0.58$
	Acetone	$14.00^{a} \pm 1.00$	$11.67^{a} \pm 0.58$	$16.67^{a} \pm 0.58$
	Methanol	$12.33^{a} \pm 0.58$	$11.33^{a} \pm 0.58$	$12.67^{b} \pm 0.58$
Melissa officinalis	Distilled water	$8.00^{b} \pm 0.00$	$9.67^{a} \pm 0.58$	$9.00^{b} \pm 1.00$
	Hot Distilled water	$8.00^{b} \pm 0.00$	$8.00^{c} \pm 0.00$	$8.00^{b} \pm 0.00$
	Acetone	$8.00^{b} \pm 0.00$	$9.33^{ab} \pm 0.58$	$14.33^{a} \pm 1.15$
	Methanol	$9.33^{a} \pm 0.58$	$8.33^{bc} \pm 0.58$	$8.67^{b} \pm 0.58$

Allium schoenoprasum	Distilled water	$8.00^{a} \pm 0.00$	$8.00^{b} \pm 0.00$	$8.00^{b} \pm 0.00$
	Hot Distilled water	$8.00^{a} \pm 0.00$	$8.00^{b} \pm 0.00$	$8.00^{b} \pm 0.00$
	Acetone	$8.00^{a} \pm 0.00$	$8.00^{b} \pm 0.00$	$14.67^{a} \pm 1.53$
	Methanol	$8.33^{a} \pm 0.58$	$9.00^{a} \pm 0.00$	$8.33^{b} \pm 0.58$
Allium tuberosum	Distilled water	$8.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$	$8.00^{c} \pm 0.00$
	Hot Distilled water	$8.00^{a} \pm 0.00$	$8.33^{a} \pm 0.58$	$10.00^{b} \pm 0.00$
	Acetone	$8.33^{a} \pm 0.58$	$8.33^{a} \pm 0.58$	$12.67^{a} \pm 0.58$
	Methanol	$8.33^{a} \pm 0.58$	$8.33^{a} \pm 0.58$	$9.33^{c} \pm 0.58$
***Laurus nobilis* L.**	Distilled water	$12.67^{b} \pm 0.58$	$9.33^{a} \pm 0.58$	$8.67^{a} \pm 0.58$
	Hot Distilled water	$8.33^{c} \pm 0.58$	$8.00^{b} \pm 0.00$	$8.00^{a} \pm 0.00$
	Acetone	$17.33^{a} \pm 0.58$	$8.00^{b} \pm 0.00$	$8.00^{a} \pm 0.00$
	Methanol	$8.33^{c} \pm 0.58$	$8.33^{ab} \pm 0.58$	$8.33^{a} \pm 0.58$
Satureja hortensis	Distilled water	$9.33^{c} \pm 0.58$	$9.67^{a} \pm 0.58$	$8.33^{b} \pm 0.58$
	Hot Distilled water	$9.33^{c} \pm 0.58$	$9.67^{a} \pm 0.58$	$8.00^{b} \pm 0.00$
	Acetone	$14.33^{a} \pm 1.15$	$10.33^{a} \pm 0.58$	$10.00^{a} \pm 0.00$
	Methanol	$11.67^{b} \pm 0.58$	$11.33^{a} \pm 1.15$	$10.67^{a} \pm 0.58$
***Origanum majorana* L.**	Distilled water	$9.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$	$8.00^{b} \pm 0.00$
	Hot Distilled water	$8.00^{b} \pm 0.00$	$8.33^{a} \pm 0.58$	$8.00^{b} \pm 0.00$
	Acetone	$8.00^{b} \pm 0.00$	$8.00^{a} \pm 0.00$	$9.33^{a} \pm 0.58$
	Methanol	$9.33^{a} \pm 0.58$	$8.67^{a} \pm 0.58$	$9.33^{a} \pm 0.58$
***Santolina chamaecyparissus* L.**	Distilled water	$9.67^{c} \pm 0.58$	$8.33^{c} \pm 0.58$	$8.33^{b} \pm 0.58$
	Hot Distilled water	$9.00^{c} \pm 0.00$	$9.33^{bc} \pm 0.58$	$9.33^{b} \pm 0.58$
	Acetone	$14.67^{a} \pm 0.58$	$10.33^{ab} \pm 0.58$	$9.67^{b} \pm 0.58$
	Methanol	$11.33^{b} \pm 0.58$	$11.33^{a} \pm 0.58$	$11.67^{a} \pm 0.58$
***Coriandrum sativum* L.**	Distilled water	$8.67^{b} \pm 0.58$	$11.33^{a} \pm 1.15$	$10.67^{a} \pm 1.15$
	Hot Distilled water	$9.67^{ab} \pm 0.58$	$10.00^{ab} \pm 0.00$	$9.67^{ab} \pm 0.58$
	Acetone	$10.67^{a} \pm 0.58$	$8.33^{b} \pm 0.58$	$8.33^{b} \pm 0.58$
	Methanol	$11.00^{a} \pm 1.00$	$11.00^{a} \pm 1.00$	$10.67^{a} \pm 0.58$
***Trachyspermum involucratum* L.**	Distilled water	$14.00^{a} \pm 1.00$	$12.00^{ab} \pm 1.00$	$10.00^{b} \pm 1.00$
	Hot Distilled water	$14.67^{a} \pm 1.53$	$14.00^{a} \pm 1.00$	$13.67^{a} \pm 0.58$

	Acetone	$10.33^{b} \pm 0.58$	$8.33^{c} \pm 0.58$	$11.00^{b} \pm 1.00$
	Methanol	$11.33^{b} \pm 0.58$	$11.67^{b} \pm 0.58$	$10.33^{b} \pm 0.58$

Notes: Data are means of three replicates (n = 3) ± standard deviation. The well diameter is 8.00 mm, if the DIZ is 8.00 mm means the extract had no activity against bacterium. Means with rows and column different letters are significantly (*p< 0.05*) different.

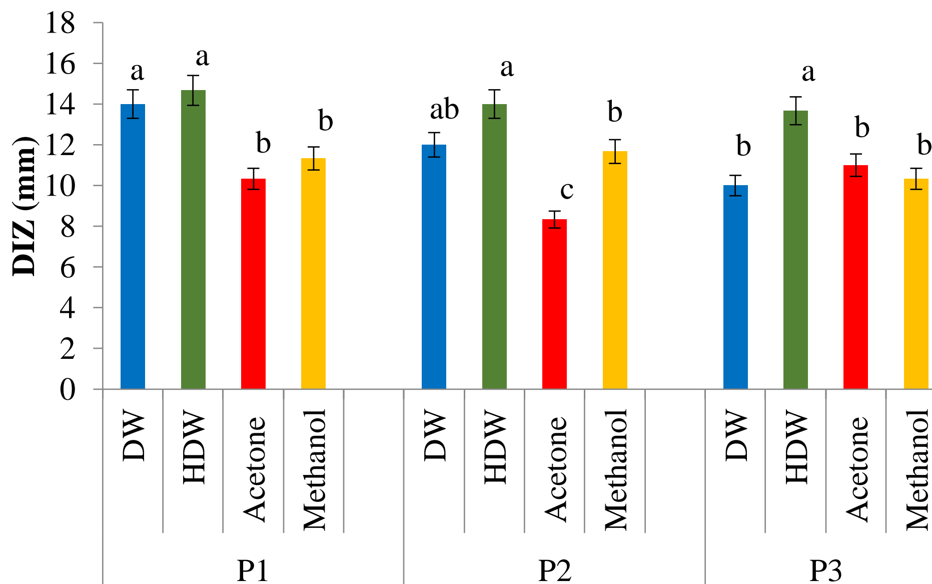

Figure 1. The diameter of the growth inhibition zone around the wells in *Trachyspermum involucratum* L. Columns with different letters are significantly (*p<0.05*) different. (P1- *E. coli* NCTC 10418, P2- *E. coli* ATCC 25922, P3- *Enterococcus faecalis*)

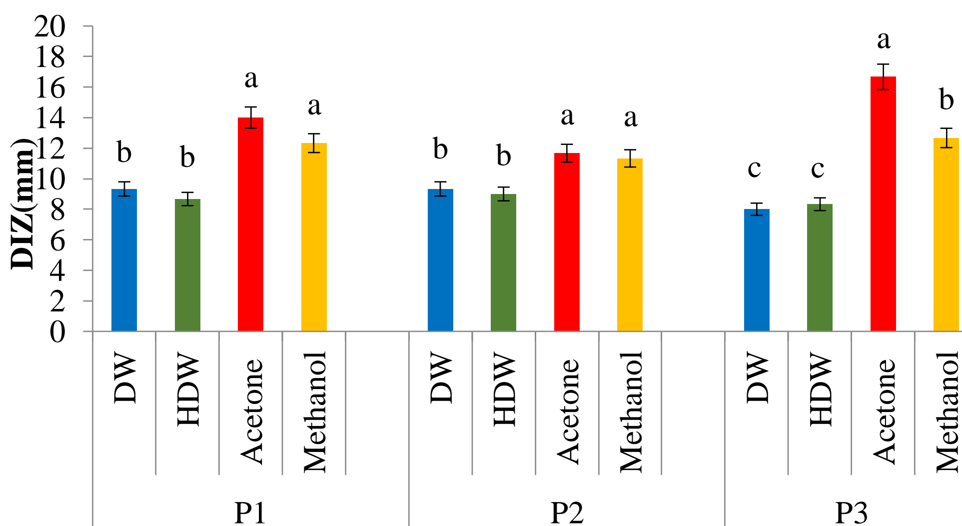

Figure 2. The diameter of the growth inhibition zone around the wells in *Rosmarinus officinalis* L. (Bush). Columns with different letters are significantly (*p<0.05*) different. (P1- *E. coli* NCTC 10418, P2- *E. coli* ATCC 25922, P3- *Enterococcus faecalis*)

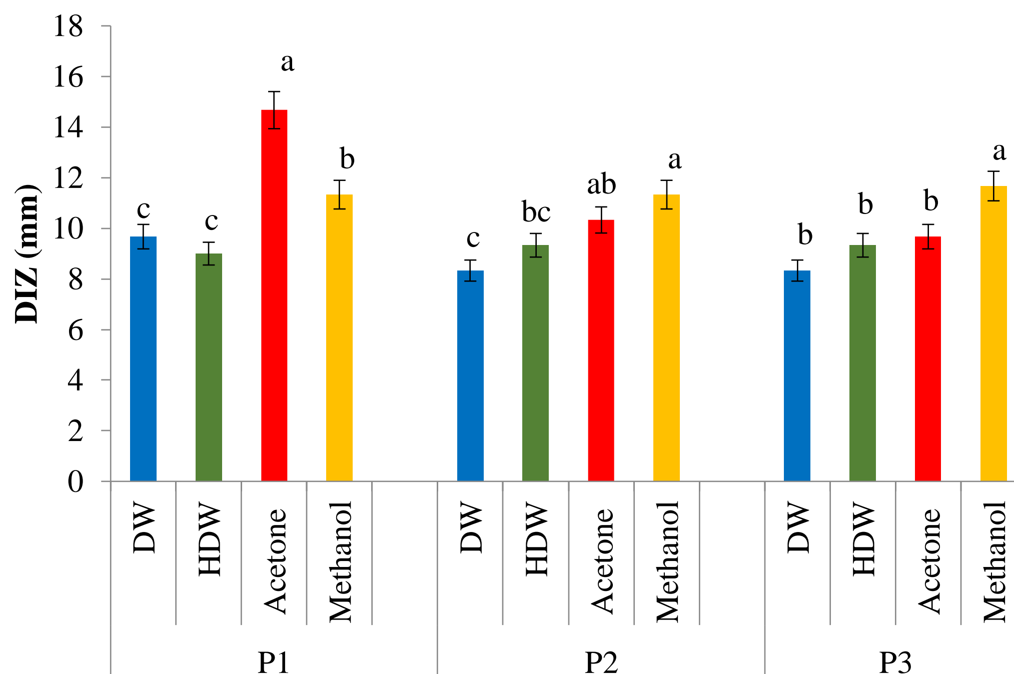

Figure 3. The diameter of the growth inhibition zone around the wells in *Santolina chamaecyparissus* L. Columns with different letters are significantly ($p<0.05$) different. (P1- *E. coli* NCTC 10418, P2- *E. coli* ATCC 25922, P3- *Enterococcus faecalis*)

Trachyspermum involucratum L. was the most effective herb that retards the growth of the three bacterial pathogens in a considerable amount for all tested extracts. The hot distilled water (80 ^{0}C) extract of *Trachyspermum involucratum* L. has significant antibacterial activity against *E. coli* NCTC 10418 (DIZ=14.67 mm) followed by *E. coli* ATCC 25922 (DIZ=14.00 mm) and *Enterococcus faecalis* (DIZ=13.67 mm) compared to other extracts of the herb (Figure 1). The bioactive compounds present in the water extract of *Trachyspermum involucratum* L. as thymol, dipentene like compounds may cause the growth reduction of the tested foodborne pathogens (Gunathilake and Ranaweera, 2016). *Trachyspermum involucratum* L. is commonly known as asamodagam which is one of the famous medicines for abdominal pain caused by different foodborne pathogenic bacteria. Normally, the seeds of asamodagam herb are used as a medicine, but the leaves of the herb also have the potential to inhibit the growth of foodborne pathogenic bacteria as shown in the results.

The acetonic extract of *Rosmarinus officinalis* L. (Bush) showed more activity against *Enterococcus faecalis* (DIZ=16.67 mm) in comparison to other extracts (methanol and water extracts). However, the methanol extract of *Rosmarinus officinalis* L. (Bush) exhibited a substantial zone of inhibition against all tested microorganisms in the present study (Figure 2). The inhibitory effect of *Rosmarinus officinalis* L. is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, and rosmanol present in the herb (Nieto *et al*., 2018).

As shown in Figure 3, the methanol and acetone extractions of *Santolina chamaecyparissus* L. showed antibacterial activity (11.33-14.67 mm) against all tested foodborne pathogenic microorganisms. The water extracts (distilled and hot distilled water) of *Santolina chamaecyparissus* L. also contributed to the inhibition of bacterial growth with a notable inhibition zone ranging from 8.33 mm to 9.67 mm. The literature search revealed as the herb contains artemisia ketone, di-hydro aromadendrene, β-phellandrene, camphor, and cubenol active compounds that proved to be

effective against various foodborne pathogens (Niu *et al.*, 2019).

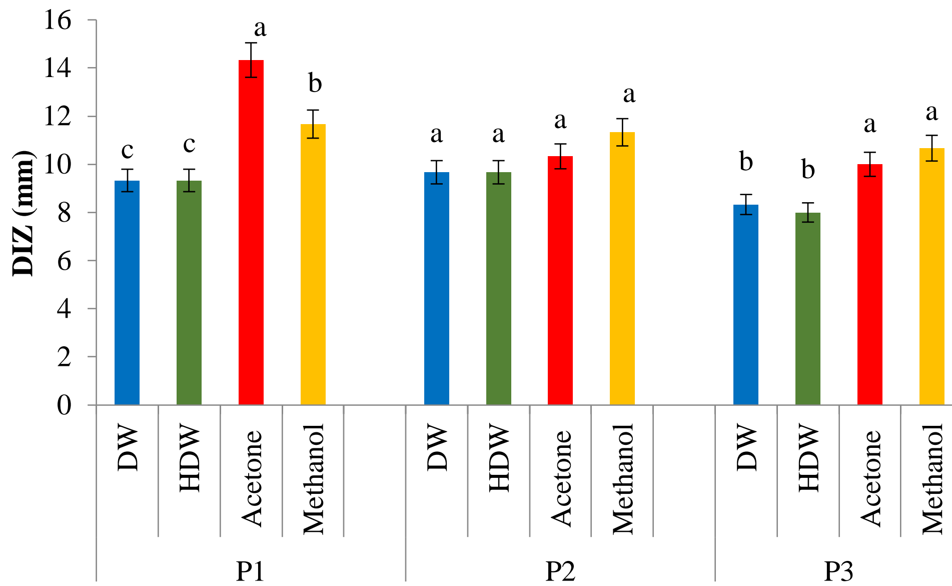

Figure 4. The diameter of the growth inhibition zone around the wells in *Satureja hortensis*. Columns with different letters are significantly (*p<0.05*) different. (P1- *E. coli* NCTC 10418, P2- *E. coli* ATCC 25922, P3- *Enterococcus faecalis*)

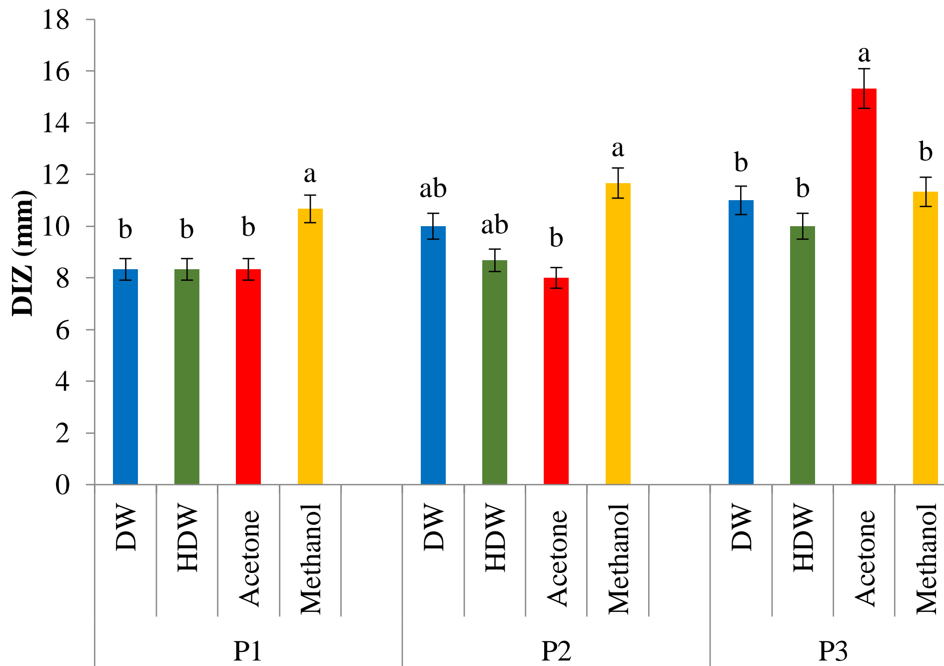

Figure 5. The diameter of the growth inhibition zone around the wells in *Origanum vulgare* L. Columns with different letters are significantly (*p<0.05*) different. (P1- *E. coli* NCTC 10418, P2- *E. coli* ATCC 25922, P3- *Enterococcus faecalis*)

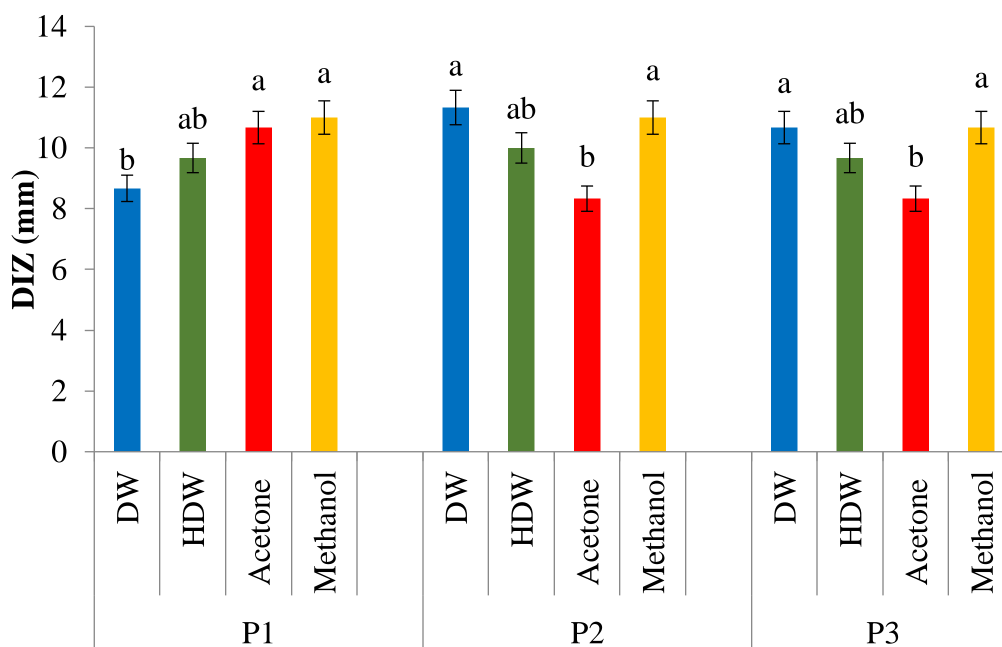

Figure 6. The diameter of the growth inhibition zone around the wells in *Coriandrum sativum* L. Columns with different letters are significantly (*p<0.05*) different. (P1- *E. coli* NCTC 10418, P2- *E. coli* ATCC 25922, P3- *Enterococcus faecalis*)

Satureja hortensis is another herb that showed antibacterial activity against tested pathogens. The acetonic extract of *Satureja hortensis* showed a higher inhibitory effect (DIZ=14.33 mm) against *E. coli* NCTC 10418 over other tested pathogens. The presence of high phenolic compounds such as thymol, carvacrol, or p-cymene caused the antimicrobial activity of *Satureja hortensis* (Mahboubi and Kazempour, 2011). In the present study, methanolic extract of *Satureja hortensis* also has antibacterial activity against all tested microbes (Figure 4).

As shown in Figure 5, all the extracts of *Origanum vulgare* L. herb displayed considerable inhibitory effect against all three pathogens. However, the methanolic extract of *Origanum vulgare* L. showed more antibacterial activity compared to the other extracts. *Origanum vulgare* L. has a wide variety of secondary metabolites, most of the phenolic compounds such as flavonoids, terpenoids, phenolic acids, and alkaloids, which are the main components responsible for its action (Teles *et al.*, 2019).

Coriandrum sativum L. is one of the famous herbs commonly used for culinary and medicinal purposes. The present study showed all the extracts of *Coriandrum sativum* L. has the potential to inhibit the growth of all three pathogens (Figure 6). The presence of a wide range of phytochemical constituents as alkaloids, flavonoids, saponins, and terpenoids can be beneficial to inhibit the growth of pathogenic microbes (Patel and Vakilwala, 2016).

As the results are shown in Table 2; *Rosmarinus officinalis* L. (Tree), *Melissa officinalis*, *Allium schoenoprasum*, *Allium tuberosum*, *Laurus nobilis* L., and *Origanum majorana* L. of all extractions (water, methanol, and acetone) showed less inhibitory effect against all tested foodborne bacterial pathogens. Among these less antibacterial active herbs, acetonic and methanolic extracts of some herbs have the notable antibacterial potential against particular microbe comparison to the water extract of that herb. As an example, the acetone extract of *Rosmarinus officinalis* L. (Tree) (DIZ=17.67 mm), *Melissa officinalis* (DIZ=14.33 mm) and *Allium schoenoprasum*

(DIZ=14.67 mm) showed antibacterial potential against *Enterococcus faecalis*. In particular, the aqueous extracts of *Allium schoenoprasum* and *Allium tuberosum* had no antibacterial activity against all of the three tested foodborne bacterial pathogens.

However, the results of the present study showed *Enterococcus faecalis* has highly inhibited the growth followed by *E. coli* NCTC 10418 and *E. coli* ATCC 25922 in the six herbs selected as the best antibacterial herbs out of the twelve herbs. But in general, those six herbs have the potential to inhibit the growth of all tested pathogens in comparison with the other herbs.

The extraction of biologically active compounds from plant material mostly depends on the sort of solvent employed in the extraction procedure. Extraction of *Rosmarinus officinalis* L. (bush) and *Origanum vulgare* L. with acetone has resulted in an exceedingly product with larger overall antibacterial activity than extraction with methanol and water. This might be the result of dissolving agent could be a sensible solvent because of its ability to dissolve in each polar and nonpolar substance, whereas other solvents can only dissolve one or the opposite. Further, methanolic extracts of *Santolina chamaecyparissus* L. and *Satureja hortensis* showed higher inhibition against *E. coli* ATCC 25922 and *Enterococcus faecalis*. The present study disclosed that organic extracts provided additional powerful antibacterial activity compared to aqueous extracts. However, a number of the herbs as *Trachyspermum involucratum* L. and *Coriandrum sativum* L. have higher antibacterial activity in the aqueous extract.

Researchers investigated the potential of herb extracts and their effective compounds as antimicrobial agents to manage the accretion of foodborne infective bacterium. Some researchers have shown the restrictive result of those herb extracts to property characters of herb extracts that modify them to react with the super-molecule (protein) of the microbial cell membrane and mitochondria distressful their structures and dynamical their porousness might disrupt of microorganism (Burt, 2004). Different researchers have urged that antimicrobial compounds of the herbal extracts move with enzymes and proteins of the microbial cell membrane, inflicting its disruption to disperse a flux of protons towards the cell exterior, that induces death or may inhibit enzymes necessary for organic compound biogenesis (Gill and Holley, 2006).

3.2. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined for six herbs that were selected as the best antibacterial herbs out of the twelve culinary herbs against three tested foodborne bacterial pathogens. The results of the MIC values of herb extracts were presented in Table 3.

The MIC results of best antibacterial active herbs demonstrated that overall organic extracts and the aqueous extracts have 0.15 g mL^{-1} and 0.2 g mL^{-1} MIC values to inhibit the growth of *E. coli* NCTC 10418, *E. coli* ATCC 25922, and *Enterococcus faecalis*. When comparing the MIC values of the tested herbs, all four extracts of *Origanum vulgare* L. and *Rosmarinus officinalis* L. (Bush) were inhibited the growth of *E. coli* NCTC 10418 and *E. coli* ATCC 25922 at the concentration of 0.2 g mL^{-1}, whereas the organic extracts of these two herbs inhibited the growth of *Enterococcus faecalis* at the concentration of 0.15 g mL^{-1}.

In particular, the organic extracts (acetone, methanol) of *Satureja hortensis*, *Santolina chamaecyparissus* L., and *Coriandrum sativum* L. inhibited the growth of the most tested pathogens at the concentration of 0.15 g mL^{-1}. However, the hot distilled water extract of *Trachyspermum involucratum* L. inhibited the growth of *E. coli* NCTC 10418, *E. coli* ATCC 25922, and *Enterococcus faecalis* at the concentration of 0.15 g mL^{-1}. It has been established that MIC results do not always correlate well with the DIZ values but, in the present investigation, the observation of MIC related with the DIZ value might be due to the adoption of well diffusion assay for both

antimicrobial activity and MIC determination of herb extracts (Dhiman *et al*., 2016).

Table 3. MIC (g mL^{-1}) of Herb Extracts in Different Solvents

Herb	**Type of Extract**	**MIC value for herb extracts (g mL^{-1})**		
		E. coli NCTC 10418	*E. coli* ATCC 25922	*Enterococcus faecalis*
***Origanum vulgare* L.**	Distilled water	0.2	0.2	0.2
	Hot distilled water	0.2	0.2	0.2
	Acetone	0.2	0.2	0.15
	Methanol	0.2	0.15	0.15
***Rosmarinus officinalis* L. (Bush)**	Distilled water	0.2	0.2	0.2
	Hot distilled water	0.2	0.2	0.2
	Acetone	0.2	0.2	0.15
	Methanol	0.2	0.2	0.15
Satureja hortensis	Distilled water	0.2	0.2	0.15
	Hot distilled water	0.2	0.2	0.2
	Acetone	0.2	0.15	0.15
	Methanol	0.15	0.2	0.15
***Santolina chamaecyparissus* L.**	Distilled water	0.2	0.2	0.2
	Hot distilled water	0.2	0.2	0.2
	Acetone	0.15	0.15	0.15
	Methanol	0.15	0.15	0.15
***Coriandrum sativum* L.**	Distilled water	0.2	0.2	0.15
	Hot distilled water	0.2	0.15	0.15
	Acetone	0.2	0.2	0.2
	Methanol	0.15	0.15	0.15
***Trachyspermum involucratum* L.**	Distilled water	0.2	0.2	0.2
	Hot distilled water	0.15	0.15	0.15
	Acetone	0.2	0.2	0.15
	Methanol	0.2	0.15	0.2

4. Conclusions

The results of the present study established that *Trachyspermum involucratum* L., *Rosmarinus officinalis* L. (Bush), *Santolina chamaecyparissus* L., *Satureja hortensis*, *Origanum vulgare* L. and *Coriandrum sativum* L. have the higher antibacterial activity out of twelve culinary herbs against all tested foodborne pathogenic bacteria. *Enterococcus faecalis* has highly inhibited the growth followed by *E. coli* NCTC 10418 and *E. coli* ATCC 25922 in the six herbs selected as the best antibacterial herbs out of the twelve herbs. Organic extracts as methanolic and acetonic extracts are the most effective extraction solvents which can use for making plant extracts. Therefore, culinary herbs proved to be potentially effective as natural antimicrobials with effective, nontoxic, and natural compounds.

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LOW-FAT ICE MILK MANUFACTURED WITH FRUITS OF NABQ (*ZIZIPHUS SPINA-CHRISTI* L.)

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ABSTRACT
Ice milk is one of the most popular frozen desserts in Egypt and nowadays consumers are interest of healthier and functional food. Therefore, the aim of this study was to produce a low- fat ice milk by replacing cream with nabq fruits pulp (NFP) at 25, 50, 75 and 100% to obtain a product with functional characteristics. The chemical composition, total phenolic compounds, antioxidant activity, mucilage content, physicochemical characteristics and sensory acceptance of the ice milk were analyzed, beside, sensory acceptance. Ice milk containing NFP had a higher concentration of phenolic compounds and posteriorly had higher antioxidant activity compared to the control samples at zero time and after 40 days for all concentration of NFP. %Overrun increased significantly in T3 compared to other treatments, whereas freezing time decreased by the increasing of replacement percentage of NFP. Also, the high level of NFP (100% replacement) led to increase viscosity as this fruit contain mucilage and fibers. Our findings purported that the best percentage of replacement was 50% for panelist's acceptance. Nabq fruit pulp potentially recorded as a natural source of antioxidants and mucilage to fortify and develop new products.

1.Introduction

At recent days, dairy factories are turning to increase production of dairy products lower in their fat content and richer in nutraceutical and functional properties. Consumers are interested in eating these products that are being acceptable in taste, flavor, appearance and cheap. Among of these products, many kinds of new regular and low fat ice cream or known as ice milk available in the markets now. Substitution of traditional ingredients in ice cream with healthful and functional food ingredients without altering taste, mouth feel, or other sensory properties was the target of various studies (Salama, et al., 2017). In Egypt and according to the Egyptian Standard 1185-3/2005, the fat content of ice milk must not less than 3% (Soad et al., 2014). Fat plays an important role in the stabilization of the ice cream structure, as partially merged fat is mainly responsible for stabilizing the air bubbles and the foam structure (Koxholt, et al., 2001).On the other hand, substitution of milk fat with fat replacers, may change both the texture and flavour profile of ice cream (Prindiville et al., 2000). Fat replacers consist

of mixtures of lipid originated fat substitutes, protein- or carbohydrate originated fat mimetic, or their combinations (Huyghebaert et al., 1996). *Zizyphus spina-christi* is a deciduous shrub which belongs to *Rhamnaceae* Family. Fruits are commonly used in folk medicine for the curing of various diseases (Abdel-Zaher et al., 2008).They are wide-spread in the Mediterranean region, Africa, China, India, Australia and tropical America (Jaeschke et al., 2006). The genus Zizyphus is famous for their high biologically active material contents such as polyphenols, exhibiting antimicrobial, antioxidant, antitumor properties. Also these fruits act as a hypoglycemic, hypotensive, anti-inflammatory, and liver protective agent and an immune system stimulant.(Said et al., 2006) and (Abdel-Zaheret al., 2005). The main important compounds characterized in this plant are flavonoids, alkaloids, triterpenoids, saponins, lipids, proteins, free sugars and mucilage (Adzu et al., 2003). Mucilage is a plant hydrocolloid, which is a polymer of a monosaccharide or mixed monosaccharide. In fact, polysaccharide mucilage is highly hydrophilic substances with high molecular weight molecules. The polysaccharides are soluble and dispersible in water due to their ability to interact with water and swell. The swelling properties are characterized by the entrapment of a large amount of water between the polymer chains and branches. Thus, mucilage can be used as one of the food additives, to modify the food quality in terms of food stability, texture and appearance properties by acting as emulsifiers, thickeners, viscosity and gelling agents or texture modifiers. So, it could be used as stabilizer in ice-cream, sauce and salad dressing (Nussinovitch, 1997 and Deogade et al., 2012). Modern life style habits cause many people to develop abnormally high levels of oxidative stress which caused mainly by free radicals(Bores et al., 1990). For the protection against free radicals, organisms are endowed with endogenous (antioxidant enzymes) and exogenous defense systems. These systems unable to protect our tissues when the generations of free radicals are significantly increased(Pakin et al., 2001). Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups, therefore, the phenolic content of plants may contribute directly to their antioxidant action (Kaneto et al., 1999 and Himesh et al., 2011). The aim of this study is to investigate the benefits of nabq which is characterized by its high nutritional value and it can be used as a fat replacer in healthy and functional ice milk to replace fat partially or completely and to examine the effects of fat replacement by the addition of nabq fruit on the ice milk quality, especially, there is a lack of information regarding analysis as well as utilization of nabq fruits in food products.

2. Materials and methods

2.1. Materials

Fresh skimmed cow's milk (8.76% Total Solids, 0.1% Fat, 3.44% Protein, 0.59% Ash, 4.63% Lactose and pH value was 6.67) was obtained from the Faculty of Agriculture, Cairo University, Giza, Egypt. Skimmed milk powder (SMP) (96.2% TS, 0.8% Fat, 33.40% Protein, 7.90% Ash, 54.10% Lactose and pH value was 6.60)(Ecoval N.V., Paris, France), Cream (64% TS, 60% Fat, 1.90 % Protein, 0.60% Ash, 1.50% Lactose and pH value was 6.62) was obtained by separating of fresh buffalo's milk using cream separator (made in Egypt) and vanilla were obtained from the local market. Commercial grade sugar cane (sucrose) was purchased from sugar and Integrated Industries Company, Giza, Egypt. High viscosity carboxy methyl cellulose (CMC) produced by TIC gums, MD, USA was used as a stabilizer. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, (Germany).Fresh full ripened Nabq fruits (*Ziziphus spina-christi*var Hozain) were purchased from the Faculty of Agriculture, Asuit University, Egypt, in March, 2020.

2.2. Methods

2.2.1. Preparation of Nabq fruit pulp (NFP).

The fruits were washed; the pulp was separated, heated with steam until reaching 85°C for 3 min to inhibit enzymatic browning reaction and then mixed using a mixer (electric mixer, Moulinex, France). The mixture was stored in a plastic bag in a freezer at -18°C, until used.

2.2.2. Manufacture of Ice Milk

According to the Egyptian standards of ice cream (Egyptian Standard (EOS, 2005), the basic ice milk mix contained 0.25% cmc, 4% fat and 12.58% milk solids non–fat (MSNF) and 14% sucrose in the mixture. Napq fruit pulp (NFP) was added to the basic ice milk mixture to replace cream content as follows: T1: control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP. The other ingredients content was kept at a stable level as presented in Table (1), except CMC, as nabq fruits pulp includes mucilage, which acts as CMC. Mixture was heated to 85 ± 1°C for about 30 seconds, then rapidly cooled to 5 ± 1°C and aged at the same temperature for 24 hrs. After ageing, 0.01 % vanilla powder was directly added to the mixes before frozen in horizontal batch freezer (Taylor Co., USA). The frozen ice milk was drawn in plastic cups (*Ca.* 120 mL) and hardened at – 26° C for 24 h before analyses. The frozen ice milk was then stored in freezer for 40 days to determine the antioxidant activity before and after storage. All treatments were of three replicates.

Table 1. Formulation of one kg low-fat ice milk containing different ratios nabq fruits pulp (NFP).

Ingredients	T1*	T2	T3	T4	T5
Cream (60% fat) (g)	65.500	49.125	32.750	16.375	0.000
Fresh skimmed cow's milk (8.76% SNF) (g)	656.500	656.500	656.500	656.500	656.500
Skim milk powder (g)	125.500	125.500	125.500	125.500	125.500
Sucrose (g)	150.000	150.000	150.000	150.000	150.000
CMC (g)	2.500	-	-	-	-
Nabq fruits (g)	-	18.875	35.250	51.625	68.000
Total (g)	1000	1000	1000	1000	1000

Vanilla was added at 0.1 g/kg,

*T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP

2.2.3.Physico- Chemical properties

Moisture, total solids, crude protein, crude fat, crude fiber and total ash contents were determined according to AOAC (2007).Total available carbohydrates were calculated by differences as described by Ceirwyn (1995). The pH values were measured using a digital Laboratory by pH meter (HI93 1400, Hanna instruments) with glass electrode that was standardized with buffers of pH 4.0 and 7.0 before pH measurements by the method described in AOAC (2005). Titrable acidity was determined by the method of AOAC (2005). Percentage of total soluble solids (TSS) were determined by the method of AOAC (2005) using Abbe Refractometer (Leica Mark

II 10481) at 25° C. Minerals content (Fe, Zn, P, Mg , K and Ca) were determined as described by Hankinson (1975) using Atomic absorption Spectrophotometer No.3300 (PerkinElmer, Us instrument Division Norwalk, CT, USA). Mucilage content% (g/100g on fresh weight) was determined by the method described by Thanatcha and Pranee (2011).

2.2.4.Determination of total phenolic content

Total phenolic content was determined colorimetrically by the Folin Ciocalteu method according to the method described by (Zheng and Wang, 2001)with some modifications as described by (Martinet al., 2012), sample extracts were dissolved in methanol to yield a concentration (w/v) of 10 mg/mL. Then, 50 μL aliquots were mixed with 1.25 mL of Folin–Ciocalteu reagent (diluted 1:10 fold) and 1 mL of 7.5% sodium carbonate solution. After 30 min, absorbance was measured by Janway model 6705 Spectrophotometer (England) at a λ=765 nm, at room temperature. The results were expressed as mg gallic acid equivalents (GAE) per 100 g nabq fruit pulp on fresh weight.

2.2.5.Determination of antioxidant activity

The 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was performed as described by (Oms-Oliue al., 2009) 250 μg /mL of methanolic solution of extract was prepared. An aliquot (10μL) of methanolic extract was mixed with 90 μL of distilled water and 3.9 ml methanolic DPPH solution (0.025 g/L), then incubated for 30 min in darkness. The absorbance was measured at 515nm against methanol as blank, using a Jenway model 6705 Spectrophotometer (England). Negative control was prepared with 10 μL methanol, 90 μL distilled water and 3.9 mL DPPH solution. The antioxidant activity was calculated using the following equation.

$$\%\ \text{inhibition DPPH} = \frac{(\text{ABS control} - \text{ABS sample})}{\text{ABS control}} \times 100 \quad (1)$$

where:
-AA is the antioxidant activity.
- Abs DPPH is the absorbance of DPPH free radical solution in methanol;
-Abs sample is the absorbance of DPPH free radical solution mixed with sample.

2.2.6. Physical properties of low- fat ice milk containing different ratios of napq fruits pulp (NFP).

The pH values were measured and Titrable acidity was determined as described above. The specific gravity of ice milk mixes and the resultant ice milk was determined as described by Arbuckle (1986).The freezing point (-°C) of ice milk mixes was also determined according to method of Marshall and Arbuckle (1996). The overrun values of ice milk were calculated mentioned by Marshall et al., (2003). Melting resistance of the resultant ice milk as samples was determined as mentioned by Segall and Goff (2002). Determination of viscosity was determined using a coaxial rotational viscometer (Rheotest II, Medingen, Germany) at shear rates ranging from 3.0 to 1312 s^{-1}. The measuring device (S1) was used as a sample volume of 30 ml per run. All samples were adjusted to 20 ± 1° C before loading in the viscometer device. Apparent viscosity was calculated at shear rate of 48.6 s^{-1}, (RPM= 20, spindle= 21). The hardness of resultant ice milk was measured by adapted the method suggested by Bourne and Comstock (1986) using fruit pressure tester (Penetrometer, Model FT 327). Samples were tempered to -19 °C in chest-type freezer for 24 h before testing. The pounds (Ib/in^2) of force required for a cylindrical probe (diameter = 0.8 cm and length = 2.65 cm) to penetrate the sample are a function of the hardness. Whipping abilities of the ice milk mix was determined using mixer at speed setting 10 with 3-cm blades (Heidolph N. 50111, Type RZRI, Germany) according to Baer et al. (1999). The mix (150 mL) was placed in a 1-Lstainless steal bowl, calibrated with known volume of water, and placed inside a 2.5 L bowl. An ice and salt mixture was

placed between the bowls to cool the mix as it was whipped. Change in volume was rotated at 5, 10, 15 and 20 min.

2.2.7.Sensory evaluation

Samples of ice milk after 24 h of hardening at -26° C were judged by ten members of Food Technology Research Institute, Agricultural Research Center. The samples were scored for flavour (out of 45 point), body and texture (out of 35 point), melting properties (out of 10 point) and colour (out of 10 point) as suggested by Arbuckle (1986).

2.2.8.Statistical analysis

All data were expressed as mean values ± SD (standard deviation). Statistical analysis system (SAS) software program (SAS Institute 2004) was performed using one-way analysis of variance (ANOVA) followed by using T tests (LSD) at $P \leq 0.05$ being considered statistically significant difference.

3. Results and discussions

3.1.Physico chemical properties of Nabq fruit pulp

The proximate chemical composition of nabq fruit pulp was calculated on fresh wt. basis as shown in Table 2. Results show that the percentages of moisture, fat, protein, ash, fibers and carbohydrate were 84.00, 0.08, 0.56, 0.29, 0.65 and 14.40% respectively. Our results were in agreement with Pareek, (2013) and Kavitha, (2013). Meanwhile results in Table 3, indicated that pH , total titrable acidity and total soluble solids of nabq fruit pulp were 4.33±0.58, 0.562±0.09 and 14.17% ±0.29,respectively.These results are near to those of Kavitha (2013) and (Gündüz and Saraçoğlu, 2014).

Table 2. Chemical composition of nabq fruits pulp on fresh weight basis

Criteria	Nabq fruits pulp
Moisture (%)	84±0.16
Fat (%)	0.08±0.03
Crude Protein (%)	0.56±0.14
Total Ash (%)	0.29±0.07
Crude fibers (%)	0.65±0.11
Total available carbohydrates (%)	14.4±0.27
pH	4.60±0.15
Total titrable acidity (as citric acid)	0.562±0.09
Mucilage (%)	0.8±0.19
TSS (%)	14.0±0.12
Antioxidant activity (%)	68.43±0.52
Total phenol (mg GAE/100g)	173.7±2.5
Fe (mg/100g)	0.52
Zn (mg/100g)	0.48
P (mg/100g)	23.6
Mg(mg/100g)	12.4
K (mg/100g)	246
Ca (mg/100g)	25.4

* Values are means of three replicates ±SD

Mucilage is a plant hydrocolloid which is a polymer of a monosaccharide or mixed monosaccharide (Deogade et al., 2012).Mucilage content in fresh nabq fruits pulp was 0.8gm/100 g (5 gm /100gm. dw),this result was in agreement with that of Thanatcha and Pranee (2011) who reported that % mucilage in the ripe stage of nabq was 5.34 on dry weight basis. In fact, polysaccharide mucilage is highly hydrophilic substances with high molecular weight molecules. The polysaccharides are soluble and dispersible in water due to their ability to interact with water and swell. The swelling properties are characterized by the entrapment of large amount of water between the polymer chains and branches. Thus, mucilage can be used as one of the food additives, to modify the food quality in terms of food stability, texture and appearance properties by acting as emulsifiers, thickeners, gelling agents or texture modifiers (Noorlaila et al., 2015).On the other hand, it could be noticed that total phenolic content constituted 173.7±2.5 mg gallic acid equivalent/100g. Our finding is in agree with Krishna and Parashar (2013) who reported that total phenolic content ranged from 48.69 to 196.34 mg GAE/100 g (F.W basis) .On the other hand, our findings were greater than those of Kaur et al., (2015) who reported that total phenolic compounds were 105.36 mg GAE /100 g fresh weight . These differences in phenolic contents may be attributed to the influence of many external factors such as soil composition, geographical location, climatic conditions and light intensity as reported by Meng et al. (2012).

Antioxidant activity of NFP was determined by DPPH assay as shown in Table 2. It recorded 68.43±0.52 and our result is in agree with Al-Jassabi and Abdullah (2013) who reported that antioxidant activity ranged from 31.76% - 90.23% . The antioxidant nature of Zizyphus is defined mainly by the presence of a β-ring chatechol group (dihydroxylated β--ring) capable of readily donating hydrogen electron to stabilize a radical species (Waggas, A. and R. Al-Hasani, 2010).

Nabq is a nutritive fruit; it is a rich source of minerals and elements. Table 2, showed that the fruit contains Fe (0.52 mg/100g), Zn (0.48 mg/100 g), P (23.6 mg/100 g), K (246 mg/100 g) and Ca (25.4 mg/100 g). Our results in agreement to those presented by USDA (2018). It could be noticed the high content of in potassium. It is noteworthy that it helps nerves to function and muscles to contract, besides, the heartbeats stay regular. It also helps move nutrients into cells and waste products out of cells. On the other hand, high potassium content in the diet could help to offset sodium's harmful effects on blood pressure (Anon., 2021).

3.2.Chemical composition of ice milk containing different ratios of napq fruits pulp (NFP)

The chemical composition of ice milk mixture presented in Table 3, indicated that the reduction of cream caused the reduction of lipids. Data in Table 3 ascertained that there are no significant differences among treatments in TS% and crude protein. On the other hand , significance ($p \geq 0.05$) appears in fat content among treatments as the replacement of cream with different % nabq fruits pulp, the lowest value appeared in T5 (100% nabq fruit pulp), it was 0.10% compared to control (4.10%), where the decrement of fat content reached to 97.6%. The results are supported by the findings of Murtaza et al., (2004),who found that ice cream without fruits (control) had the highest fat contents however ice cream with fig in treatments showed gradually lower levels according to the fat replacement. Table 3, also shows the significant ($p \geq 0.05$) decrement in crude fibers for the control (T1), it was the lowest value (0.202%), but the highest value was in T5, it might be due to high fibers content in of nabq fruits pulp (NFP) that raised the fibers content for other treatments. Also, there was significant increment in total carbohydrates with the increasing of NFP

concentration. The lowest ash contents were observed in control ice milk followed by T2, while it increased significantly (p≥0.05) in other treatments (Table 3).

Table 3. Chemical composition (%) of low-fat ice milk mixes containing different ratios of napq fruits pulp (NFP) (on fresh wet basis).

Property (%)	T1*	T2	T3	T4	T5
Total solids	32.600 ^{a} ±0.12	32.620 ^{a} ± 0.15	32.650 ^{a} ± 0.05	32.670 ^{a} ± 0.16	32.690 ^{a} ± 0.10
Crude protein	5.460 ^{a} ± 0.10	5. 440 ^{a} ± 0.18	5.240 ^{a} ± 0.15	5.360 ^{a} ± 0.05	5.330 ^{a} ±0.19
Crude Fat	4.100 ^{a} ± 0.03	3.111 ^{b} ± 0.04	2.100 ^{c} ± 0.10	1.110 ^{d} ±0.09	0.100 ^{e} ± 0.07
Crude fibers	0.202 ^{e} ±0.005	0.212 ^{d} ±0.002	0.221 ^{c} ±0.0.004	0.233 ^{b} ±0.001	0.240 ^{a} ±0.002
Total ash	0.970 ^{b} ±0.22	0.990 ^{b} ± 0.21	1.080 ^{a} ± 0.12	1.100 ^{a} ± 0.11	1.110 ^{a} ±0.01
Total available carbohydrates**	21.870 ^{e} ± 0.005	22.870 ^{d} ± 0.015	24.010 ^{c} ± 0.015	24.870 ^{b} ± 0.0.017	25.910 ^{a} ± 0.01

*T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP.
**Total available Carbohydrates content was determined by difference.
Values are means ± standard deviations of triplicate determinations.
Means in the same row with different superscript (a,b,c…..) are significantly different (p≥0.05).

3.3.Physicochemical properties of ice milk mixes containing different ratios of napq fruits pulp (NFP)

Total acidity in T5 (100% replacement of cream by nabq fruits) recorded the highest value and decreased gradually for the other treatments under investigation. Moreover, the results of pH values were in inversely proportional with the results of total acidity. As the pH increased, total acidity decreased for all the tested samples. They were statistically significantly (p≥0.05) difference. Data in Table 4, showed that the control (ice milk containing 3% cream) showed the highest freezing point, whereas the addition of nabq fruits affected significantly on freezing point. The mixes showed lower freezing point gradually with the increasing replacement of cream by nabq fruits. Our findings were in agreement with Khalil and Blassy (2015) who reported that full fat ice cream exhibited the highest freezing point. Freezing point reduction affects on the initial and gradual growth of the formed ice crystals and also their native thermodynamic instability (Hartle, 2001). Also Ohmes et al. (1998) and El-Kholy (2005) reported that, when fat is removed from ice cream and is replaced with non-fat milk solids or other dissolved substances the freezing point is lowered. Data in Table 4 also showed that replacing fat by NFP, increased significantly (p≥0.05) the specific gravity of mixes, in a positive way with the rate of substitution. Regarding apparent viscosity of ice milk mix as presented in (Table 4), it could be observed that it increased accordance with replacing fat by nabq fruit. There was a direct proportional between viscosity and the ratio of replacement (Table 4). A certain level of viscosity in ice milk mixes is needed for proper whipping and retention of air cells. Our results were in accordance with El-Kholy and Abbas (2015) who reported that apparent viscosity increased in appositive way with the replacement of fat by pumpkin. This may be due to TSS and fibers contents which were responsible for gel forming viscous, as well as particle size and high water holding capacity of fibers (Vani and Zayas, 1995 and Hassan, 2005).

Table 4. Physicochemical properties of low-fat ice milk mixes with different ratios of nabq fruits pulp (on fresh wet basis).

Properties	T1*	T2	T3	T4	T5
Total acidity (%)	0.40 ^{e} ±0.03	0.44 ^{d} ±0.01	0.46 ^{c} ±0.02	0.48 ^{b} ±0.01	0.50 ^{a} ±0.01
pH value	6.43 ^{a} ±0.04	6.21 ^{b} ±0.04	6.08 ^{c} ±0.04	5.99 ^{d} ±0.03	5. 92 ^{e} ±0.03
Freezing point (−°C)	-2.31 ^{a} ±0.02	-2.44 ^{b} ±0.03	-2.56 ^{c} ±0.03	-2.60 ^{d} ±0.04	-2.64 ^{e} ±0.03
Specific gravity	1.0852 ^{c} ±0.0076	1.1014 ^{b} ±0.0017	1.1039 ^{b} ±0.0005	1.1048 ^{a} ±0.0005	1.1050 ^{a} ±0.0.0005
Apparent viscosity (mPas)	159.0±0.62 ^{e}	194.0±0.99 ^{d}	227.0 ^{c} ±0.41	270.0 ^{b} ±0.87	322.0 ^{a} ±0.91

* T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP
Values are means ± standard deviations of triplicate determinations.
Means in the same row with different superscript (a,b,c…..) are significantly different (p≥0.05)

3.4.Effect of storage time and treatments of low-fat ice milk mixed with different ratios of nabq fruits on total phenols and antioxidant activity Both total phenols and antioxidant activity significantly increased among the nabq fruits ice milk treatments in direct proportion with increasing the concentration of nabq fruits, this finding may be due its content of phenols. Whereas, they decreased with the increasing storage time among treatments (T3, T4 and T5). This may be due to freezing that affect phenols. Thus, it may be concluded that the decrement in total phenol in ice cream treatments (Table 5) is closely related to the decline in their antioxidant capacity. Our results were in accordance with Vital et al. (2018), who reported that there was degradation of the phenolic compounds appeared in the ice creams supplemented with grape juice residue during storage.

Table 5. Total phenols and antioxidant activity of low-fat ice milk mixed with different ratios of nabq fruits pulp (NFP) during storage.

Treatments	Total phenols (mg/100g)		Antioxidant activity (%) by DPPH	
	Zero time	40 days	Zero time	40 days
*T1	9.40 ^{eA} ±0.27	9.17 ^{eA} ±0.31	1.96 ^{eA} ±0.80	1.50 ^{dA} ±0.70
T2	15.11 ^{dA} ±1.39	13.47 ^{dA} ±0.84	8.35 ^{dA} ±0.40	7.78 ^{cA} ±0.45
T3	20.43 ^{cA} ±0.68	19.11 ^{cB} ±0.37	15.27 ^{cA} ±1.38	9.76 ^{cB} ±0.25
T4	24.56 ^{bA} ±1.14	22.06 ^{bB} ±1.15	22.38 ^{bA} ±0.30	15.13 ^{bB} ±0.07
T5	27.13 ^{aA} ±0.56	24.53 ^{aB} ±0.63	30.66 ^{aA} ±0.57	24.57 ^{aB} ±3.01

*T1:control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP
**A& B: The means with the different capital (A& B) superscript letters within the same raw indicate significant (LSD at 5%) differences between the two periods of storage.
*** a, b,c...,: The means with the different small (a, b,…) superscript letters within the same column indicate significant (LSD at 5%) differences among treatments.The data are means of 3 replicates ± SD.

3.5.Mineral content of low fat ice milk mixed with different ratios of nabq fruits pulp (NFP).

Milk and milk products are considered poor sources of Fe, so addition of nabq fruit to ice milk will be useful. Results in Table 6, showed that the iron content of substituted cream with nabq fruit varied between 0.09 – 0.33 (mg/100g), compared to 0.05 (mg/100g) in control treatment. Also the addition of nabq fruits to ice milk was accompanied by high level of potassium content because of the high level of it in nabq fruits. On the other hand, adding of nabq fruits increased the level of calcium, because nabq fruits contains high amount of calcium (25 mg/100g on fresh weight basis).Each of Zn and P increased in ice milk in direct proportional to the addition of nabq fruits.

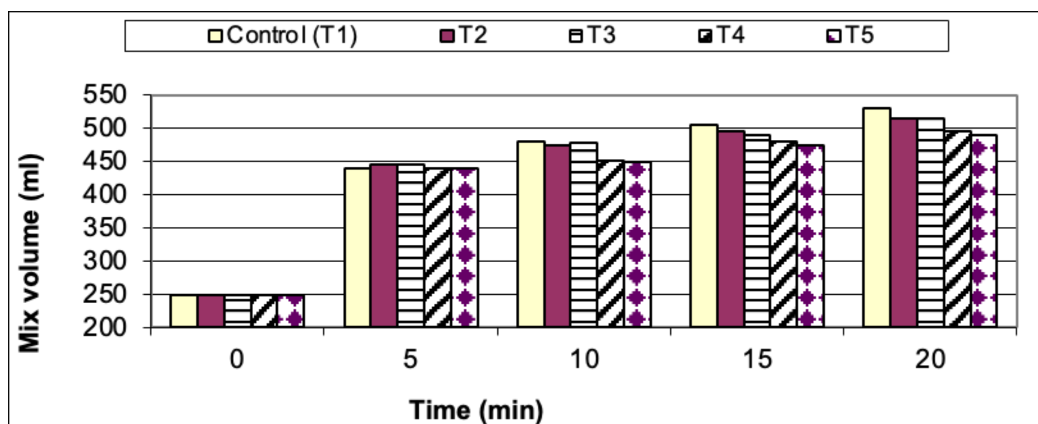
Table 6. Mineral content (mg/100g) of low-fat ice milk mixes with different ratios of napq fruits pulp (NFP).

Minerals content (mg/100g)	T1*	T2	T3	T4	T5
Fe	0.05	0.09	0.18	0.27	0.33
Zn	33.95	34.99	34.15	34.19	34.25
Mg	33.6	35.9	37.6	40.6	42.9
K	200	222	254	276	311
Ca	142.12	150.51	156.21	162.81	166.71
P	95.12	99.52	105.71	109.14	113.10

* T1: control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP

3.6.Whipping ability

Figure 1, shows the whipping ability (increase the volume with the time) of ice milk mixes containing nabq fruit. The values were in directly proportional increased with the time for each treatment. The results revealed that the control (without nabq fruit) recorded the highest whipping ability after 20 minutes followed by T2 and T3 (milk mixes containing 25% and 50% nabq fruits of cream respectively) whereas T5(milk mixes containing 100% nabq fruits of cream) recorded the lowest whipping ability after 20 min. These results could be attributed to the high ratio of nabq fruit in T5 that had higher viscosity, which prevent air incorporation. High viscous systems do not favour foaming capacity but do favour foam stability (Stanley et al., 1996). As nabq fruit contains mucilage, it is concluded that at the same shear rate, the shear stress and viscosity of mucilage solution at high concentration were higher than that of lower concentration (Thanatcha and Pranee, 2011).Camacho et al. (2001) suggested that hydrocolloids cause kinetic hindrance to the cream foaming which could be referred to not only the increase in the liquid-phase viscosity but also to stabilizer-protein interactions that could partially inhibit the foaming properties of milk proteins.



T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP

Figure 1. Whipping ability of low-fat ice milk mixes with different ratios of fruits of Nabq.

3.7.Properties of the resultant low-fat ice milk mixes with different ratios of fruits of Nabq.

Table 7, shows the significant differences of specific gravity values among treatments. The highest value was in T5 (100% replacement of cream by nabq fruits) comparing with T1 (control without nabq fruits). The increments were observed by the increasing ratio of nabq fruit in ice milk mixes. Also, significances appeared in values of overrun % for all treatments. These results may be due to the increase in viscosity and / or reduction in freezing point (Khalafalla et al., 1975 and Kebary, 1996).From Table 7 we noticed that the highest value of overrun was in T3 compared to T1(control) followed by T2.On the contrast T4 and T5 had adverse effects on the overrun of resultant ice milk. Improvement in overrun in T3 followed by T2 may be attribute to mucilage in nabq fruit. Marshall *et al*. (2003) reported, as the viscosity increases, the resistance to melting and the smoothness of texture increases but the rate of whipping decreases.

Table 7. Effect of using different percentages of nabq fruits pulp (NFP) on the physical properties of the resultant low-fat ice milk

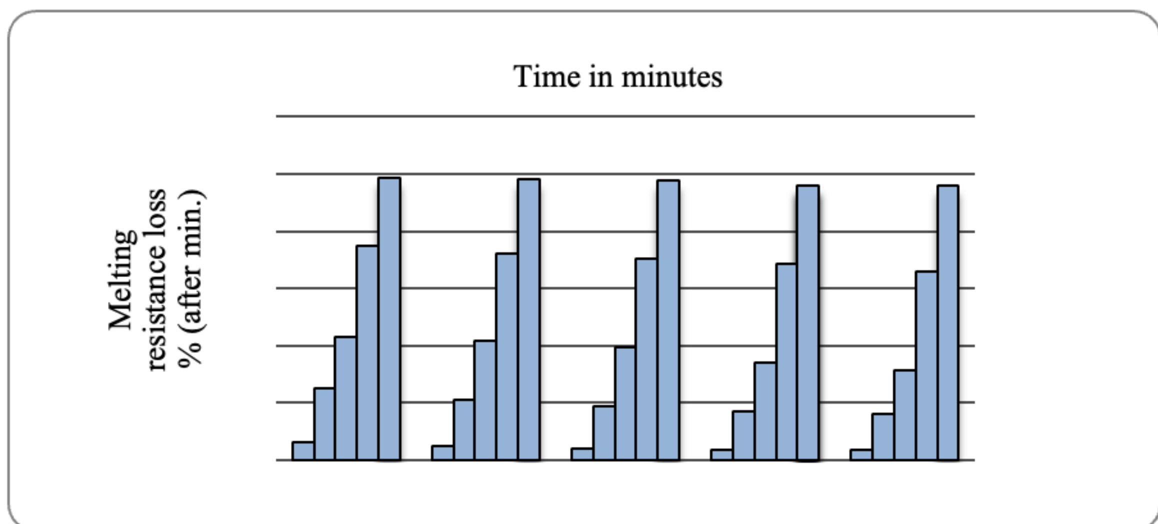
Properties	T1	T2	T3	T4	T5
Specific gravity	$0.660^{b} \pm 0.003$	$0.655^{b} \pm 0.003$	$0.649^{c} \pm 0.004$	$0.657^{b} \pm 0.002$	$0.670^{a} \pm 0.003$
Overrun (%)	$63.51^{d} \pm 1.57$	$73.8^{ab} \pm 1.65$	$75.12^{a} \pm 1.12$	$72.42^{bc} \pm 1.12$	$69.90^{c} \pm 1.45$
Freezing time (min.)	$17.25^{a} \pm 0.75$	$16.00^{b} \pm 0.50$	$15.60^{c} \pm 0.75$	$15.5^{c} \pm 0.75$	$15.10^{c} \pm 0.75$
Hardness	$9.28^{a} \pm 0.09$	$9.22^{a} \pm 0.10$	$9.15^{a} \pm 0.11$	$9.10^{a} \pm 0.15$	$9.00^{a} \pm 0.13$

* T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP
Values are means ± standard deviations of triplicate determinations.
Means in the same row with different superscript (a,b,c…..) are significantly different ($p \geq 0.05$).

Our results were in accordance with Abd El-Aziz et al., (2015) who concluded that the volume increase (overrun) requires a certain level of viscosity and that depends on the type and proportions of ingredients in the ice cream mixture. Our finding is in agreement with Akesowan, (2008) who reported that hydrocolloids enhance emulsion stability binding free water. Also Marshall and Arbuckle (1996) argued that air cells in ice cream are stabilized by surface active components such as proteins, phospholipids and stabilizers. From Table 7, it could be observed that the time required for freezing ice milk samples decreased with the increment of nabq fruits concentration. In addition, hardness of ice milk is not affected by addition of nabq fruit in ice milk, it could be noticed in Table 7 that there were nonsignificant differences among treatments.

3.8.Melting resistance

As shown in Figure 2, The control of ice milk scored the highest level of melting resistance but it decreases in sequence way with the increment of nabq fruits ratio. Melting resistance of ice milk was expressed as the loss in weight percent of the initial weight of the tested formula during 90 min. The increase of melting resistance of nabq ice milk attributed to nabq fruit ratio addition especially in T2 and T3, whereas there was a higher melting in T4 and T5 than other treatments. These were in accordance with Sofjan and Hartel (2004), who stated that ice cream which has a low overrun value will melt faster. On the contrary, ice cream which has high overrun will have a better resistance to melting properties.



T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP

Figure 2. Melting resistance (loss % after min.) of low-fat napq fruits ice milk

3.9.Sensory evaluation

From Table 8, it could be noticed significances (p ≥0.05) differences in all tested samples concerning flavour parameter, T3 (50% nabq fruit of cream) scored the highest level , whereas there were no significant differences in treatments T1, T2 and T3(control, 25 and 50% nabq fruit of cream) and decreased significantly in T4 and T5 (75 and 100 % nabq fruit of cream, respectively).The same sequence was observed in melting properties, this means that the replacement of cream up to 50% with nabq fruits maintain sensory properties of ice milk.

On the other hand, colour was more acceptable in T5 than that of control because its colour was light cafe and this colour was due to the slight enzymatic browning in nabq during processing. So, T5 was preferable by panelists and this appeared through total score that there were no significant differences among the first three treatments (T1, T2 and T3).

Table 8. Sensory properties of low-fat nabq fruits ice milk samples

Parameter	T1	T2	T3	T4	T5
Flavour(45)	42.00 ^{d} ±1.41	43.60 ^{c} ± 1.17	44.40 ^{a} ±0.84	44.10 ^{b} ± 1.44	43.90 ^{e} ±0.87
Body &texture (35)	33.00 ^{a} ±1.33	33.00 ^{a} ± 1.49	33.00 ^{a} ±1.41	29.60 ^{b} ± 2.17	29.00 ^{b} ±1.39
Melting properties(10)	9.10 ^{a} ±1.05	9.00 ^{a} ± 1.56	9.50 ^{a} ± 0.82	8.40 ^{b} ± 1.49	8.00 ^{b} ± 1.49
Colour(10)	8.70 ^{c} ± 1.05	9.00 ^{b} ± 1.56	9.60 ^{a} ± 0.82	9.00 ^{b} ± 1.49	8.60 ^{d} ± 1.49
Total score (100)	92.80 ^{a} ±3.36	94.60 ^{a} ± 3.13	96.50 ^{a} ± 2.01	91.10 ^{b} ± 3.03	89.50 ^{c} ±3.57

T1 : control (3% cream without replacement), T2: 25% of cream was replaced with NFP, T3: 50% of cream was replaced with NFP, T4: 75% of cream was replaced with NFP and T5: 100% of cream was totally replaced with NFP
Values are means ± standard deviations of triplicate determinations.
Means in the same row with different superscript (a,b,c…..) are significantly different ($p \geq 0.05$).

4.Conclusions

Low-fat ice milk with nabq fruits has high nutritional value and has good physical and organoleptic properties especially at 50% replacement of fat in the mix. The final products can be considered as a functional and healthy ice milk.

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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF FOODBORNE BACTERIAL PATHOGENS ISOLATED FROM *BAGHLAVA* AN IRANIAN EXPORTING PASTRY SWEET

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ABSTRACT

Baghlava, a traditional pastry sweet product, are manufactured in Iran and exported to different countries around the world known as a tasty confectionery. The aim of this study was to investigate the prevalence and antibiotic resistance pattern of foodborne pathogens isolated from *Baghlava* samples. *E. coli*, *C. sakazakii*, *Salmonella* spp., *C. perfringens* and *S. aureus* were isolated and identified using PCR assay for detection of virulence factor gene in *Baghlava* samples. All pathogens except *Salmonella* spp. were detected in samples. Total contamination rates of *E. coli*, *C. sakazakii*, *C. perfringens* and *S. aureus* were observed 8.92, 7.14, 1.78 and 2.67%, respectively. Multidrug resistance properties to amoxicillin and ampicillin have been found in all strains; however, all isolates were susceptible to ciprofloxacin. Hierarchical clustering and contamination patterns of pathogens showed that the prevalence of each pathogen is significantly higher in the southern and northern regions of the city than central areas in which these products were produced.

1.Introduction

Baghlava is a traditional sweet product categorized as a hard confectionery mostly are just produced with an especial formulation in Qazvin and Kermanshah cities in Iran but consumed around the world as a tasty sweet confectionary (Mahmoodi Sadr et al., 2019). Also, this sweet is produced with a different formulation in some other areas of the world such as Turkey and Arabian countries (Krondl, 2011). The principle ingredients for *Baghlava* producing in Qazvin with specific formulation are nuts powder including pistachio and almond, egg, milk, sugar, crushed saffron and cardamom powders. After crushing all components, the initial pastes of the *Baghlava* is formed then punched layer by layer (consisting of pistachio, almond and cardamom pastes separately) into the specific pan containers following cooking process at 180 °C for 30 min in the oven. Finally, it is cut into the small pieces then covered with concentrated sugar syrup for being fresh during the storage time (Gharibzahedi, 2018). This traditional product is consumed in Iran and also exported to many countries in Asia and Europe (Mahmoodi Sadr et al., 2019). Using different ingredients in *Baghlava* manufacturing make it sensitive to spoilage and increase the risk of foodborne pathogen transmission to consumers

as a vehicle. On the other hand, cooking process and higher osmotic pressure as a key characteristic of this product reduce the probability of bacterial viability, growth and activity (Lee et al., 2011).

Foodborne bacterial pathogens are the strains of bacteria transmitted to humans by food consumption leading to intestinal and extraintestinal diseases and disorders. Consumption of contaminated food with foodborne pathogens is associated with enteric infections and several outbreaks annually around the world (Paudyal et al., 2017). There are many foodborne pathogens identified in traditional and ethnic pasty sweets and confectionaries. Staphylococcus aureus, Escherichia coli serotype O157: H7 and *Salmonella* spp. have the most prevalence reported by researchers in traditional products (Grace, 2015). Other foodborne pathogens such as Clostridium perfringens and Cronobacter sakazakii also have recently been detected in pastry ethnic confectionaries (Matheus et al., 2016). Some of these pathogens can survive during thermal processing and low water activity condition of sweet products; Consequently, the transmission risk of these pathogens, unfortunately, threaten the health of consumers (Mahmoodi Sadr et al., 2019). Presence of virulence factor encoded genes is crucial for risk assessment and evaluation the prevalence of pathogens isolated from food samples. Ordinarily, identification and confirmation of foodborne pathogen presence in food samples are implemented by detection of the most important virulence factor gene known for each pathogen using polymerase chain reaction (PCR) procedure (Law et al., 2015). It is worth pointing out that the region of traditional food production and sample collection significantly affect the prevalence of isolated pathogens as previously described by many researchers. The contamination rates reported significantly different for traditional food samples collected from manufacturers and local markets located in southern, northern and central regions of the city (Owusu-Kwarteng et al., 2017).

Multidrug resistance characteristics of pathogens isolated from food samples attract the attention of researchers in recent decades. Several studies investigated and detected phenotypic properties of antimicrobial resistance in pathogens isolated from food samples representing serious concern for the public health (Deak et al., 2016). Due to the extensive use of antibiotics in medical cares and agricultural process, development of resistant foodborne pathogens has been increased rendering less effective treatment for infections in human (Baym et al., 2016). Investigation of antimicrobial susceptibility pattern of isolated pathogens can help to develop the strategies for figuring out ways to solve these problems (Porse et al., 2016). This research has been done to investigate the prevalence and antimicrobial susceptibility pattern of some foodborne pathogens isolated from *Baghlava* sweet samples collected from manufacturers and local markets located in southern, northern and central regions of Qazvin city, Iran.

2. Materials and methods

Totally 112 *Baghlava* samples were randomly collected from local markets located in different areas of Qazvin, Iran; from May to September 2018. Samples were immediately transported under cool condition to the laboratory of Health Products Safety Research Center, Qazvin University of Medical Science and stored at 4 °C until primary microbial isolation procedures. All *Baghlava* samples showed normal organoleptic and physical properties consisting of colour, odour and consolidation.

2.1.Antibiotic susceptibility testing

Antibiotic resistance pattern of isolates was investigated using Kirby-Bauer antibiotic testing method as described by the Clinical Laboratory and Standards Institute (CLSI, 2016). As described in the procedure, confirmed isolates colonies were transferred and grown on Muller-Hinton agar (Promedia, Spain) for disk diffusion technique. Antibiotic susceptibility of isolates was studied against ampicillin (AM)(10

µg/disk), amoxicillin (AMX)(30 µg/disk), chloramphenicol (C)(30 µg/disk), tetracycline (TE)(30 µg/disk), ciprofloxacin (CP)(5 µg/disk) and ceftriaxone (CRO)(30 µg/disk). The diameters of inhibition areas (mm) were measured for evaluation antibiotic susceptibility profile of the strains. The results were reported as resistant (R), intermediate sensitive (I) and complete sensitive (S) according to the CLSI interpretation guidelines. Reference organisms for quality control of the antimicrobial susceptibility testing procedure were employed, including Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922.

2.2. Culture-base microbial isolation and identification

At the present study, *Escherichia coli, Cronobacter sakazakii, Salmonella spp., Clostridium perfringens, and Staphylococcus aureus* were initially isolated and identified through collected samples using culture-based isolation and identification methods to detect presumptive isolates describing in Microbiological Examination methods of Food and water. For sample preparation, twenty-five grams of each sample was homogenised and blended for 1 min using Stomacher BagMixer Lab-blender (Interscience, France) then mixed with 225 mL of sterile buffered peptone water (BPW, ProMedia, Spain); subsequently, they were subjected to initial isolation based on culture methods (Da Silva et al., 2018).

2.3. DNA extraction

For extraction of genomic DNA, presumptive confirmed single colonies of bacterial isolates were picked up from each isolation medium for inoculation into 5 mL of Bovine Heart Infusion broth (BHI-broth, ProMedia, Spain) for 24 h at 37 °C using 120 rpm shaking. Incubated medium tubes were centrifuged at 5000g for 15 min. After supernatant separation, the biomass pellets were subjected to DNA extraction by commercial kit. Cinnagen DNA extraction kit for gram-negative bacteria (Cinnagen Co. Iran) was used in accordance with the manufacturer instructions. NanoDrop spectrophotometer (ThermoFisher Scientific Co., USA) was employed for quantity and quality evaluation of extracted strain genomes. DNA samples were stored at -20 °C until the PCR assay.

2.4. PCR assay

PCR assay was employed to confirm the presence of *E. coli*, *C. sakazakii*, *Salmonella*, *C. perfringens* and *S. aureus* between the strains isolated and presumptively identified from *Baghlava* samples by culture base method. Species-specific primers for detection of rfb (Hu et al., 1999), *ompA* (Kilonzo-Nthenge et al., 2012), sty (Kim et al., 2006), *plc* (Abildgaard et al., 2010) and *nuc* (Kim et al., 2001) genes, present in *E. coli*, *C. sakazakii*, Salmonella, *C. perfringens* and *S. aureus* respectively, were used at the present study. Primer sequences and thermal cycling procedures for each primer are provided in Tables 1 and 2. PCR mixtures consisting of 200 µM of each dNTP, 10 mM $(NH_4)_2$ SO_4, 2.5 U taq polymerase, 3 µM $MgSO_4$, 10 mM Tris-HCl (Cinnagen Co. Iran), 70 ng DNA template, 3 µM of each primer and addition of deionized sterile water reaching the final volume of 25 µL were used for each PCR reaction. PCR products were characterised using electrophoresis at 100 V for 1 h on a 1.5% agarose gel containing 0.005% v/v safe staining dye (Ampliqon, Denmark).

Table 1. Primer sequences for PCR assay

Primers	Accession Number	Sequence	Amplicon size (bp)	Reference
rfbEF	S83460.1	GTGTCCATTTATACGGACATC CATG	292	Hu et al. 1999
rfbER		CCTATAACGTCATGCCAATATTGCC		
ompAF	NZCP011047.1	GGATTTAACCGTGAACTTTTCC	369	Kilonzo-Nthenge et al. 2012
ompAR		CGCCAGCGATGTTAGAAGA		
sty1F	U25352.1	TGGTATGGTTAAGCGGAGAATGG	424	Kim et al. 2006

sty1R		GAGAGTCATAGCCCACACCAAAG		
plcF	NC008261.1	GCTAATGTTACTGCCGTTGA	325	Abilgaard et al. 2010
plcR		CCTCTGATACATCGTGTAAG		
nucF	AF400161.1	CGAAAGGGCAATACGCAAAG	310	Kim et al. 2001
nucR		CGTAAGCCACGTCCATATT		

Table 2. Thermal cycling procedures of PCR assays

Foodborne pathogen	Primer	Thermal cycling program
E. coli O157: H7	rfbE	Initial denaturation: 5 min at 94°C, followed by 35 cycles: 40 s at 94°C, 60 s at 59°C and 60 s at 72°C; finally, 4 min at 72°C as final extension step
C. sakazakii	ompA	Initial denaturation: 5 min at 95°C, followed by 30 cycles: 60 s at 95°C, 60 s at 55°C and 45 s at 72°C; finally, 5 min at 72°C as final extension step
Salmonella spp.	sty	Initial denaturation: 5 min at 94°C, followed by 40 cycles: 40 s at 95°C, 30 s at 61°C and 35 s at 72°C; finally, 7 min at 72°C as final extension step
C. perfringens	plc	Initial denaturation: 3 min at 94°C, followed by 40 cycles: 60 s at 94°C, 60 s at 57°C and 50 s at 72°C; finally, 3 min at 72°C as final extension step
S. aureus	nuc	Initial denaturation: 5 min at 95°C, followed by 45 cycles: 60 s at 95°C, 60 s at 58°C and 60 s at 72°C; finally, 6 min at 72°C as final extension step

2.5. Statistical analysis

Fisher`s exact and Chi-square tests were carried out for evaluating the significant differences ($P < 0.05$) between contamination rates using SPSS software version 22.0.1 (Chicago, IL, USA). Heatmap and hierarchical clustering were performed by RStudio version 1.2.1335 and R package version 2.8.1 available on www.rstudio.com and www.r-project.org websites respectively. All statistical and experimental measurements were performed in triplicate.

3.Results and discussions

3.1. Prevalence of foodborne pathogens

Identification of pathogenic *E. coli*, *C. sakazakii*, *Salmonella* spp., *C. perfringens* and *S. aureus* strains were implemented by culture-based methods in 11, 10, 0, 6 and 5 samples respectively confirmed by the morphology of typical colonies and biochemical tests. As they are provided in the Figures 1-4, presence of rbfE, *ompA*, *plc* and *nuc* genes was confirmed by PCR assay in 10, 8, 2 and 5 *E. coli*, *C. sakazakii*, *C. perfringens* and *S. aureus* isolates respectively. Consequently, total contamination rate (including all samples collected from different areas of the city) of confirmed *E. coli*, *C. sakazakii*, *Salmonella* spp., *C. perfringens* and *S. aureus* strains isolated from *Baghlava* samples were determined 8.92 (2.67% from southern, 3.57% from northern and 2.67% from central areas of the city), 7.14 (3.57% from southern, 2.67% from northern and 0.89% from central areas of the city), 0, 1.78 (0.89% from southern and same contamination rate from central areas) and 2.67% (0.89% from northern and 1.78% from central areas) respectively (Fig. 5). Total contamination rate by *E. coli* and *C. sakazakii* were significantly higher than that for other foodborne pathogens in collected *Baghlava* samples from different areas of the city. As it is illustrated in Fig 5, *C. sakazakii* and *E. coli* for samples collected from southern and northern areas; *S. aureus* and *E. coli* for central areas had significantly higher contamination rate than other pathogens in samples collected from each areas of the city. As it can be seen in the Fig. 6, the contamination rate pattern of the southern and northern areas of the city was

significantly categorised in one group (cluster 2). Indeed; because of higher hygienic inspection of the manufacturers located in the central areas of the cities, lower microbial contamination rate generally observed in samples collected from this area as described by other researchers. Yang et al. in the year 2016 found that the prevalence of foodborne pathogens in food samples collected from northern areas is higher than other regions. Also, the prevalence patterns of *E. coli* and *C. sakazakii* as gram negative foodborne pathogens (considering absence of *Salmonella* spp.) clustered in the same group (cluster A) showing significant correlation between prevalence of these pathogens in *Baghlava* samples.

Baghlava is a sweet product with lower water activity and moisture content led to declination in growth rate and pathogenicity risk of a broad spectrum of foodborne pathogens; however, presence and transmission of some pathogens including *Salmonella*, Clostridium, Staphylococcus, Escherichia coli and Cronobacter species are still probable and previously reported by some researchers in the same sweet products. At the present study, prevalence and antimicrobial susceptibility pattern of these probable foodborne pathogens were evaluated. All foodborne pathogens except *Salmonella* spp. were detected in 112 *Baghlava* samples.

Prevalence of *Salmonella* spp. is more reported in poultry and seafood products (Issa et al., 2017); however, detection and survival of this pathogen have been dispatched by many researchers in sweet and confectionary products recently (Nascimento et al., 2018). Negative results for detection of *Salmonella* spp. in *Baghlava* samples reveal this product is safe regarding the absence of the most dangerous foodborne pathogen. Also, according to the national food safety standards of Iran, *Salmonella* spp. must be absent from twenty-five grams of any food and drink samples (Mahmoodi Sadr et al., 2019). *Salmonella* spp. usually transmit to the confectionary and sweet products by some ingredient and additives consisting of nuts and its powder (Woh et al., 2017).

Regarding use of almond and pistachio nut powder as raw materials in *Baghlava* formulation, contamination of these components with *Salmonella* spp. can be transmitted to the final product effectively. Farakos et al. (2017) and Harris et al. (2016) reported considerable prevalence and levels of *Salmonella* spp. contamination in almond and pistachio nut samples respectively (Farakos et al., 2017, Harris et al., 2016). Cooking process during *Baghlava* manufacturing should be a probable reason for not detecting of pathogens (Manios & Skandamis, 2015).

E. coli and *C. sakazakii* as gram negative foodborne pathogens were detected in *Baghlava* samples collected from different areas of Qazvin city, Iran. Contamination with *E. coli* and *C. sakazakii* ordinarily occurs in poor hygienic condition of manufacturing process or failure in thermal process (Saeedi et al., 2017) considering significant correlation between contamination patterns clustered in one group (Fig. 6). Detection of *rfbE* (O157) gene showed that isolated *E. coli* strains were characterized as serotype O157 categorized in Enterohemorrhagic *E. coli* (EHEC) pathotype having the potential of making gastrointestinal disease and hemorrhagic uremic syndrome (HUS). It is worthwhile pointing that the source and reservoir of *E. coli* serotype O157 is animal and associated products which are not using in *Baghlava* as raw materials; consequently, probable cross-contamination after production should be occurred to explain the presence of this serotype (Heiman et al., 2015). Also, Davidson et al. (2015) detected *E. coli* serotype O157: H7 in prevalence investigation of some foodborne pathogens in walnut samples as a non-animal-derived food product (Davidson et al., 2015). Outer membrane protein (*ompA*) gene was detected in *C. sakazakii* strains isolated from our samples characterised for invasion of gastrointestinal epithelial cells and passing through blood-brain barrier leading to diarrheal symptoms and meningitis. Presence of this gene as an important virulence factor of *C. sakazakii*

showed the strength of pathogenicity in our isolated strains (Singh et al., 2017). Usually prevalence of *E. coli* serotype O157: H7 and *C. sakazakii* are evaluated by detection of *rfbE* and *ompA* genes respectively from food samples by researchers (Li et al., 2016).

Prevalence of *S. aureus* and *C. perfringens* as gram positive foodborne pathogens were confirmed in *Baghlava* samples. Because of shape formation and somewhat manufacturing with hands, *Baghlava* products have the potential of contamination with *S. aureus* (Mahmoodi Sadr et al., 2019); although, we detected it in our samples. Isolation of *S. aureus* is quite common in bacterial evaluation of traditional food products because of manufacturing process by hands as reported previously by many researchers in sweet, confectionary and other traditional food products (Demirci et al., 2017). Presence of *S. aureus* was confirmed by primer-specific detection of *nuc* gene encoding the thermostable nuclease of *S. aureus*. With regard to presence of sugar as a dry powder ingredient in *Baghlava* formulation and heat stability of *C. perfringens*, prevalence of this pathogen in this sweet product is probable as it was detected and identified in our samples; however, transmission of this pathogen to traditional products is likely by food handlers (Lee, 2016). Isolation and identification of *C. perfringens* from many traditional foods such as dried beans and rice products have been implemented (Wang et al., 2016). Because of spore-forming characteristic of this pathogen, it can survive in a wide range of processed and dried foods (Luo et al., 2017). Phospholipase C (*plc*) gene encoding alpha-toxin as the main virulence factor belonging to pathogenic *C. perfringens* usually is used for detection and identification of this pathogen in food samples (Hernández et al., 2017). As we identified *plc* gene in our isolates, pathogenic potential of the isolated *C. perfringens* from our samples is discernible. It is needed for more investigation of other virulence factors encoded genes in these isolates because of transmission risk of opportunistic and nosocomial isolated pathogens to human by consumption of contaminated *Baghlava* sweet products. However, using hygienic manufacturing process is necessary to reduce the spreading probability of these opportunistic pathogens from food to consumers leading to decrease the human health threat potential (Paudyal et al., 2017).

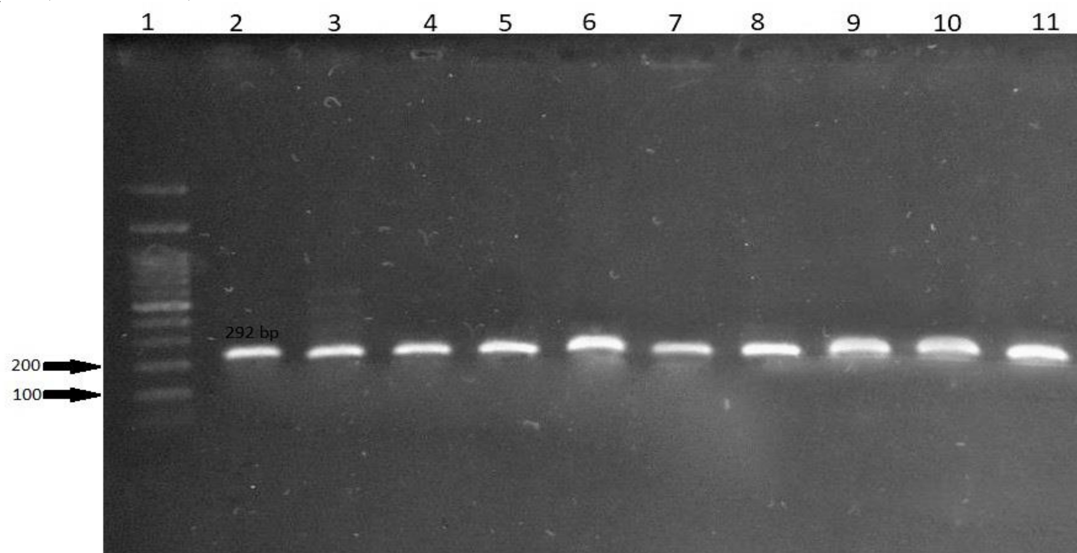


Figure 1. PCR amplification of *rfbE* gene and *E. coli* identification including 100-bp marker (lane 1) and positive samples (lanes 2-11)

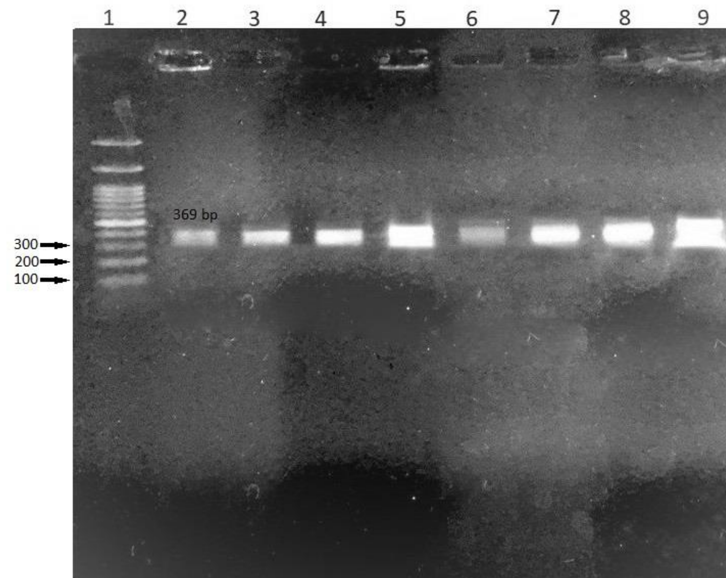


Figure 2. Identification of *C. sakazakii* by PCR amplification of *ompA* gene, lane 1 is 100-bp marker and lanes 2-9 are positive samples

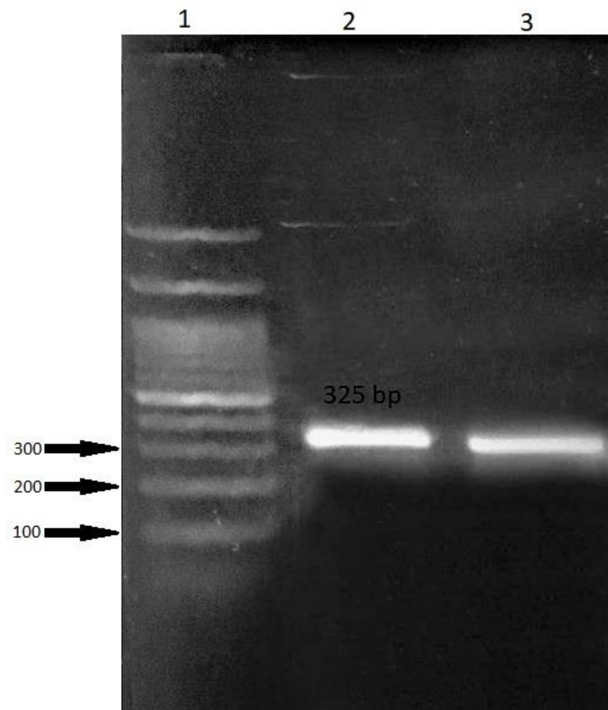


Figure 3. Detection of *plc* gene for confirmation of presumptive *C. perfringens* isolates by PCR assay, lane 1 is 100-bp marker and other lanes are positive samples

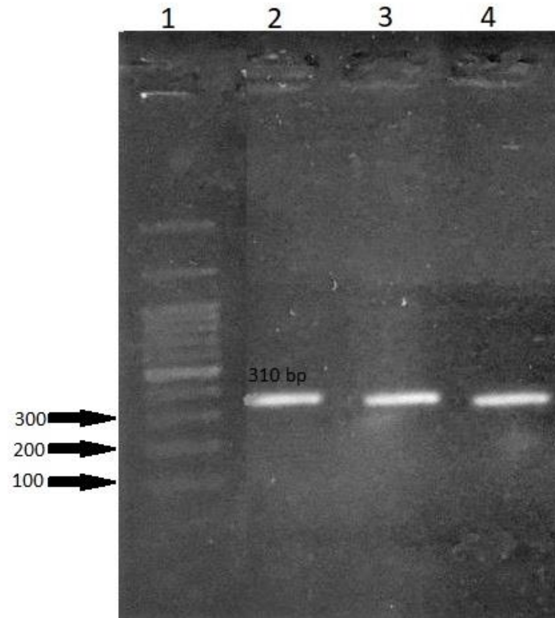


Figure 4.PCR amplification of *nuc* gene for identification of pathogenic *S. aureus* in *Baghlava* samples consisting of lane 1 as 100-bp marker and lanes 2-4 as positive samples

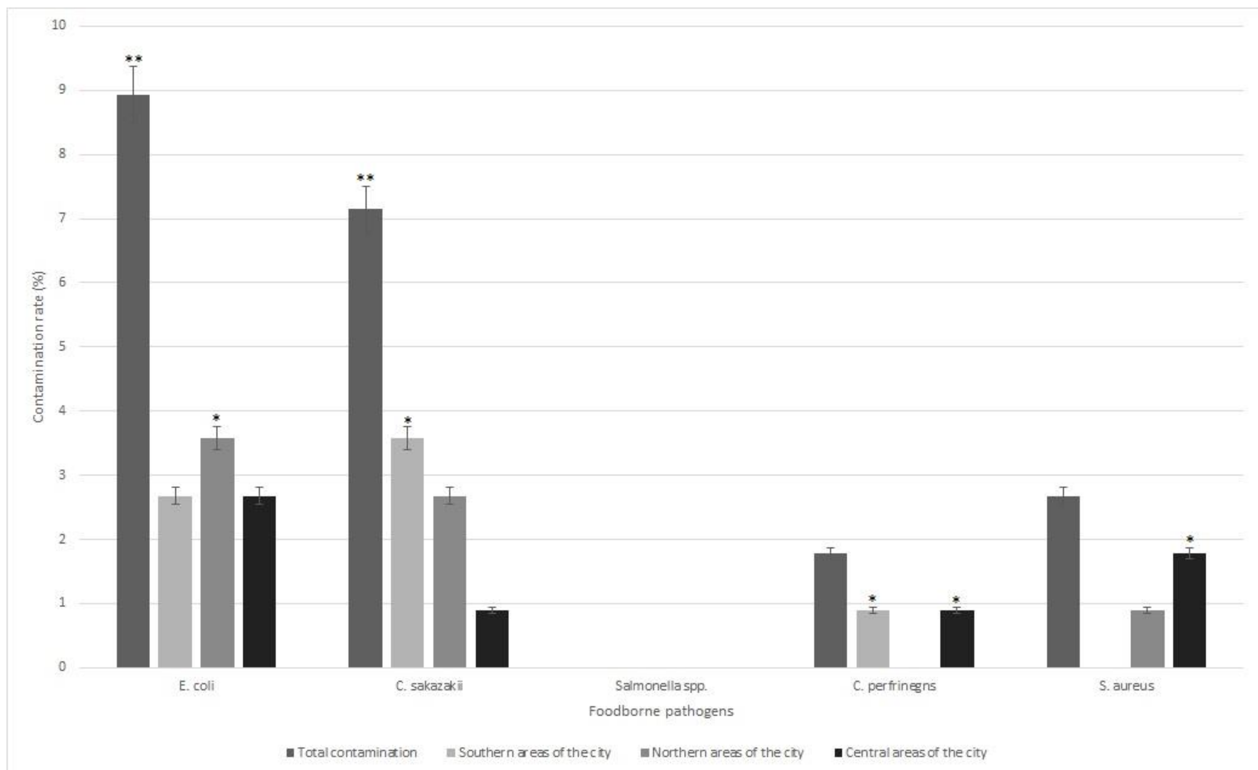


Figure 5. Contamination rate (%) of *E. coli, C. sakazakii, Salmonella spp., C. perfringens* and *S. aureus* by PCR assay including strains isolated from *Baghlava* samples collected from southern, northern and central regions of the Qazvin city, Iran.
** and * indicate statistically significant differences ($P < 0.05$) between results of total contamination rate and contamination rates in areas of the city for each pathogen separately based on Fisher`s exact test.

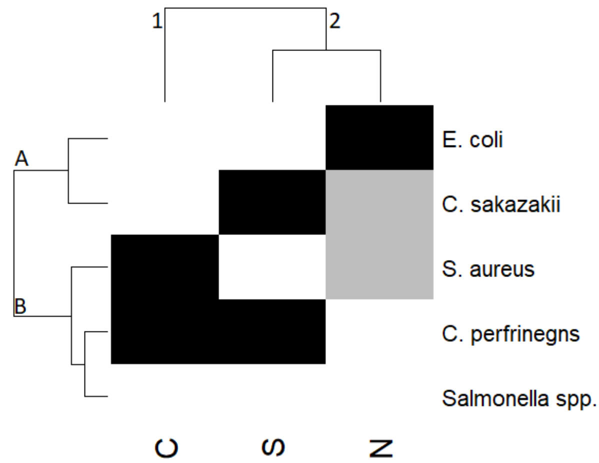
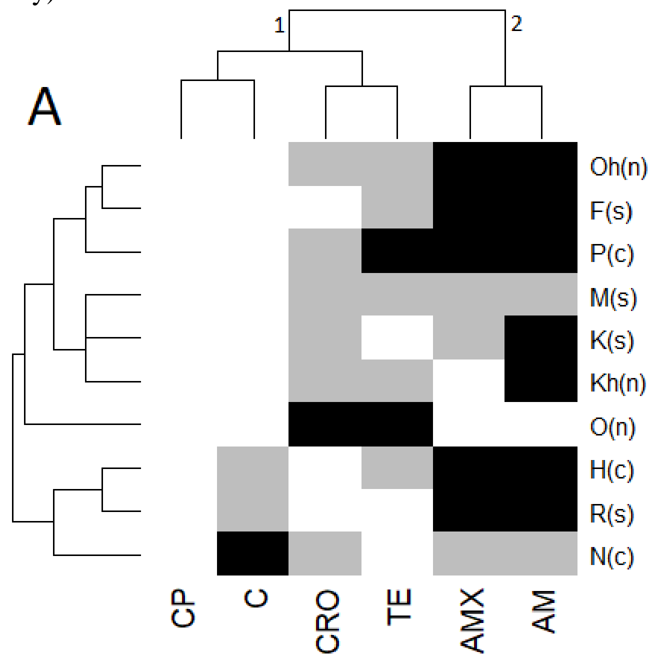
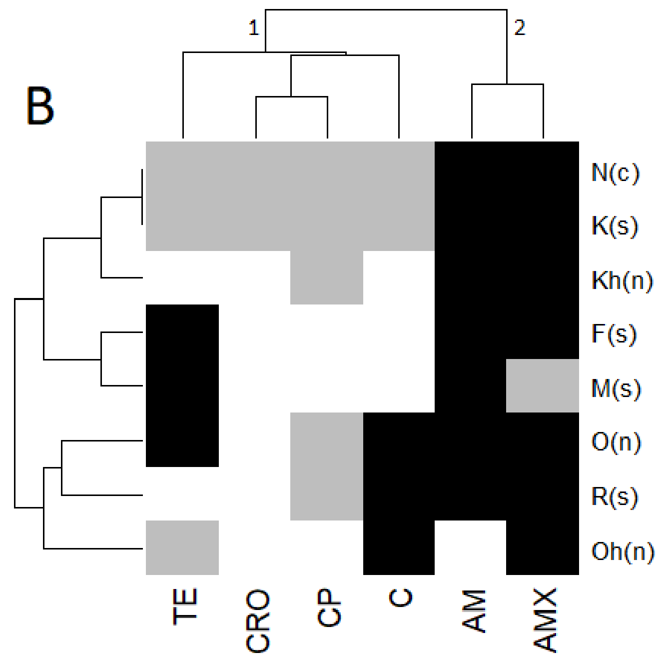


Figure 6. Hierarchical clustering (groups 1-2 for sampling locations and A-B for foodborne pathogens) for contamination rate and prevalence patterns of pathogen strains (White, grey and black colors as low, medium and high contamination rate respectively; rows as isolated pathogens and columns as regions of the sample collection) isolated from *Baghlava* samples collected from different areas of Qazvin, Iran (southern (S), northern (N) and central (C) areas of the city)

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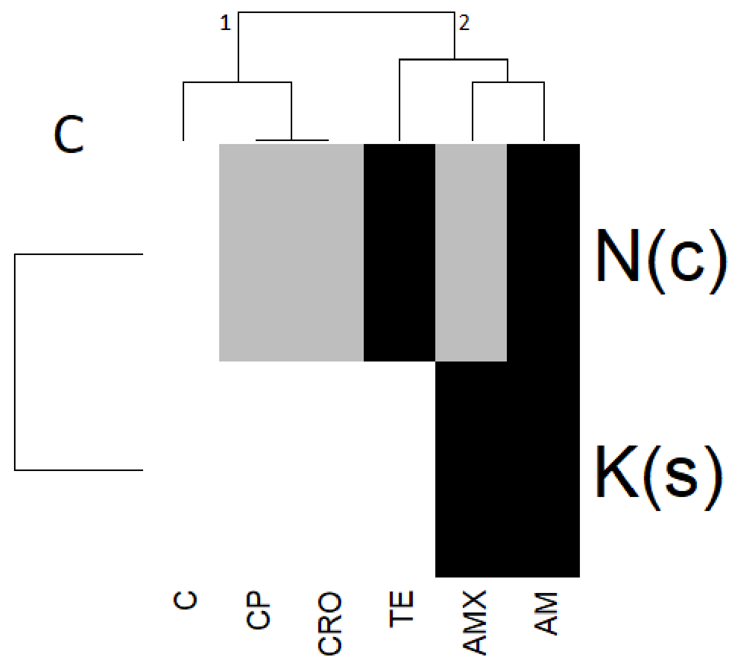
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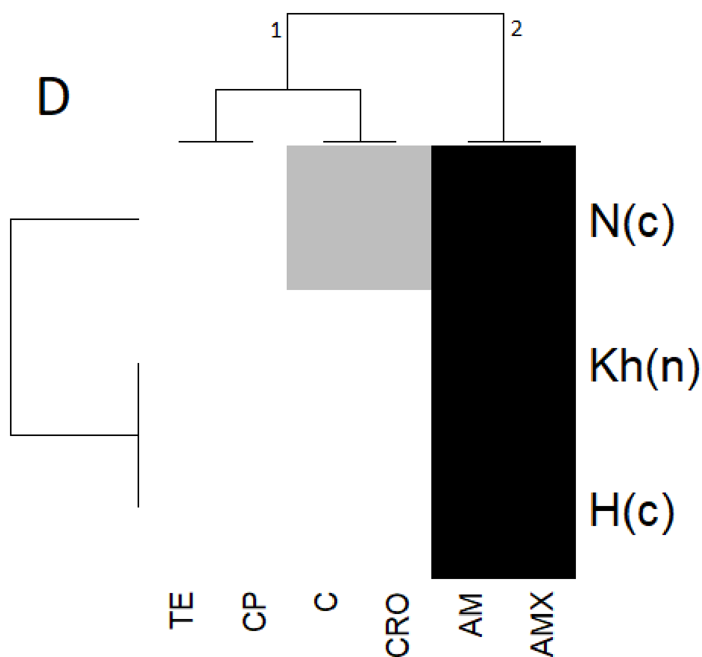

Figure 7. Heatmap and hierarchical clustering of antimicrobial susceptibility phenotypic characteristics of *E. coli* (A), *C. sakazakii* (B), *C. perfringens* (C) and *S. aureus* (D) strains (White, grey and black colors as susceptible, intermediate and resistant to antibiotics respectively; rows as name of the samples and columns as antibiotics) isolated from *Baghlava* samples collected from different areas of the Qazvin, Iran (southern (S), northern (N) and central (C) areas of the city)

3.2. Antimicrobial susceptibility of the isolated strains

Figure 7 A-D provide hierarchical clustering and heatmap of antimicrobial susceptibility properties of the detected pathogens isolated from *Baghlava* samples including *E. coli* (A), *C. sakazakii* (B), *C. perfringens* (C) and *S. aureus* (D). Figure 7 A reveal that isolated *E. coli* strains were more resistant to amoxicillin and ampicillin than other antibiotics visibly illustrated with hierarchical clustering in a significant different group. However, all *E. coli* strains were observed susceptible to ciprofloxacin. Antibiotic resistance pattern of isolated *C. sakazakii* strains (Fig. 7 B) showed strong resistance to amoxicillin and ampicillin as they were categorized in significant different cluster.

On the other hand, susceptibility to ceftriaxone can be distinguished higher than other antibiotics through the AMR patterns of C. sakzakii. Considering low positive samples, most resistance properties for isolated *C. perfringens* and *S. aureus* strains (Fig. 7 C and D) were also observed for amoxicillin and ampicillin antibiotics clustering in the different and significant group as arose from result of gram-negative pathogen AMR patterns. It is worthwhile to note that, susceptibility to chloramphenicol; and tetracycline and ciprofloxacin were detected for *C. perfringens* and *S. aureus* isolates respectively. It is important emphasising that there is not any reasonable relationship between hierarchical clustering of sample types (collected from southern, northern and central areas) in AMR patterns of isolated pathogens. As can be seen in all AMR pattern figures (Fig. 7 A-D), simultaneous presence of amoxicillin and ampicillin AMR phenotypic properties were detected in all gram positive and negative isolated foodborne pathogens according to the results obtained from hierarchical clustering.

Antimicrobial susceptibility profiles of isolates showed resistance to ampicillin and amoxicillin for all isolated foodborne pathogens in this

study, including both gram positive and negative bacteria (Fig. 7 A-D). Several researchers have found phenotypic properties of amoxicillin and ampicillin resistance in bacterial pathogens isolated from food samples (Economou & Gousia, 2015). Ampicillin and amoxicillin resistance properties usually observed in beta-lactamase resistant Enterobacteriaceae family (Bryce et al., 2016) as they were detected in *E. coli* and *C. sakazakii* strains isolated from our samples. Should be considered as multidrug resistant foodborne pathogens, all isolated gram-negative pathogens were resistance to more than one antibiotic illustrated in Fig. 7. However, wide range of antibiotics should be tested on isolated gram-positive pathogens for evaluation of multidrug resistance characteristics (Matuschek et al., 2018). Several researchers showed multidrug resistance properties and complete or intermediate susceptibility to ciprofloxacin for pathogens isolated from food samples (Dan et al., 2015); however, the same results were observed at the present study for all strains isolated from *Baghlava* samples. Multidrug resistance properties of *E. coli* and *C. sakazakii* isolates indicate that antibiotic therapy would not generally be effective for treatment of intestinal and extraintestinal infections and diseases such as bloody-diarrhoea, HUS and meningitis caused by these pathogens (Turnidge, 2015). Also, these results revealed that use of antibiotics must be restricted in agriculture, human and animal health care in Iran.

4. Conclusions

(In the present study, the prevalence of foodborne bacterial pathogens and antibiotic resistance pattern of the strains isolated from *Baghlava* samples manufactured in Qazvin, Iran have been investigated for the first time. Presence of all pathogens except *Salmonella* spp. were confirmed in samples. Prevalence percent of isolated and identified *E. coli*, *C. sakazakii*, *C. perfringens* and *S. aureus* strains were 8.92, 7.14, 1.78 and 2.67%, respectively. For each pathogen, most virulence factor encoded gene was detected by PCR assay and the specific primers. All isolates were resistance to amoxicillin and ampicillin considering multidrug resistant foodborne pathogen isolated from *Baghlava*.

On the other hand, based on phenotypic properties of antimicrobial susceptibility testing, all isolated strains were completely and intermediately sensitive to ciprofloxacin. The contamination rate of all pathogens was observed significantly higher in samples collected from southern and northern areas of the city than central region because of more inspection of manufacturers located in central areas of the city by the health monitoring systems. For more safe and higher microbial quality production of *Baghlava*, hygienic manufacturing process and multidrug resistant bacterial evaluation of raw ingredients are suggested.

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RHEOLOGICAL, PASTING, THERMAL AND GEL PERMEATION CHROMATOGRAPHIC CHARACTERISTICS OF GUAR GUM ACID HYDROLYSATES

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ABSTRACT

Guar gum was partially hydrolyzed with HCl for 30 (GGH_{30}) and 60 (GGH_{60}) min to obtain its acid hydrolysates. Guar gum and its acid hydrolysates were studied for hydration, pasting, rheological and thermal properties. Acid hydrolysis significantly lowered the intrinsic viscosity and molar mass of the hydrolysates. The steady shear properties of guar gum and its hydrolysate (GGH_{30}) studied at 25°C revealed a shear thinning behavior while GGH_{60} displayed a nearly Newtonian behavior. Dynamic measurements revealed a typical biopolymer viscoelastic gel behavior for NGG and GGH_{30}. The FT-IR study indicated no structural changes in the hydrolysates as no additional functional groups were added in the molecular structure after acid hydrolysis of the intact guar gum. The differential scanning calorimetry results displayed lowered thermal stability of the hydrolysates relative to its native counterpart. The present study suggested that partial hydrolysates could be used suitably in different food products as a soluble dietary ingredient.

1. Introduction

Guar gum is a high molar mass polymer comprising of a linear backbone of (1→4) linked β-D- mannopyranosyl units linked with (1→6) α-D-galactopyranosyl side chain residues having mannose and galactose units in 1:2 ratio. The key features such as molar mass, mannose/galactose ratio (M/G) and the distribution of galactose units over the mannose backbone influence the technical applications of the polysaccharide. Guar gum has been employed as a thickening, stabilizing agent and also as a dietary fiber in food industry due to its viscous nature which in turn maintains the rheology of food systems (Ellis *et al*., 2001).

However, guar gum has some drawbacks such as high-water insoluble content, low dissolution rate, tough to regulate consistency and vulnerable to microbial growth (Hongbo *et al*., 2013). The sensorial attributes of foods containing guar gum also tend to be poor because of its high viscosity and conjointly limits its addition in foods at high concentrations (Cui *et al*., 2007). To overcome these drawbacks, guar gum needs to be modified to attain desired characteristics like increased water solubility, solution clarity, and ionic character and increased shelf life depending on the specific application. Degradation of guar gum is vital for several food applications with an objective to raise the dietary fiber amount in foods as low molar mass or partially hydrolyzed gum is regarded as ample source of dietary fiber and has remarkable health implications (Yoon *et al*., 2008). Partially hydrolyzed guar gum is considered as a prebiotic ingredient which can play beneficial role in improving the gastro-intestinal health (Li and Hu, 2018). The guar gum especially partial degraded guar gum finds its food applications in juice, ice creams, sauces and salad dressing etc. (Krishnaiah *et al*., 1998;

Tuohy *et al*., 2001). Prebiotic potential of guar gum hydrolysate has been reported in biscuits (Tuohy *et al*., 2001). Acid hydrolysis is considered as a convenient method for the degradation of guar gum into various low molar mass units allowing its addition in liquid food such as beverages at a concentration effective enough to provide the beneficial health effects of guar gum.

Rheology has been employed to investigate the potential applications of guar gum in food products. However, still the rheological characterization of guar gum is unclear due to its complicated nature that cannot be described by a single model (Wientjes *et al*., 2000). Also, there has been too little studies discussing about the pasting profiles of gum solutions. RVA has been mainly associated with the starch, the primary reason behind the less applicability of RVA as an analytical tool for gum solutions. Mainly controlled shear stress devices have been used to analyze the rheological behavior of gum solutions but RVA can be used as a powerful tool to distinguish gums on basis of their viscous behavior in respect of the temperature. So, an attempt has been made to analyze the pasting, rheological and thermal behavior of guar gum and its acid hydrolysates to expand its applicability in various food products.

2. Materials and methods

2.1. Materials

Food grade guar gum was procured from Hindustan Gums & Chemicals Ltd., Bhiwani and other reagents and chemicals in the study were of analytical grade.

2.2. Acid hydrolysis of guar gum

Guar gum was depolymerized by the procedure as proposed by Chauhan *et al.* (2009) with minor changes. 10g guar gum was dispersed in 80% methanol (200ml) consisting HCl (5% w/v). The reaction mixture was then heated for 30 and 60 min separately, at 65ºC. The gum hydrolysis was terminated by neutralization with NaOH (1N) and hydrolysates were filtered, washed with absolute ethanol, freeze dried and milled.

2.3. Intrinsic Viscosity

Intrinsic viscosity was analyzed at ambient temperature (25º C) with the aid of an Ostwald's viscometer. Gum solutions (0.02-0.1% w/v) were prepared by sprinkling gum in a vortex with water and left overnight for complete dissolution. Relative viscosity was determined using the following equation:

$$\eta_r = \frac{t}{t_0} \quad (1)$$

where t is the flow time of gum solution and t_0 is the flow time of pure solvent. Specific viscosity was determined from relative viscosity using the equation

$$\eta_{sp} = \eta_r - 1 \quad (2)$$

Reduced viscosity was then obtained from specific viscosity using the correlation

$$\eta_{red} = \frac{\eta_{sp}}{C} \quad (3)$$

Intrinsic viscosity (η) was analyzed by measuring reduced viscosity at different concentrations and extrapolating to concentration C = 0.

2.4. Molar mass determination

The molar mass was analyzed from the viscometry results as well as chromatographic results.

2.4.1. Molar mass determination with viscometry

Viscosity average molar mass (M_v) was measured from intrinsic viscosity using Mark-Houwink's equation,

$$[\eta] = KM_v^{\alpha} \quad (4)$$

where K= 5.13 x 10^4 and α= 0.72 (Beer *et al*., 1999).

2.4.2. Gel permeation chromatography

Number average molar mass (M_n), weight average molar mass (M_w) and polydispersity index (PDI) (M_w/M_n) of guar gum and its

hydrolysates were assessed using a gel permeation chromatography system (Viscotek GPC max, Malvern, UK) with an aqueous column (A6000, 300x8 mm) connected to a RI detector. Guar gum and its hydrolysates (0.5 mg/ml) were dissolved in $NaNO_3$ (0.05 M), filtered through 0.22 µm nylon filter prior injection and eluted at a flow rate of 0.7 ml/min at 35° C. The GPC was calibrated with the known molar mass standards of pullulan (342 - 7,10,000 Da, Fluka, USA). Molar mass was analyzed using linear regression equation obtained for pullulan standards.

2.5. Degree of Polymerization

Viscosity average molar mass of gums was used to determine the degree of polymerization using the following equation:

$$\text{Average DP} = \frac{\text{molecular weight of polymer}}{\text{molecular weight of monomer}} \quad (5)$$

Molar mass of guar gum monomer used was 270 (Mahammad *et al*., 2006).

2.6. Hydration properties of gums

Water binding capacity (WBC) of guar gum and its hydrolysates was measured according to the standard AACC method (2010) using 0.1 g sample. The WBC was determined in g/g as the amount of water bound to per gram of the sample. Swelling power and solubility of gums were determined by the procedure described by Bae *et al.* (2009) by using 0.1 g sample. Swelling power was calculated as the ratio of the wet precipitate weight to the dry sample weight whilst solubility was measured as the dry supernatant weight to dry sample weight.

2.7. Rheological properties of gums

2.7.1. Solution preparation

The appropriate amount of gum sample was dissolved in deionized water to get the final concentration of 1, 2 and 3%. The dispersions were vigorously mixed for 1 h at room temperature and subsequently heated at 80° C in a shaking water bath for 30 min to achieve complete hydration.

2.7.2. Dynamic and steady shear rheological properties

Dynamic and steady flow measurements of aqueous gum solutions were performed at 25° C with a dynamic rheometer (MCR 102, Anton Paar GmbH, Germany) equipped with a cone and plate geometry (40 mm diameter, 0.08 mm gap and 1° cone angle). Frequency sweep measurements were conducted in the range (0.1-100 rad/s) at 1% constant strain within the linear viscoelastic range as observed by amplitude sweep test.

Steady shear tests were also conducted at 25° C to obtain flow curve data with the increasing shear rates from 0 to $100s^{-1}$. The data obtained was fitted to the following models:

Power law model,

$$\tau = K\gamma^{\cdot n} \quad (6)$$

Herschel-Bulkley model,

$$\tau = \sigma_o + K\gamma^{\cdot n} \quad (7)$$

where τ is the shear stress (Pa), σ_o is the yield stress (Pa), γ is the shear rate (s^{-1}), n is the flow behavior index (dimensionless) and K is the consistency coefficient (Pa s^n).

2.8. Rapid visco analysis

The viscosity profiles of gums were studied by Rapid Visco Analyzer (RVA). Sample dispersions (1 and 2% w/w) were prepared in a canister and positioned in the instrument. In the beginning of the test, samples were stirred at 960 rpm for 1 min succeeded by 160 rpm all over the test. The temperature was held at 80°C for 2 min and subsequently cooled to 30°C at a wage of 4°C/min.

2.9. FT-IR spectroscopy

The structural variations among guar gum and its hydrolysates were analyzed using FT-IR spectroscopy. The guar gum samples were directly placed on to sampling unit and spectra were recorded between 4000 and $400cm^{-1}$ with a FT-IR spectrophotometer (Bruker Alpha, Platinum ATR, Germany).

2.10. Thermal properties

The thermal properties of guar gum and its hydrolysates were assessed with a differential scanning calorimeter (DSC 25 TA instruments, USA). The powder samples (3.5 mg±0.1) were weighed in an aluminum pan and loaded in the instrument with a blank pan as a standard. The scans were performed in the temperature range from 50-450°C with a wage of 10° C/min and different thermal parameters including onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH) were measured.

2.11. Statistical analysis

The statistical variations were assessed by one-way Analysis of Variance (ANOVA) using SPSS version 19.0 with a significant level ($p < 0.05$). All determinations were made in triplicates and the data was expressed as mean values ± standard deviation.

3. Results and discussion

3.1. Intrinsic viscosity and viscosity average molar mass of gums

Intrinsic viscosity measures the hydrodynamic size engaged by the molecules present in the solution and is directly linked to their molar mass and shape (Gupta *et al*., 2015). Acid hydrolysis led to the cleavage of glycosidic bonds of the polymer chain and molar mass of guar gum leading to a drop in the intrinsic viscosity. The η of the NGG was found to be greater (11.99 dL/g) in comparison to its hydrolysates, GGH_{30} (4.98 dL/g) and GGH_{60} (2.01 dL/g) respectively (Table 1). High molar mass guar gum chains were disintegrated into low molar mass fragments which indicated weakened aggregations in hydrolyzed gums and thus low molar mass fractions can be obtained to achieve specific applications (Cheng *et al*., 2002). Molar mass was observed to be a function of duration period of acid hydrolysis as it decreased from 1.06×10^6 Da to 3.19×10^5 Da and 9.02×10^4 Da after 30 and 60 min duration of acid hydrolysis, respectively (Table 1).

Table 1. Molar mass and intrinsic viscosity of guar gum and its hydrolysates

Sample	η (dL/g)	M_v (Da)	DP	M_w (Da)	M_n (Da)	PDI
NGG	11.99± 0.02[c]	1.06×10^6 ± 0.01[c]	3925.92± 6.11[c]	1.02×10^7 ±0.02[c]	2.39×10^6 ±0.01[c]	4.26± 0.01[c]
GGH_{30}	4.98± 0.03[b]	3.19×10^5 ± 0.01[b]	1181.48± 3.51[b]	3.11×10^6 ±0.01[b]	8.16×10^5 ±0.02[b]	3.80± 0.02[b]
GGH_{60}	2.01± 0.01[a]	9.02×10^4 ± 0.02[a]	334.26± 3.02[a]	2.79×10^5 ±0.01[a]	1.05×10^5 ±0.01[a]	2.64± 0.01[a]

NGG - Native guar gum, GGH - Guar gum hydrolysate, η - intrinsic viscosity, M_v - viscosity average molar mass, DP - degree of polymerization, M_w - weight average molar mass, M_n - number average molar mass, PDI - polydispersity index

3.2. Gel permeation chromatography (GPC)

The GPC chromatograms of guar gum and its acid hydrolysates are shown in Figure 1. The GPC profiles of acid hydrolysates were moved towards the right side in retention time as compared to NGG revealing their low molar masses. The weight average molar mass (M_w) and number average molar mass (M_n) were observed to be higher for NGG (1.02×10^7 and 2.39×10^6 Da) when compared to its acid hydrolysates, GGH_{30} (3.11×10^6 and 8.16×10^5 Da) and GGH_{60} (2.79×10^5 and 1.05×10^5) respectively (Table 1). The value for molar mass determined by GPC were on the higher side in comparison to those obtained from intrinsic viscosity molar weight. Similar observations have been observed for hydrolysed galactomannan from *C. pulcherrima* (Buriti *et al*., 2014). Polydispersity index was found to be lower for GGH_{30} (3.80) and GGH_{60} (2.64) in comparison to NGG (4.26) indicating a high proportion of high

molar mass molecules in native guar gum. The hydrolysates showed narrow molar mass distribution (MWD) reflecting the homogeneity of low molar mass molecules. Acid hydrolysis might have disrupted the glycosidic bonds resulting into a decrease in the molecule weight of gum molecules. The lower degree of polymerization of hydrolysates in contrast to its native gum also confirmed that acid hydrolysis had significantly broken the bonds between mannose units leading to decrease in molar mass and hence lower degree of polymerization. Molar mass and concentration are the key factors that affect the functional properties of polysaccharides, and the above results indicate that guar gum can be degraded to various M_w with narrow MWD to achieve desired characteristics.

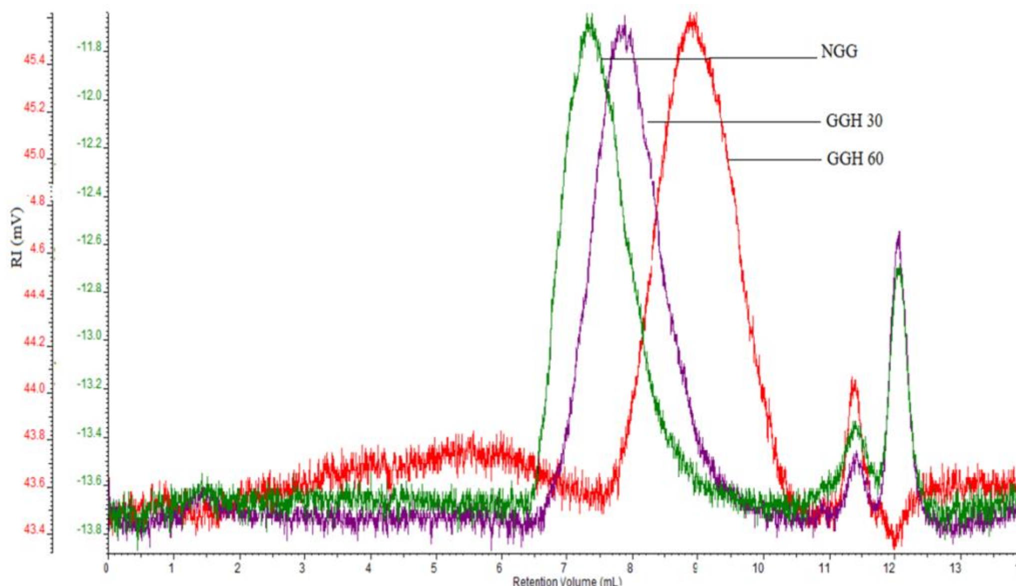


Figure 1. GPC chromatograms of guar gum and its acid hydrolysates.

Table 2. Hydration properties of guar gum and its acid hydrolysates

Sample	Water binding capacity (g/g)	Swelling power (g/g)	Solubility (%)
NGG	21.44 ± 1.42^{c}	66.52 ± 2.13^{c}	30.86 ± 4.16^{a}
GGH_{30}	6.81 ± 0.48^{b}	20.42 ± 3.71^{b}	74.64 ± 3.79^{b}
GGH_{60}	3.56 ± 0.29^{a}	4.21 ± 0.25^{a}	87.86 ± 0.14^{c}

Values are mean ± S.D of triplicates.
Values in the same column with different letters are significantly different ($p<0.05$)

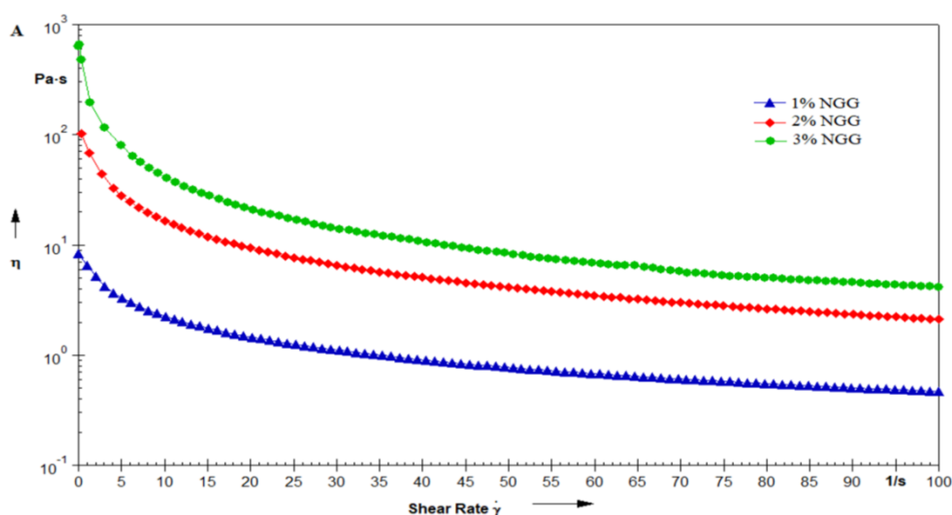
3.3. Hydration properties

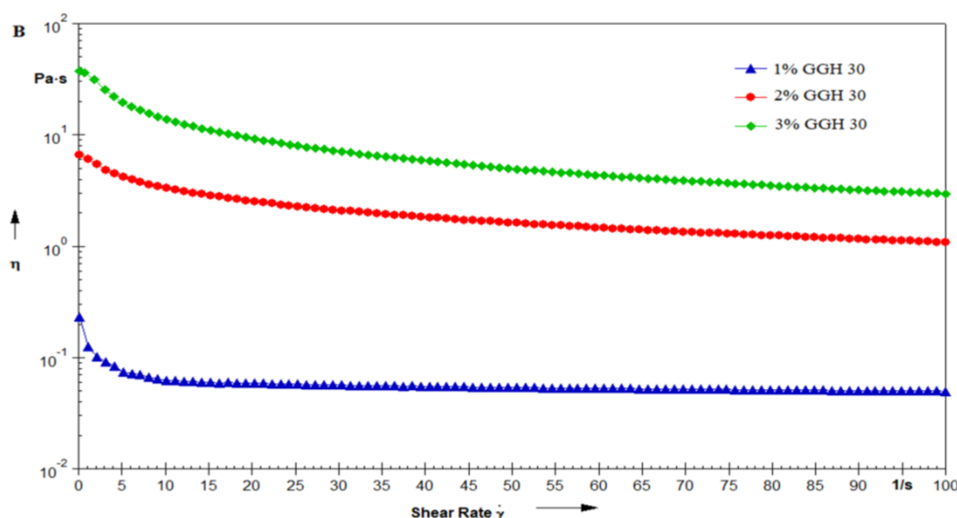
Hydration properties of food components are very crucial in food industry as it influences the overall product quality, stability, and shelf life of food products. Highest water binding capacity was observed for NGG, followed by GGH_{30} and GGH_{60}, respectively (Table 2). The greater affinity of native guar gum towards water is directly related to its molecular structure owing greater number of OH groups allowing more water interactions via hydrogen bonding. The hydrolysates showed less affinity towards water in comparison to NGG because of a smaller number of hydroxyl groups resulting from the reduction in chain length during acid hydrolysis. Similarly, swelling power was also significantly ($p < 0.05$) higher in case of NGG in contrast to its acid hydrolysates (Table 2). Molecular structure plays a crucial part in regulating the functionality of gums in foods. Higher the swelling power, lesser will be the commercial

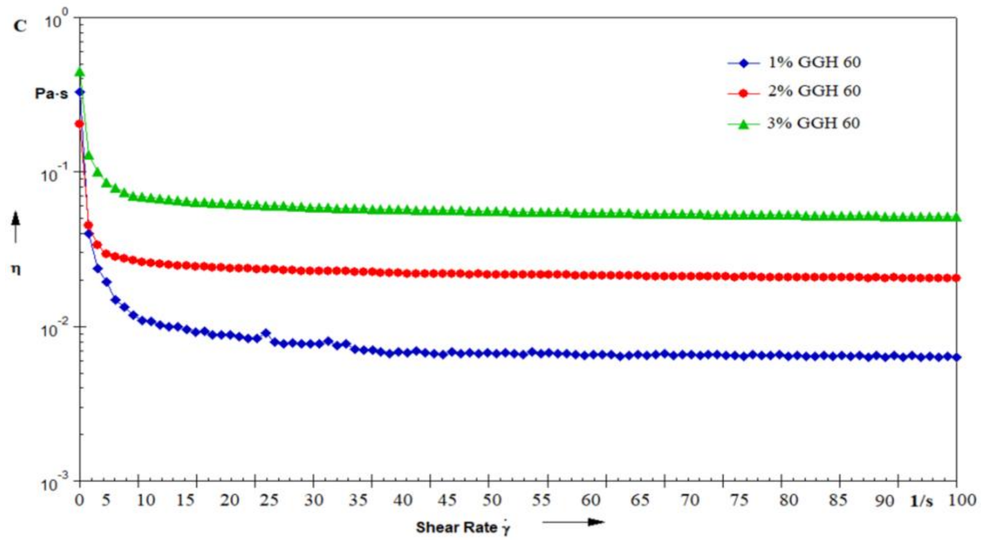
applications (Dodi et al., 2011). So, acid hydrolysis can be used to increase the potential applications of guar gum in foods. A significant increment ($p < 0.05$) in solubility was noticed for hydrolysates, being maximum for GGH_{60} (87.86%) and minimum for NGG (30.86%). Solubility increased by 141% after 30 min hydrolysis and by 184% after 60 min hydrolysis. Formation of low molar mass fragments through disintegration and depolymerization of guar chains after acid hydrolysis might be responsible for the increased solubility of hydrolysates as small monosaccharaides have higher hydration ability as compared to native gum. These results implied that guar gum hydrolysates can be used a soluble dietary ingredient in food products.

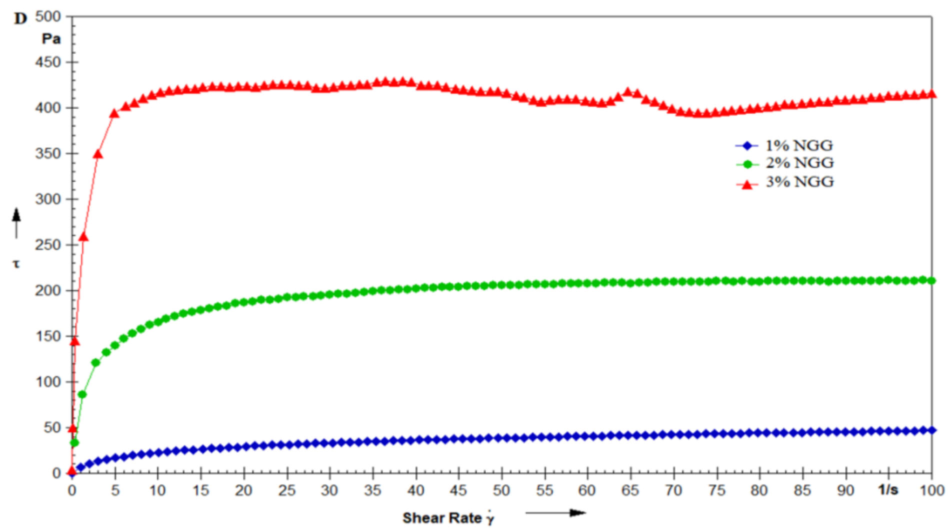
3.4. Rheological properties of gums
3.4.1. Steady shear properties

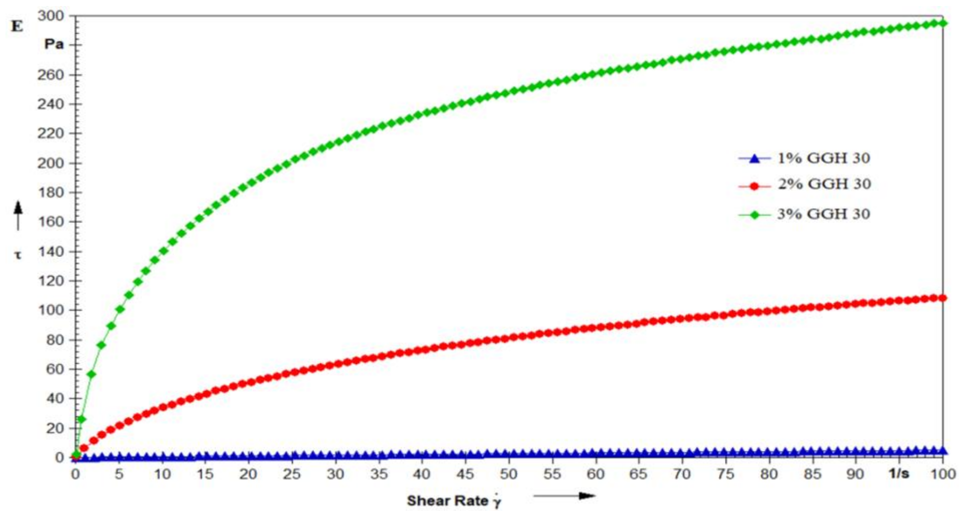
Rheology illustrates the flow behavior of polysaccharides and determine their potential applications in foods. The solutions of guar gum and its acid hydrolysates prepared with different concentrations of 1, 2 and 3% exhibited a non-Newtonian shear-thinning behavior in the shear range from 0-100s^{-1}. As shown in viscosity curves given in Figure 2 (a, b and c), viscosity decreased with the growing shear rates, which could be attributed to the reason that disruption dominated over the formation of new entanglements with increasing shear rates and molecules aligned in the direction of flow resulting into lowering of the viscosity (Hussain *et al*., 2015).






C
Pa·s
η
1% GGH 60
2% GGH 60
3% GGH 60
Shear Rate $\dot{\gamma}$
1/s


D
Pa
τ
1% NGG
2% NGG
3% NGG
Shear Rate $\dot{\gamma}$
1/s


E
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1% GGH 30
2% GGH 30
3% GGH 30
Shear Rate $\dot{\gamma}$
1/s

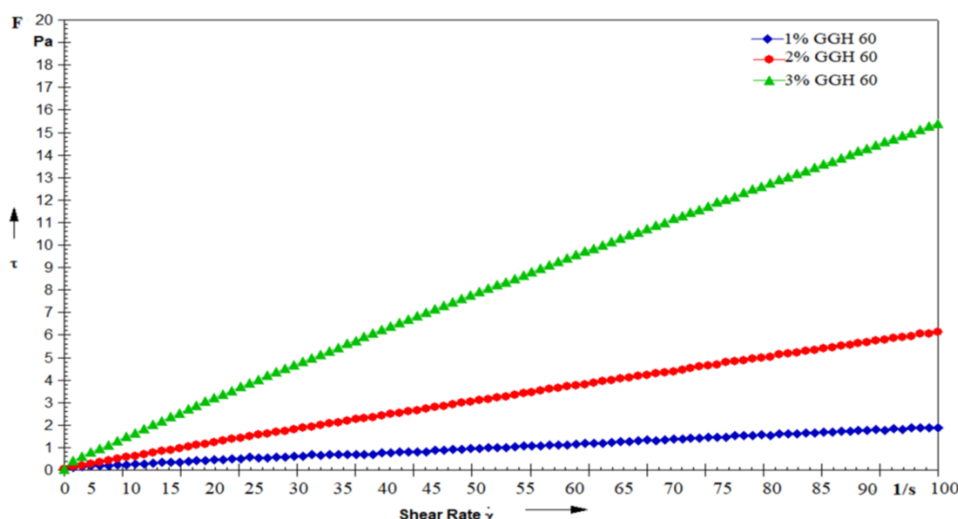


Figure 2. Viscosity curves (a, b, c) and flow curves (d, e, f) of NGG (native guar gum), GGH_{30} and GGH_{60} (guar gum hydrolysate) at different concentrations.

These outcomes also reflected the concentration dependency of viscosity of the guar gum and its hydrolysate which increased with increasing concentrations. Guar gum and its hydrolysates have hydroxyl groups within their chemical structure due to which entanglement occurrs at higher concentrations resulting in higher viscosities (Gong *et al.*, 2012).

The flow curves shown in Figure 2 (d, e and f) depicts the flow properties of gum solutions with different concentrations at 25° C. The flow behavior as studied by using using Power law and Herschel-Bulkley model also indicated a non-Newtonian pseudoplastic behavior. Significant variations were observed ($p < 0.05$) in the values of 'σ', '*n*' and '*K*' for NGG and its acid hydrolysates (Table 3).

Table 3. Flow parameters of guar gum and its acid hydrolysates determined at different concentrations.

Sample	Power	Law	Model	Herschel	Bulkley	Model	
	n	*K* (Pa.s^n)	R^2	σ(Pa)	*n*	*K* (Pa.s^n)	R^2
1% NGG	0.31±0.01^a	10.48±0.32^b	0.97	5.75±0.58^d	0.12±0.01^c	46.73±4.34^c	0.98
2% NGG	0.25±0.06^a	75.63±18.6^d	0.74	32.53±1.16^e	0.06±0.01^b	548.78±58.93^e	0.94
3% NGG	0.24±0.03^a	127.48±4.46^e	0.00	48.81±2.04^f	0.01±0.00^a	2701.56±425.08^f	0.00
1% GGH_{30}	0.80±0.02^d	0.11±0.15^a	0.98	0.08±0.02^b	0.86±0.02^f	0.09±0.03^a	0.99
2% GGH_{30}	0.61±0.01^d	7.53±0.66^b	0.93	0.74±0.11^b	0.52±0.01^e	11.30±1.14^b	0.98
3% GGH_{30}	0.48±0.03^b	36.09±0.12^c	0.82	2.36±0.43^c	0.34±0.02^d	70.61±1.30^d	0.97
1% GGH_{60}	0.62±0.01^c	0.04±0.01^a	0.93	0.03±0.02^a	0.88±0.02^f	0.04±0.02^a	0.98
2% GGH_{60}	0.77±0.03^c	0.06±0.01^a	0.97	0.05±0.03^a	0.94±0.01^g	0.08±0.01^a	0.99
3% GGH_{60}	0.81±0.01^c	0.13±0.01^a	0.98	0.12±0.04^a	0.92±.002^g	0.10±0.04^a	0.99

Values are mean ± S.D of triplicates.
Values with different letters in the same column are significantly different ($p<0.05$)

Highest yield stress was observed for 3% NGG (48.81) while lowest for 1% GGH_{60} (0.03). This was indicative of the presence of some entagled networks in the samples which should be disintegrated to initiate the flow and it also reflected the strong associated network of NGG in comparison to its hydrolysates. Acid hydrolysis imparted a drastic change in the flow properties of guar gum as the magnitude of shear stress values for GGH were much lower than that of the NGG. Also, a decrease in the pseudoplasticity was observed with increase

in the degree of hydrolysis. The flow behavior index (*n*) shows the Newtonian or non-Nwetonian behavior of the polymers and '*n*' value near to 1 indicates the Newtonian behavior while '*n*' values less than 1 reflects the pseudoplastic behavior f the polymers. The '*n*' values decreased with rising concentration for NGG and GGH_{30} indicating that the pseudoplastic behavior enhanced at higher concentrations while an opposite trend was found for GGH_{60} where '*n*' values increased with increasing concentration suggesting increasing concentrations led to the Newtonian like behavior of GGH_{60}. The consistency index for hydrolysates was much lower in comparison to NGG suggesting a much drop in viscosity due to decrease in the molar mass and chain length by acid hydrolysis. A near Newtonian behavior was observed for GGH_{60} and almost negligible viscosity values were obtained. It can be concluded from the flow results that guar gum hydrolysates can be used in food products like beverages to enhance their dietary fibre content without modifying their viscosity. The flow results were also suggestive of the fact that NGG should be added upto 2% concentration in the food products, as the concentration beyond this may deteriorate the textural properties of foods. Evidently, NGG at 3% concentartion showed a very high consistency and no regression coefficient was found which suggested a very high viscosity making it unsuitable for the textural properties of foods. Also an interrupted flow was achieved which might be due to the high viscosity of the system.

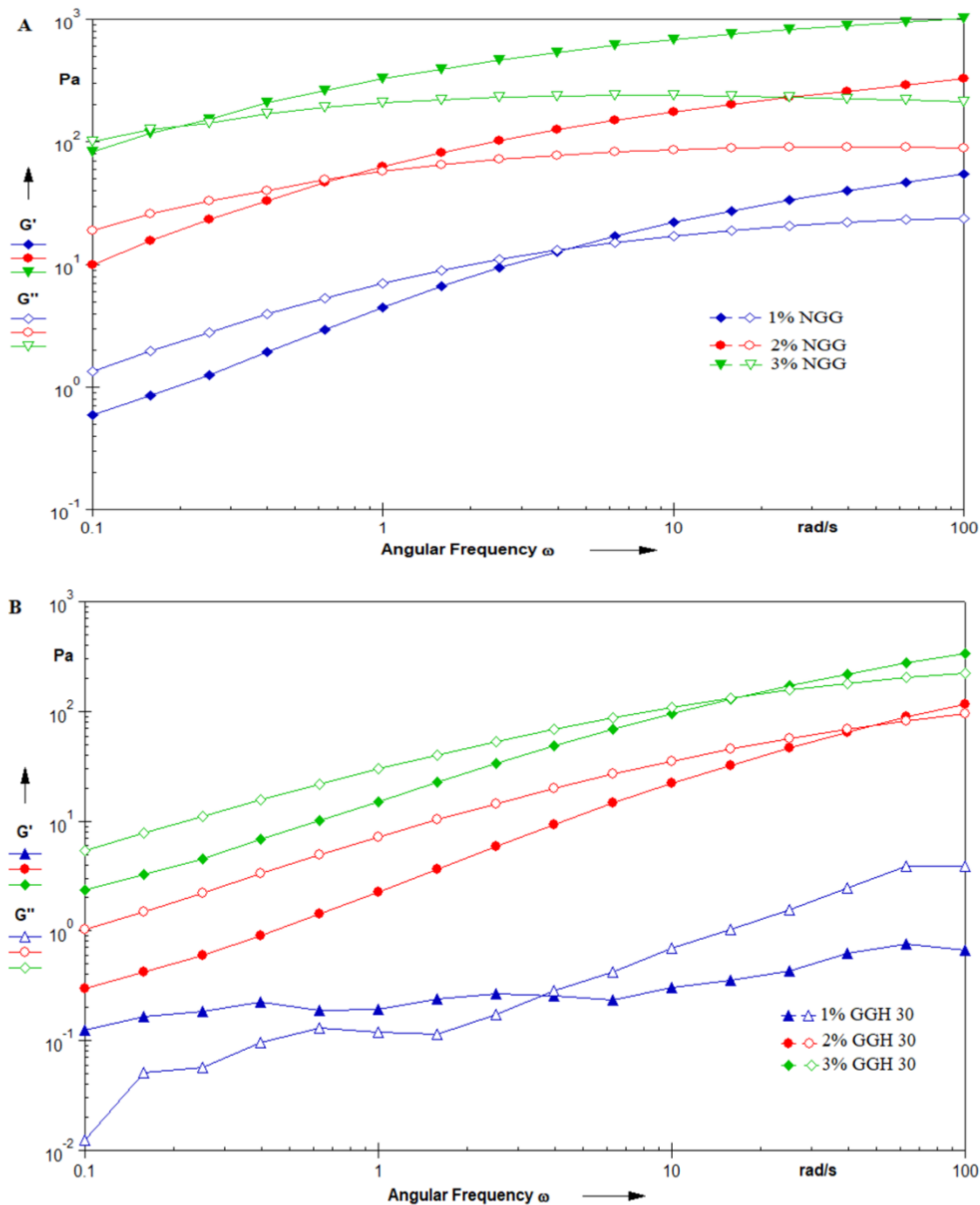
3.4.2. Oscillatory experiments

The viscoelastic characteristics of guar gum and its acid hydrolysates at different concentrations were analyzed by performing frequency sweep tests which determines the storage (*G'*) and loss modulus (*G"*). The dynamic mechanical spectra of guar gum and its acid hydrolysates analyzed at 25° C are shown in Figure 3 (a and b). Dynamic measurements were only conducted on the NGG and GGH_{30} because GGH_{60} showed a near Newtonian behavior and was having very less viscosity even at the highest concentration used and the LVE range could not be reached as the strain value was too low to be determined by the instrument. Therefore, only flow properties were analyzed for GGH_{60}. The rheograms for NGG and GGH_{30} revealed that values of both *G'* and *G"* shifted upwards with rising frequency (ω). The frequency sweep for various gum solutions showed the classic characteristics of macromolecular solutions where *G"* was higher than *G'* at low frequencies while at higher frequency range, *G'* was predominant. Similar behavior for various galactomannans and disordered random-coil polysaccharides have reported by different researchers (Bourbon *et al*., 2010; Oblonsek *et al*., 2003).

The extent of *G'* and *G"* was found to be greater for NGG as compared to GGH_{30} which increased with increasing gum concentrations. These results revealed that the solutions showed a liquid like character till crossover occurred after which elasticity prevailed due to highly structured nature of the polysaccharide. It was found that GGH_{30} showed more liquid like behavior while NGG exhibited more entangled behavior as *G'* dominated over the G" at higher frequencies. Similar findings were also reported by Pollard *et al*. (2010) and Thombre and Gide (2013). It was observed that the crossover frequency decreased as the concentration of NGG was increased, which might be a consequence of increased relaxation times resulting into entanglement of polymeric chains in solution (Torres *et al*., 2014). However, in case of GGH_{30}, the crossover frequency increased as the concentration increased from 1-2% but it decreased at a concentration of 3%. These results indicated that elasticity improved with increasing concentrations indicating the strong associations among the molecules in the polymer solutions.

Tan δ distinguishes polymers based on their viscoelastic character which is the ratio of *G'* versus *G"*. Tan $\delta > 1$ shows the prominence of viscous nature while tan $\delta < 1$ reflects the

elastic nature. As can be seen from Figure 3 (c and d) NGG displayed an elastic characteristic at higher frequencies while viscous characteristics at lower frequencies as tan δ values increased in the lower frequency range and were beyond 1. Similar trend was revealed by GGH_{30} but NGG presented more elasticity in comparison to its acid hydrolysate which was obviously due to highly structured network of the native gum because of its greater branching and molar mass. Tan δ values decreased with increasing gum concentrations in both NGG and GGH_{30}. Low tan δ values suggested that higher stress values are needed to the break the intermolecular associations between gum molecules. These results are also consistent with the flow behavior as described above reflecting higher yield stress values in case of NGG in comparison to its hydrolysates. Overall, these dynamic results showed that guar gum and its hydrolysates had elastic behavior at high concentrations and viscous behavior at low concentrations.



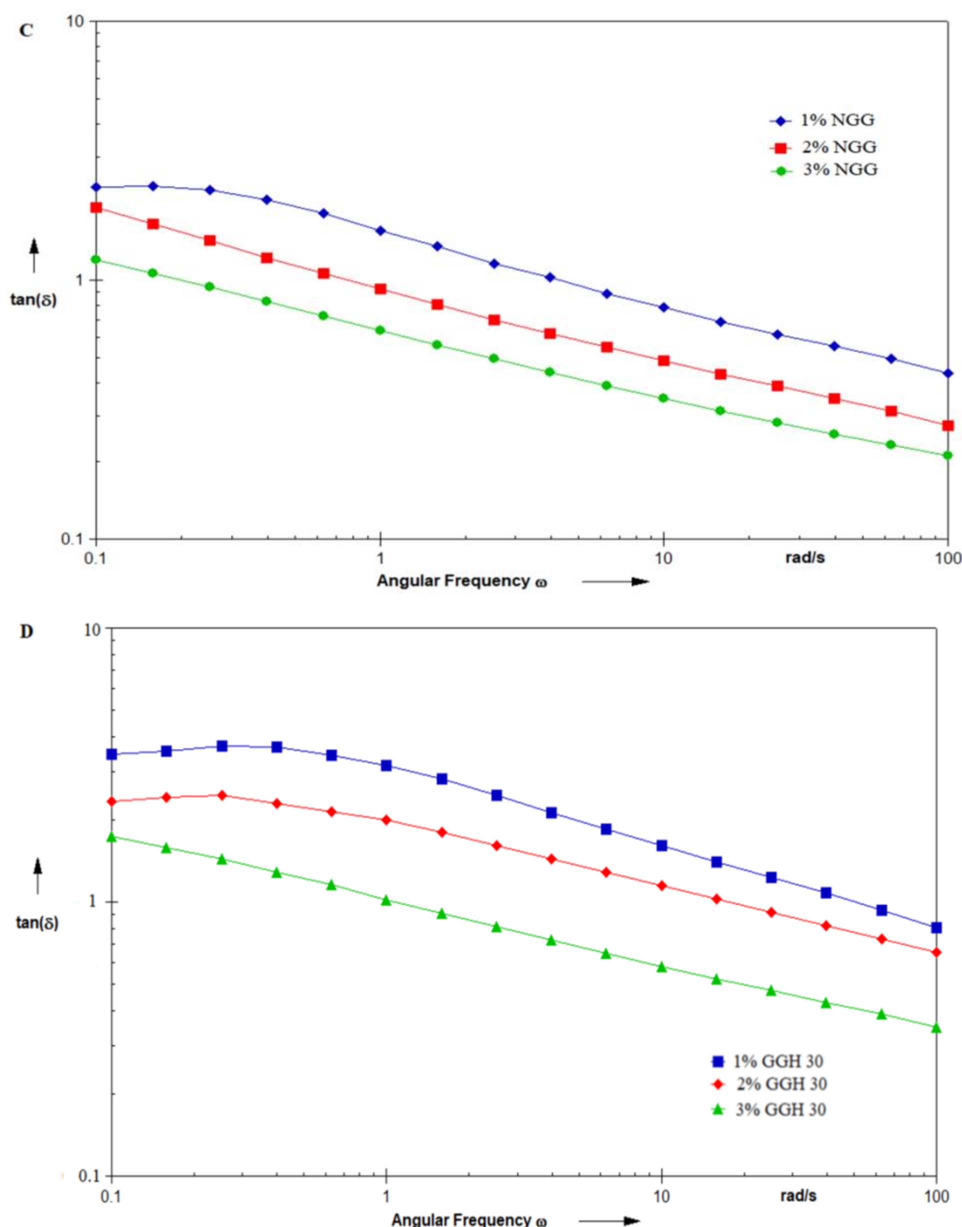


Figure 3. Dynamic rheological properties (a, b) mechanical spectra and (c, d) loss tangent (tan δ) of NGG and GGH_{30} at different concentrations measured at 25°C.

3.5. Rapid visco analysis

The viscosity profiles of guar gum and its hydrolysates were studied at two levels (1 and 2%) with the aid of RVA. Guar gum is a cold-water soluble polymer whose final viscosity relies on its molar mass and dissolution temperature. As can be seen from Figure 4 the decrease in viscosity continued till temperature persisted at 80°C. However, as the temperature dropped, the viscosity began to rise reaching the final viscosity. This behavior reflects that viscosity was reliant on temperature and gum amount. It has been reported that gum molecules move faster at higher temperatures due to enhanced thermal vibrations and eventually results into lower viscosities, while at low temperatures, molecules associate to make entanglements via intra molecular hydrogen bonding due to reduced mobility of gum molecules (Morris, 1990). Significant difference ($p < 0.05$) was observed between the viscosities of guar gum and its acid

hydrolysates. Highest final viscosity was observed for 2% NGG (3265cp) while lowest for 1% GGH_{60} (22cp). 3-fold reduction in viscosity was observed when guar gum was hydrolyzed for 30 min succeeded by 64-fold reduction in 60 min hydrolysis at 2% level. Guar gum and its hydrolysates revealed their specific cooling profile which was obvious due to changes in the molar mass of gums after acid hydrolysis. Viscosity is often related to molar mass and chain length; greater the molecular weight, greater will be the viscosity. These results suggest that RVA can be used to distinguish the hydrocolloids on basis of their viscosity and to assess their suitability for food purposes.

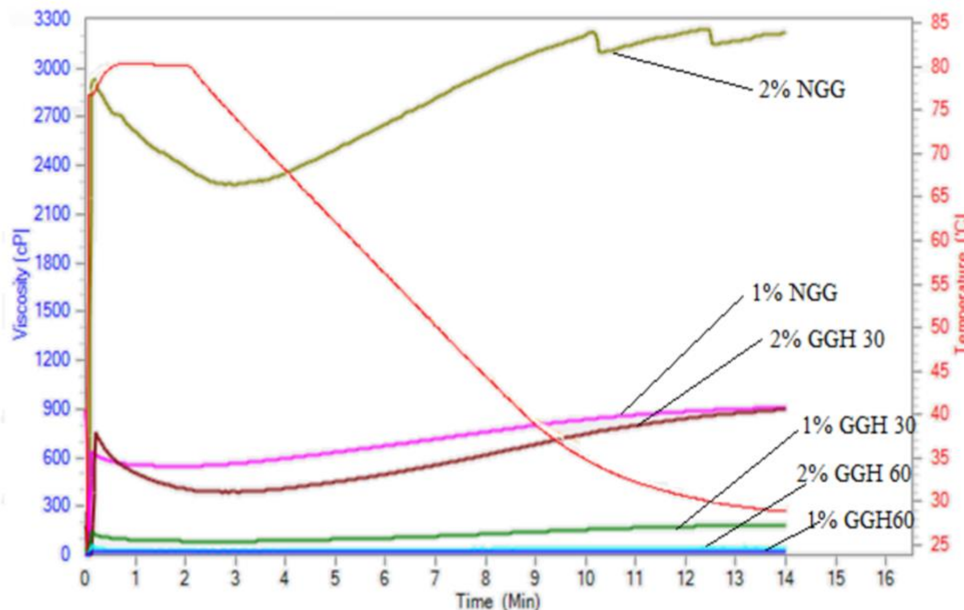


Figure 4. RVA profiles of guar gum and its hydrolysates at different concentrations.

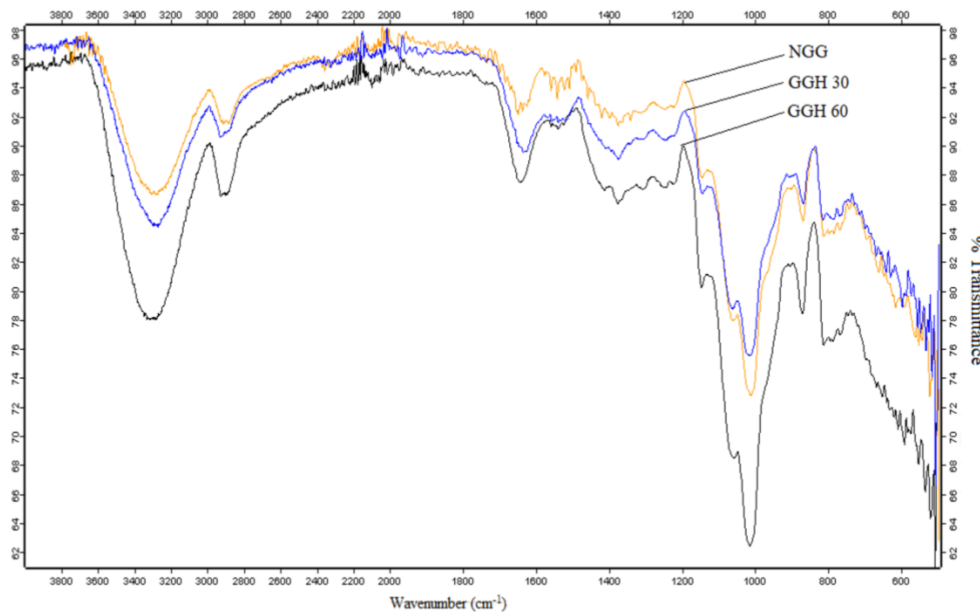


Figure 5. FT-IR spectra of guar gum and its acid hydrolysates.

3.6. FT-IR

Acid hydrolysis might have brought some chemical changes in the structure of guar gum due to the disintegration of glycosidic bonds. So, FT-IR was performed to analyze the structural variations in the guar gum after acidic depolymerization. It can be seen that both hydrolysates had the similar superimposable spectra to that of NGG, which indicated that no structural changes occurred on account of acid hydrolysis (Figure 5). FT-IR results also revealed that no functional groups were induced and the depolymerization of polymer was basically due to cleavage of glycosidic bonds between the guar molecules. Peaks in the spectral region of 3200-3400 cm^{-1} are attributed to O-H stretching of the polymer and water involved in hydrogen bonding and peaks in the region near 2900 cm^{-1} corresponds to –C-H stretching mode (Shobha *et al*., 2005). The bands near 800 and 1200 is due to the stretching vibrations of C–C–O, C–OH and C–O–C polysaccharide backbone respectively. The peak around 1600 is attributed to the bound water and in the spectral region around 1400 corresponds to $–CH_2$ bending. Earlier studies also reported no structural changes after partial hydrolysis of galactomannans (Mudgil *et al*., 2012; Prajapat *et al*., 2015).

3.7. Thermal properties

Thermal stability of gums is considerable in determining the applicability of polymers in foods where food is thermally processed such as baking, sterilization, and pasteurization etc. (Hussain *et al*., 2018). Two distinct peaks were observed for NGG and GGH_{30} while three peaks were observed for GGH_{60}. The first peak at temperature around 100° C for NGG and GGH_{30} and around 150º C for GGH_{60} corresponds to the early endothermic events associated with the dehydration of water. The second endothermic peak observed for GGH_{60} at around 200°C could be due to the early disintegration of mannose and galactose units from the guar gum backbone resulting from the acidic depolymerization. The presence of low molecular fragments in the acid hydrolysate, GGH_{60} could be responsible for the early decomposition of the guar molecules. The later peaks correspond to the exothermic transitions showing the commencement of combustion due to the random breakdown of glycosidic bonds arising from dehydration, pyrolytic decomposition and vaporization and elimination of volatile substances at higher temperatures (Cerqueira *et al*., 2011; Mudgil *et al*., 2012). Wider peaks were observed in case of native guar gum when compared to its hydrolysates. The presence of wider peaks in case of intact guar gum was indicative of broad range of molar mass distribution in comparison to hydrolysates. These observations were consistent with the observations on the polydispersity index made during the GPC study.

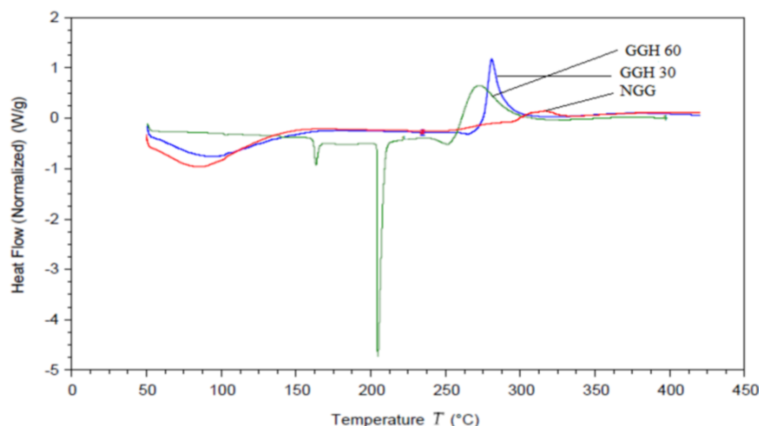


Figure 6. Thermal scans of native guar gum and its acid hydrolysates

Thermograms of guar gum and its hydrolysates are shown in Figure 6. The onset, peak and conclusion temperature shows the temperature range in which thermal transitions occur in the gum molecules. Onset temperature describes the initiation of thermal transitions while conclusion temperature denotes the termination of the transitions occurring in the polymer molecules. In first endothermic event, there was a shift in onset, peak and conclusion temperatures towards higher side while ΔH decreased for acid hydrolysates in comparison to NGG suggesting that molecular structure of guar gum particles was destroyed due to acid hydrolysis (Hongbo *et al*., 2013). However, in the second exothermic event an opposite trend was found where T_o, T_p and T_c shifted towards lower temperature side and enthalpy increased for GGH_{30} and GGH_{60}. The exothermic peak temperatures decreased by 11 and 14% for GGH_{30} and GGH_{60}, respectively. The acid hydrolysis caused a decrease in the decomposition temperatures of hydrolysates as long guar gum chains were degraded to shorter chains which was also evidenced by molar mass determination indicating less thermal stability than native gum. Similar results have been earlier reported for partially hydrolyzed guar gums (Min *et al*., 2013).

4. Conclusions

NGG was observed to be more viscous having high molar mass in contrast to GGH_{30} and GGH_{60}. Acid hydrolysis substantially declined the intrinsic viscosity and molar mass in a time dependent manner. Water binding capacity and swelling power decreased but solubility increased after acid hydrolysis. FT-IR results indicated that no structural variations after depolymerization and thermal stability was reduced consequent upon acid hydrolysis of guar gum. Flow behavior results revealed a pseudoplastic behavior for NGG and GGH_{30} whereas a near Newtonian behavior for GGH_{60} suggesting the applicability of GGH_{60} especially in liquid food products. Dynamic results showed an elastic behavior at higher frequencies while viscous nature at lower frequencies depicting a viscoelastic behavior. It could be proposed from this study that acidic depolymerization provides an easy approach to prepare hydrolysates of various M_w having specific applications.

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CHEMICAL COMPOSITION, PHYSICAL AND SENSORY PROPERTIES OF DEHYDROFROZEN YAM CHIPS AS INFLUENCED BY PRE-DRYING CONDITIONS

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ABSTRACT

This study investigated the effect of pre-drying conditions on the quality of dehydrofrozen yam chips. Yam tubers were processed into yam chips and pre-dried using two methods, microwave and hot-air drying. Microwave pre-drying was carried out at microwave power 252, 406 and 567 W for 12.5, 7.0 and 5.0 min, respectively, and hot air pre-drying at temperatures 50ºC, 70ºC and 80ºC for 60, 40 and 20 min, respectively. Pre-dried yam chips were frozen for 48 h, thawed and analysed for chemical composition and physical properties. Dehydrofrozen yam chips were deep-fried and subjected to sensory evaluation. Moisture, sugar and amylose contents of chips were 63.59-60.21%, 5.43-7.89% and 23.5-38.03%, respectively. Drip loss of dehydrofrozen yam chips decreased with increase in pre-drying time. Measures of lightness, yellowness/blueness, hue angle, chromaticity and colour intensity were higher in chips pre-dried at high microwave power and air temperature. This study showed that pre-drying conditions affected the quality of dehydrofrozen yam chips.

1. Introduction

White yam (*Dioscorea rotundata*) is a staple food in many African countries where close to 90% of total global output is produced (Etim et al., 2013). In the last few decades, there has been a steady growth in global yam production output from 39.55 million metric tons in 2000 to 58.75 million metric tons in 2012 (Verter and Becvarova, 2015). Yam is richer than many tropical root/tuber crops in carbohydrate, dietary fibre and minerals (Kouassi et al., 2010). According to Alozie et al. (2009), yam makes substantial contribution to protein in the diet than the widely grown cassava.

The moisture content of yam is in tune of 80% or more and this accounts for its high rate of deterioration and spoilage (Torres et al., 2012). It is therefore imperative to convert yam into stable forms such as chips, flour and starch (Karim et al., 2013). Furthermore, yam is relished by its consumers after it is subjected to operations such as boiling, steaming, pounding, frying and roasting (Abioye, 2012; Otegbayo et al., 2012).

In recent times, there have been concerted efforts toward improving the acceptability of yam products at the world market through value addition and development of new products (Kouadio et al., 2013). One of the least explored areas in this context is in the production of dehydrofrozen yam chips.

Dehydrofreezing is a food freezing technique that involves pre-treatment of vegetables by dehydration to an appropriate moisture level prior to freezing (Ramallo and Mascheroni, 2010). Consequently, there is reduction in ice crystal formation in the cell during freezing, therefore, post thawing quality of products such as colour, texture, flavour and nutrients are preserved (Ando et al., 2011). Due to their lower moisture content, less energy is

required to freeze partially dehydrated foods compared to energy required for fresh products (Marani *et al.,* 2007).

Successes have been recorded in the use of dehydrofreezing techniques for the production of French fries from potato (*Solanum tuberusum*) (Sanz et al., 2007; Garmakhany et al., 2010) and many fruit and vegetable products (Blanda et al., 2009; Romalo and Mascheroni, 2010; James et al., 2014). The potential of yam in the production of dehydrofrozen yam chips has also been reported (Quansah et al., 2010). However, there is dearth of information on effects of pre-drying conditions on the quality of dehydrofrozen yam chips, hence, the focus of this study. The objective of this study was to determine effects of microwave and hot air pre-drying conditions on the chemical composition, physical and sensory properties of dehydrofrozen yam chips.

2. Materials and methods

2.1. Materials

White yam tubers *(Dioscorea rotundata* Poir*), Ogoja* cultivar, were obtained from the Teaching and Research Farm of Federal University Wukari, Wukari, Nigeria. Average moisture, total sugar, starch and fat contents of the tubers were 83.37%, 4.17%, 70.98% and 0.30%, respectively. Vegetable oil (Golden Penny, Lagos, Nigeria) was procured at PJD Stores, Ilorin, Nigeria. All chemicals used for analyses were of analytical grade.

2.2. Methods

2.2.1. Yam chips preparation

The method described by Quansah et al. (2010) was employed for the production of yam chips. Yam tubers were washed, peeled and cut into rectangular strips of 1 × 1 × 6 cm. The chips were blanched at 80ºC for 2 min, cooled to room temperature (28±2ºC) and packaged in polyethylene bags (Ziploc, Yantai Bagmont, Shandong, China).

2.2.2. Production of dehydrofrozen yam chips

Two pre-drying methods, microwave oven and hot-air oven, were used. Microwave pre-drying was done based on the method described by Priyadarshini et al. (2013) with modification in microwave power and pre-drying time. Yam chips were pre-dried at different microwave power and time as optimised in a preliminary study (data not presented). Microwave pre-drying was carried out at microwave power 252, 406 and 567 W for 12.5, 7.0 and 5.0 min, respectively. This was done using a laboratory microwave oven (NX-802, Nexus, Beijing, China). Hot air pre-drying was done based on the methodology outlined by Agnieszka et al. (2007) with little modification in temperature and time. Yam chips were pre-dried at different temperature and time as optimised in a preliminary study (data not presented). Hot air pre-drying was carried out at temperature 50, 70, 80ºC and time 60, 40 and 20 min, respectively, in a hot-air oven (NL-9023A, Genlab Ltd., Widnes, Cheshire, England). Pre-dried chips were cooled in desiccators.
Freezing of pre-dried yam chips was carried out as described by Agnieszka et al. (2007). Pre-dried yam chips were arranged on a tray and froze at -20ºC for 48 h. The frozen chips were thawed at room temperature (28 ± 2ºC) for 1 h.

2.2.3. Frying of dehydrofrozen yam chips

Frying of dehydrofrozen yam chips was carried out as described by Agnieszka et al. (2007). Chips were fried in a deep-fat frier (S-616, Saisho, Osaka, Japan) at 170ºC for 5 min. Oil was changed after each frying operation. The fried chips were gently blotted with the aid of a fluffy kitchen towel (Fidson, Lagos, Nigeria) to remove surface oil. The chips were cooled to room temperature (28 ± 2˚C) and packaged (Ziploc, China).

2.3. Analyses

2.3.1. Chemical and physical analyses

Moisture, total sugar, starch, amylose, amylopectin and fat contents of dehydrofrozen yam chips were determined using standard methods (AOAC, 2000). Drip loss of dehydrofrozen yam chips and browning index of yam fries were determined using methodologies described by Garmakhany et al. (2010).

A colorimeter (CR-410, Minolta, Osaka, Japan) was used to determine colour order properties of dehydrofrozen yam chips. The

equipment was standardized based on the manufacturer's instructions. Subsequently, colour properties were measured and expressed as L* (measure of lightness), a* (measure of redness and greenness), b* (measure of blueness and yellowness), hue angle, chromaticity and colour intensity.

2.3.2. Sensory analysis

Sensory attributes of yam fries were evaluated according to Agnieszka et al. (2007). Fifty panellists who comprised staff and students of the Department of Food Science and Technology, Federal University Wukari were employed. Selection of panellists was based on their familiarity with fries. They were requested to rate the samples based on crispiness, texture, oiliness, taste, colour and general acceptability. Scoring was based on a 9-point hedonic scale where 1 and 9 represented dislike extremely and like extremely, respectively. The analysis was conducted in a well-lighted room with separate booths for each of the panellists.

2.3.3. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using the statistical packages for social science (version 23, Stat-Ease Incorporated, Minneapolis, USA). Difference between means were determined by Duncan Multiple Range Test and significance level was defined at $p < 0.05$.

3. Results and discussion

3.1. Effect of Pre-drying Conditions on Chemical Composition of Dehydrofrozen Yam Chips

Chemical composition of dehydrofrozen yam chips at different pre-drying conditions is presented in Table 1. Moisture content was 60.21-63.59%. This shows that all the pre-drying conditions considered in this study were able to reduce the moisture content of chips to an acceptable level. Krokida et al. (2001) had suggested moisture content of chips to be between 60 and 65% prior to freezing. Dry matter content differs significantly ($p<0.05$) between samples with chips pre-dried at 567 W for 5.0 min and 252 W for 12.5 min having the highest (39.79%) and lowest (36.40%), respectively. Dry matter varied significantly ($p<0.05$) among the chips, depending on their moisture content. Total sugar content of chips ranged from 5.43 to 7.89%, with chips pre-dried at 70°C for 40 min and 567 W for 12.5 min having the lowest and highest, respectively. This could be due to increased hydrolysis of starch to sugar at high temperature. Tocci and Mascheroni (2008) also reported differences in sweetness of fries pre-dried at different conditions and time. Lower starch content was recorded for samples pre-dried at high microwave power and temperature compared to those pre-dried at lower microwave power or air temperature. This could be connected to loss of starch because of intense heat treatment. High amylose and low amylopectin contents were recorded for yam chips pre-dried at elevated air temperature and microwave power. This suggests better starch digestibility of dehydrofrozen yam chips pre-dried at low microwave power and air temperature. According to Sanz et al. (2007), starch digestibility increases with decreasing amylose content. Fat content of dehydrofrozen chips was 0.14-0.25% and varied significantly ($p<0.05$) among the samples. Fat content was lower in chips pre-dried at low heating conditions. This agreed with the report by Oyeyiola et al. (2014) who reported decrease in oil content of chips with increasing pre-drying treatment.

Fat content of dehydrofrozen yam fries is presented in Figure 1. There were variations in fat content of the fries. This result is similar to the findings of Pedreschi and Moyano (2005) who reported variation in oil content of French fries subjected to different pre-drying conditions. Variation in fat content of fries could be due to differences in level of disruption of internal organs caused by differing pre-drying conditions (Pedreschi and Moyano, 2005). Fat content of fries pre-dried at 567 W for 5.0 min, 70°C for 40 min and 80°C for 20 min were 11.8, 11.6 and 11.2%, respectively. This suggests good quality of the fries pre-died at these stated conditions. Agnieszka (2014) had reported that French

fries with oil content of 10-12% were of good quality and high consumer acceptability. Higher oil content could result in an oily taste while lower oil content could result in dryness (Agnieszka, 2014).

Figure 2 shows browning index of dehydrofrozen yam fries. Results obtained showed variation in browning index of the fries. Highest browning index was obtained for fries pre-dried at 80°C for 20 min, followed by 70°C for 40 min and 406 W for 7.0 min. This could be due to increased browning reaction at these conditions (Rincon and Ker, 2010). Lowest browning index (11) was recorded for fries pre-dried at 567 W for 5.0 min and this could imply a reduction in browning reaction at this condition.

Table 1. Effect of pre-drying conditions on the chemical composition of dehydrofrozen yam chips

Pre-drying methods	Pre-drying condition	Moisture content (%)	Dry matter (%)	Total sugar (mg/g)	Starch (g/100 g)	Amylose (g/100 g)	Amylopectin (g/100 g)	Fat content (%)
Microwave radiation	252 W for 12.5 min	63.59^{a}±0.9	36.40^{d}±0.10	7.68^{b}±0.28	68.88^{a}±0.42	23.59^{d}±0.42	45.29^{a}±0.83	0.20^{b}±0.09
	406 W for 7 min	61.41^{c}±0.10	38.59^{b}±0.01	5.68^{e}± 0.14	56.81^{b}±0.45	30.65^{b}±2.08	26.29^{b}±1.63	0.18^{c}±0.01
	567 W for 5 min	60.21^{e}±0.10	39.79^{a}±0.01	7.89^{a}±0.14	55.12^{c}±0.02	38.03^{a}±0.04	17.09^{c}±0.06	0.16^{d}±0.01
Hot air oven	50°C for 60 min	62.02^{b}±0.20	37.98^{c}±0.08	6.88^{d}±0.14	50.50^{d}±0.45	23.00^{d}±0.41	27.50^{b}±0.04	0.25^{a}±0.01
	70°C for 40 min	61.39^{c}±0.10	38.61^{b}±0.01	5.43^{f}±0.14	43.26^{e}±1.65	29.15^{c}±1.12	14.11^{d}±0.52	0.17^{d}±0.01
	80°C for 20 min	60.52^{d}±0.02	39.48^{a}±0.08	7.15^{c}±0.06	34.24^{f}±0.89	30.38^{b}±0.54	3.86^{d}±0.36	0.14^{e}±0.01

Values are means ± standard deviations of triplicate scores. Means with different superscripts in column were significantly ($p<0.05$) different.

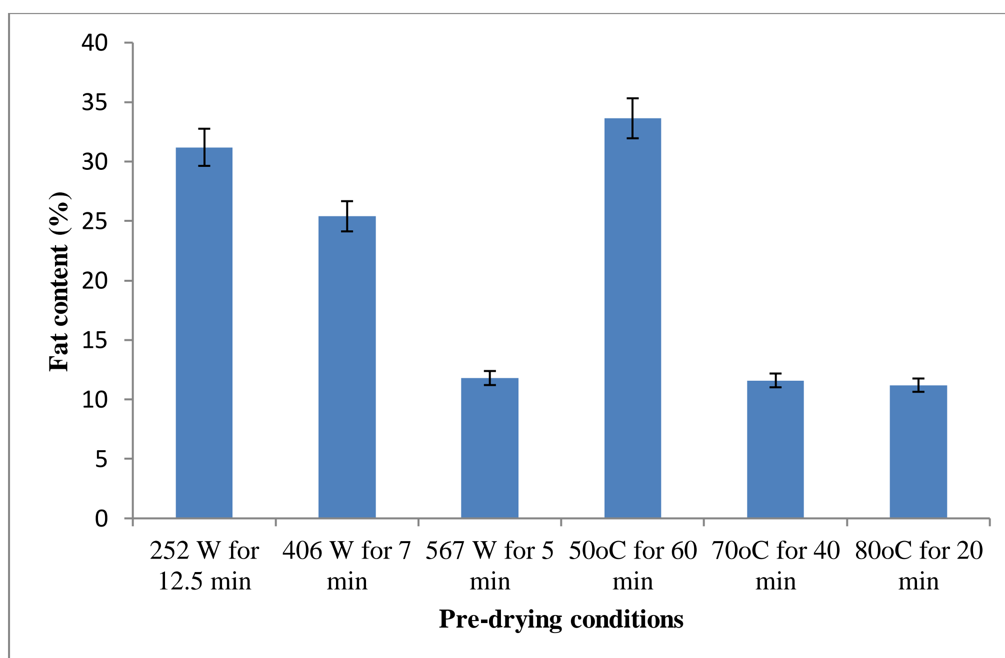

Figure 1. Effect of pre-drying conditions on fat content of dehydrofrozen yam fries

3.2. Effect of pre-drying method on the physical properties of dehydrofrozen yam chips

Percentage drip loss of dehydrofrozen yam chips is presented in Figure 3. There was variation in drip loss of dehydrofrozen yam chips due to different pre-drying conditions. This variation could be due to differences in mechanical behaviour of the chips as a result of differing pre-drying conditions. Marani et al. (2007) had demonstrated variation in mechanical behaviour of fruit and vegetables as a result of differences in pre-drying conditions. From the results obtained, yam fries pre-dried at 567 W for 5.0 min showed highest resistance to mechanical rupture, while chips pre-dried at 252 W for 12.5 min showed least resistance. Low drip loss (18%) recorded for the chips pre-dried at 567 W for 5.0 min could imply reduced cellular damage by microwave radiation at short treatment time. Dehydrofrozen yam chips pre-dried using hot air oven had high drip loss, and hence, low resistance to mechanical rupture. Ramallo and Mascheroni (2010) had reported that microwave pre-drying was more efficient than hot air pre-drying for the preservation of cellular integrity of dehydrofrozen products.

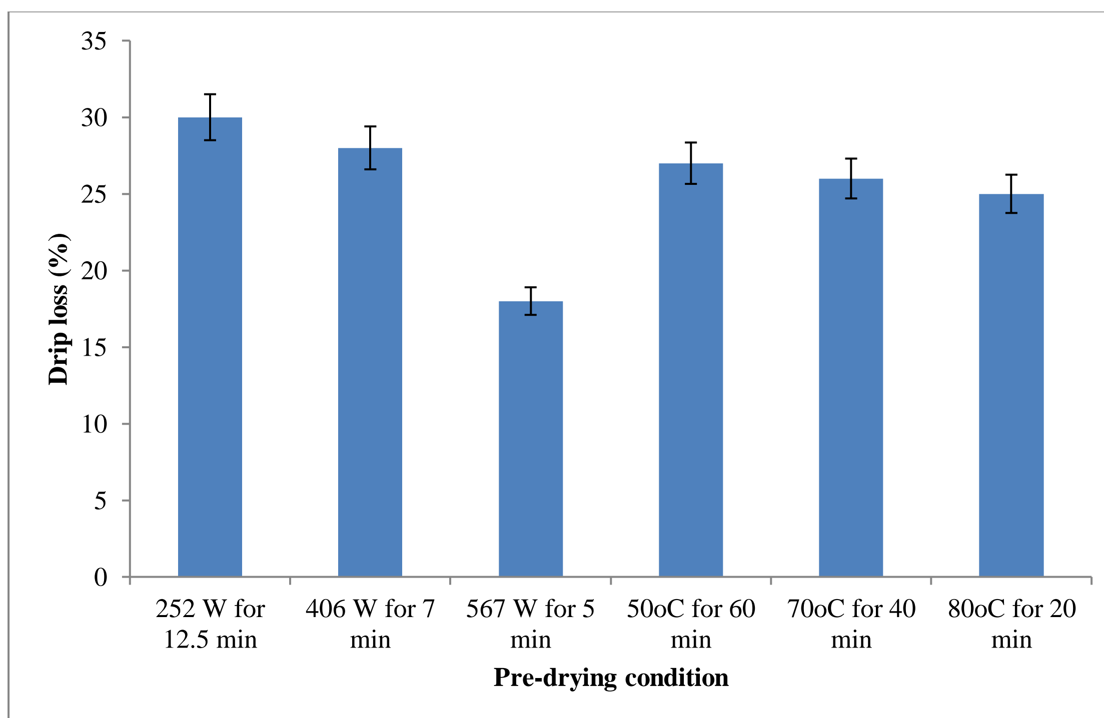

Figure 3. Effect of pre-drying conditions on drip loss of dehydrofrozen yam chips

Colour order properties of dehydrofrozen yam chips are shown in Table 2. Measure of lightness, a* and b* were 60.30-69.35, 1.89-2.52 and 6.36-9.34, respectively. Significantly ($p < 0.05$) higher L* was obtained for chips pre-dried at 567 W for 5.0 min and 50°C for 60 min. This result was consistent with low browning index reported for the chips at these conditions (Section 3.1). Significantly ($p < 0.05$) lower L* was recorded for chips pre-dried at 406 W for 7.0 min, 70°C for 40 min and 80°C for 20 min, and this could probably be due to higher rate of browning at these conditions. Significantly ($p<0.05$) higher a* was recorded for chips pre-dried at 567 W for 5.0 min (2.46) and 406 W for 7.0 min (2.52). This result corroborates the findings of Garmakhany et al. (2010) who reported high a* for pre-dried potatoes strips at high pre-drying temperature. Significantly higher b* was obtained for chips pre-dried at 567 W for 5.0 min (9.34) and 80°C for 20 min. This implies development of golden yellow colouration, which is highly preferred by consumers of fries (Sanz et al., 2007). Hue angle, chromaticity and colour intensity of the chips were 74.92-78.51,

3.89-8.04 and 30.32-47.79, respectively. Chips pre-dried at 567 W for 5.0 min and 406 W for 7.0 min had the highest and lowest hue angle, respectively. Hue angle above 70° indicates clear transition from red to yellow; hence, chips with high hue angle suggest high development of golden yellow colouration. According to Garmakhany et al. (2010), hue angle is an important parameter in potatoes fries and implies development of golden yellow colour that is highly relished by fried chips consumers. Highest chromaticity and colour intensity were recorded for chips pre-dried at 567 W for 5.0 min. This implies high colour purity of the sample (Correia et al., 2016). Low values obtained for chips pre-dried at 252 W for 12.5 min and 50°C for 60 min indicated low colour purity for chips pre-dried at low temperature and long time. Rincon and Ker (2010) also reported low colour intensity for frozen mango pre-treated for long time.

Table 2. Effect of pre-drying conditions on colour properties of dehydrofrozen yam chips

Pre-drying method	Pre-drying condition	L*	a*	b*	Hue angle (^{o})	Chromaticity	Colour intensity
Microwave radiation	252 W for 12.5 min	64.25^{b}±0.32	1.89^{c}± .03	8.79^{b}±0.06	77.89^{b}±0.12	5.97^{c}±0.07	30.32^{e}±0.31
	406 W for 7 min	60.57^{c}±1.31	2.52^{a}±0.16	8.37^{b}±0.62	74.92^{d}±0.06	6.78^{b}±0.62	40.00^{b}±1.18
	567 W for 5 min	69.35^{a}±0.28	2.46^{a}±0.01	9.34^{a}±0.02	78.51^{a}±0.42	8.04^{a}±0.07	49.79^{a}±0.20
Hot air oven	50°C for 60 min	68.69^{a}±0.72	1.67^{d}±0.09	6.36^{d}±0.14	75.29^{d}±0.45	3.89^{e}±0.08	32.49^{d}±0.73
	70°C for 40 min	62.93^{c}±0.28	1.91^{c}±0.08	7.56^{c}±0.16	75.83^{d}±0.27	4.93^{d}±0.18	33.42^{d}±0.28
	80°C for 20 min	60.30^{c}±0.16	2.17^{b}±0.01	9.31^{a}±0.22	76.89^{c}±0.27	6.56^{b}±0.19	34.32^{c}±0.1

Values are means ± standard deviations of triplicate scores. Means with different superscripts in column were significantly ($p<0.05$) different. L* (measure of lightness), a* (measure of redness and greenness), b* (measure of blueness and yellowness)

3.3. Effect of pre-drying on sensory properties of dehydrofrozen yam fries

The sensory properties of fries as influenced by pre-drying conditions are shown in Table 3. Chips pre-dried at high microwave power and air temperature for short duration were most preferred in terms of crispiness and texture. This might be due to the effect of case hardening during pre-drying operation. Result obtained was in agreement with the findings of Sanz et al. (2007) who reported that French fries pre-dried at high temperature were crispier than those pre-dried at lower temperature. Quansah et al. (2010) also reported that yam fries pre-dried at high temperature for short time produced fries with desired texture. Highest oiliness preference was also recorded for chips pre-dried at high microwave power and air temperature. This could be due to good oil absorption capacity of the samples (Section 3.1). According to Agnieszka (2014), oiliness, which is a perception of oil content of foods, is correlated with oil absorption capacity. Highest taste score was recorded for chips pre-treated at 567 W for 5.0 min. The preference for taste was lowest in chips pre-dried at 80°C for 20 min. A similar trend was observed in panellists' preference for chips colour and general acceptability.

Table 3. Effect of pre-drying conditions on sensory properties of dehydrofrozen yam fries

Pre-drying method	Pre-drying condition	Crispiness	Texture	Oiliness	Taste	Colour	General acceptability
Microwave radiation	252 W for 12.5 min	$7.00^{c}\pm1.00$	$6.88^{a}\pm1.05$	$6.64^{bc}\pm1.66$	$6.68^{b}\pm1.03$	$5.84^{c}\pm1.86$	$6.84^{b}\pm1.67$
	406 W for 7 min	$7.32^{b}\pm1.22$	$7.08^{a}\pm0.81$	$6.72^{bc}\pm1.43$	$6.96^{b}\pm1.02$	$6.28^{c}\pm2.05$	$7.28^{b}\pm0.94$
	567 W for 5 min	$7.44^{a}\pm1.66$	$7.12^{a}\pm1.39$	$7.32^{ab}\pm1.18$	$7.56^{a}\pm0.65$	$8.12^{a}\pm1.13$	$8.24^{a}\pm1.05$
Hot air oven	50°C for 60 min	$6.16^{e}\pm2.06$	$6.04^{b}\pm1.69$	$5.96^{c}\pm2.09$	$5.32^{d}\pm1.75$	$6.04^{c}\pm1.67$	$5.72^{c}\pm1.88$
	70°C for 40 min	$6.84^{d}\pm1.52$	$6.64^{ab}\pm1.63$	$6.84^{abc}\pm1.82$	$6.84^{b}\pm1.46$	$7.64^{ab}\pm1.04$	$7.32^{b}\pm1.14$
	80°C for 20 min	$7.44^{a}\pm1.19$	$7.04^{a}\pm1.24$	$7.72^{a}\pm1.34$	$6.16^{c}\pm0.69$	$7.12^{b}\pm0.78$	$7.24^{b}\pm0.78$

Values are means ± standard deviations of fifty scores. Means with different superscripts in column were significantly ($p<0.05$) different.

4. Conclusions

This study showed that the properties of yam chips varied based on pre-drying conditions. High heat treatment (microwave radiation and air temperature) of yam chips for short duration favoured nutrient retention, physical and sensory properties. Particularly, yam chips pre-dried at 567 W microwave power for 5.0 min showed good results and therefore, recommended.

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FUNGAL HOME-MADE ENZYMATIC COCKTAILS FOR APPLE JUICE CLARIFICATION

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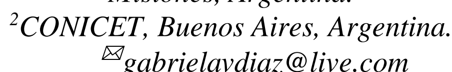

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ABSTRACT
Sugarcane bagasse and cassava bagasse are lignocellulosic industrial residues that can be used as cheap substrates for organisms' growth. These lignocellulosic residues are also suitable for inducing enzyme secretion that can be applied in different bioprocesses such as juices clarification. The goal of our work was to use *Aspergillus niger* LBM 134 to produce xylanases, characterize them and employ these enzymes in the clarification of apple juice and pulp. *A. niger* LBM 134 was isolated from a natural environment and grown on sugarcane bagasse and cassava bagasse. The highest endoxylanase and β-xylosidase activity were 144 ± 5,65 and 0,74 ± 0,05 U mL^{-1}, respectively ($p<0.05$). The optimum activity and high stability at acidic pH values make these enzymes suitable for biotechnological applications in juice industries. The enzymatic cocktails produced by the fungus grown on cassava bagasse reached the major clarification of apple juice (36,66 ± 4,01%) and pulp (60,15 ± 5,63%) ($p<0.05$). These clarification percentages were due to the hydrolysis of hemicellulosic material carried out by the xylanases from *A. niger* LBM 134.

1.Introduction

The high demand for natural fruit juice during the last years, caused by growing health and nutrition consciousness, the development of modern processing technologies for improvement of juice quality in a cost-effective manner is inevitable (Kharazmi et al. 2020). The main cause of quality loss of fresh fruit juices is the presence of pectin, starch, cellulose, and hemicellulose as colloidal dispersion which form a cloudy, viscous, and turbid suspension (Lee et al. 2006). The cloudy juices contain fewer yields, are difficult to pasteurize and concentrate, and shorten the longevity of the membrane used in industrial scales. Therefore, these polysaccharides must be removed before commercialization to produce clarified fruit juices with high-level quality (Lee et al. 2006; Kharazmi et al. 2020).

The utilization of enzymes has been significantly extended in juice processing industries. Treatment of fruit juices with enzymes promotes the degradation of polysaccharides, for maximum yield, juice clarity, safety, shelf life, and storage stability (Rosmine, et al 2017). Xylanases are broadly used in the clarification of fruit and vegetable juices (Rosmine et al. 2017). Endo-1,4-β-xylanases (EXs, EC 3.2.1.8) are one of the most important xylanases; they hydrolyze the xylan, the main hemicellulosic polysaccharide, to small molecules such as xylooligosaccharides and xyloses (Lee et al. 2006; Salupi & Meryandini). In the food industry, particularly, in juice clarification, the main desirable biochemical

properties for xylanases are optimum activity and high stability at acidic pH values (Polizeli et al., 2005). Moreover, the use of enzymatic cocktails including these enzymes and other enzymes such as pectinases, cellulases and amylases, improves the juice clarification.

On the other side, the high cost of available commercial enzymatic cocktails urges bioprospecting and manipulation of microbes for higher productivity and enhancement of existing commercial enzymes (Kumar et al. 2014; Khusro et al. 2016). Therefore, numerous investigations on varying culture conditions for optimal xylanase production include fungal strains of *Trichoderma, Penicillium,* and *Aspergillus* (Rosmine et al. 2017). In this context, the aim of this study was to biochemically characterize the EXs present in the enzymatic cocktails from the fungus *Aspergillus niger* LBM 134 grown on agroindustrial wastes and to clarify the apple pulp and juice employing these home-made enzymatic cocktails.

2. Materials and methods

2.1. Fungal culture and enzyme production

A. niger LBM 134 is deposited in the Collection of the Laboratory of Molecular Biotechnology (LBM, from Spanish *Laboratorio de Biotecnología Molecular*) of the Instituto de Biotecnología Misiones. The fungus was cultivated in potato dextrose agar (PDA, 39 gL^{-1}) and incubated at 28 ± 2 °C for 5 days. A spore suspension of 10^7 spores mL^{-1} was obtained from the fungal culture and 1 mL of this suspension was used for inoculating Erlenmeyer flasks containing previously optimized media (Díaz *et al*., 2019). Then, media were filtrated and centrifugated at 10.000 g, 4 ºC for 15 min to obtain supernatants for enzyme xylanase characterization and juice and pulp clarification.

2.2. Determination of EX activity

EX activity was determined according to Bailey et al. (1992) through the quantification of released reducing sugars using beechwood xylan (Sigma-Aldrich, USA. Reducing sugars were measured by 1,3-dinitrosalicylic acid (DNS) assay (Miller, 1959) using xylose as the standard curve. Absorbance was measured at 540 nm. EX activity was expressed as international units (U), defined as the amount of enzyme needed to produce 1 µmol of xylose per min at 50 °C.

2.3 Effect of temperature and pH on EX activity

To study the effect of the temperature on EX activity, both crude enzymatic cocktails of *A. niger* LBM 134 grown on SCB and CB were incubated at pH 4.8 at 4, 10, 20, 30, 40, 50, 55, 60, 65, 70 and 80 °C. The effect of pH on EX activity in both crude extracts was studied at different pH values (3.0, 4.0, 4.8, 5.0, 6.0, 7.0, 8.0, 9.0 and 10) at 50 °C. EX activity was determined and expressed as described previously.

2.4. Thermostability and pH stability of EX activity

The thermostability of EX activity was evaluated by incubating both crude enzymatic cocktails at different temperatures (4, 30, 40, and 50 °C) during different intervals (6, 12, 24, 48, 72, and 96 h). To determine the pH stability of EX activity, the crude enzymatic cocktails were incubated at pH 5.0, 6.0, and 7.0 at 50 °C during different periods (6, 12, 24, 48, 72, and 96 h). The buffer solutions used were: 0.05 M citrate buffer for pH 3.0; 0.05 M sodium acetate buffer for pH 4.0, 4.8 and 5.0; 0.05 M sodium phosphate buffer for pH 6.0, 7.0 and 8.0; and 0.05 M Tris-glycine buffer for pH 9.0 and 10.0. EX activity was determined as described previously. Thermostability and pH stability was expressed as residual activity in percentage, taking the initial enzymatic activity as 100%.

2.5. Zymography

Previous to the zymography, cocktail supernatants were clarified by Chromafil Xtra PET-20/25 (0.20 µm) filters (Macherey Nagel; Düren, Germany) to obtain the cell-free enzymatic cocktails. Also, supernatants were clarified with a 0.1% Tween 80 aqueous solution, in a 2:1 ratio, to precipitate polysaccharides. Polysaccharides quantification

was determined by the phenol-sulphury technique (DuBois et al. 1956). Electrophoresis was performed in gels containing 7.5% (w/v) acrylamide; 2% (w/v) of beechwood xylan (Sigma-Aldrich, USA) was added into the separating gel. About 20 µg of proteins were applied to the gel. For that, proteins were determined following the Bradford method (Bradford 1976) employing Bradford Protein Assay (Bio-Rad, Hercules, California). Electrophoresis was conducted at 100 V for 2 h. A molecular weight marker (Phage Ruler pre-stained protein ladder (Fermentas, Thermo Scientific, USA) was added to the same gel and separated under the same electrophoresis conditions. Then, the gel was divided in half. One-half of the gel, containing the samples, was fixed with solution methanol:acetic acid: water (4:1:5), immersed in 0.05 mM sodium acetate buffer pH 4.8 and incubated at 50 °C for 60 min. The gel was then stained with 0.1% (w/v) Congo red solution and washed with distilled water and 1 M NaCl. Light yellowish activity bands were visible on deep red background. The other half of the gel, containing the molecular weight marker was stained with 2 g L^{-1} $AgNO_3$ to detect the proteins profile. The native molecular mass of the endoxylanases was estimated by using the molecular weight marker.

2.6.Apple juice and pulp obtention

Apple was washed and macerated using a blender to obtain the pulp. Apple fruit was peeled; separated from the seeds and macerated using a hand blender. A minimum amount of water was added to facilitate the maceration process as well as to help extract more juice from the pulp. The maceration process was repeated four times to get a smooth-textured puree.

2.7. Apple juice and pulp clarification by the supernatants from *A. niger* LBM 134

Apple juice and pulp were clarified by supernatants from *A. niger* LBM 134 grown on SCB and CB. The ratio used was supernatant:pulp or juice, 1:1. Two incubation conditions were assayed: 45 ° for 120 min and 50 °C and 60 min. After this period the samples were boiled for 5 min for enzyme inactivation and centrifuged at 21,000 g for 15 min.

The supernatant (juice) was used for determining juice clarity by recording transmittance at 650 nm, taking distilled water as the blank. Controls were carried out using apple juice and distilled water (1:1) and pulp:destilled water (1:1). Clarification was calculated according to Rosmine et al. 2017:

$$\% \text{ Clarification} = Tt - Tc/Tc \times 100 \qquad (1)$$

whereas, Tt is the transmittance of test; Tc is the transmittance of control.

2.8.Statistical analysis

The experimental results were analyzed and graphed with the software GraphPad Prism 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

3.Results and discussions

3.1. Biochemical characterization of proteins with EX activity in supernatants from *A. niger* LBM 134

Different enzymes have been employed for fruit juice clarification with the main purpose to generate a final product that is clear and visually attractive. The use of xylanases leads to an increase in the juice extraction yield and production efficiency and quality (Bajaj and Manhas 2012; Ahmed et al. 2016). The major factors influencing the enzyme activity are temperature, incubation period, pH, and concentration (Dhiman *et al.,* 2008). The EXs presents in both homemade enzymatic cocktails from *A. niger* LBM 134 showed a mesophile behavior; the optimal EX activity occurred at middle temperature and pH values. The effect of temperature and pH on the stability of the enzyme activities was studied in the crude enzymatic extracts of *A. niger* LBM 134 (Fig. 1). The maximal EX activity ($p<0.05$) was yielded when both extracts were incubated at 50 °C, reaching 108 ± 7.58 and 176 ± 1.79 U mL^{-1} in extracts from fungus grown on SCB (Fig. 1a) and CB (Fig. 1b), respectively. Regarding the effect of pH, the highest EX activity ($p<0.05$) of *A. niger* LBM 134 was reached when both crude

enzymatic extracts were incubated at pH 4.8 and 5 without a statistical difference being 119.5 ± 5.79 U mL^{-1} of EXs in the case the extract from the fungus grown on SCB (Fig. 1c) and 177.96 ± 1.32 U mL^{-1} in the case of CB (Fig. 1d). The EX activity was optimal at 50 °C and at higher temperatures, the enzyme activity dramatically decayed. This occurred since most of the globular enzymes such as xylanases are denaturalized under temperatures higher than 60 – 70 °C (Lehninger *et al*., 1976). Also, the enzyme activity depends on the pH of the media. In this case, the optimal pH EX activity was in the range of 4 to 5. At minor and higher pHs, the EX activity was very poor probably due to conformational changes in the enzymes or variation at the ionization states of their active sites, leading to no functional isoforms (Lehninger *et al*., 2006). Our findings agreed with other studies reported for fungal EXs with optimal catalysis at 40 - 60 °C and pH 4 to 7 (Wong *et al.,* 1988; Kulkarni *et al.,* 1999; Cuyvers *et al.,* 2011; Díaz *et al*., 2015; Cayetano-Cruz *et al*., 2016; Barchuk, 2017; Dhiman & Mukherjee, 2018).

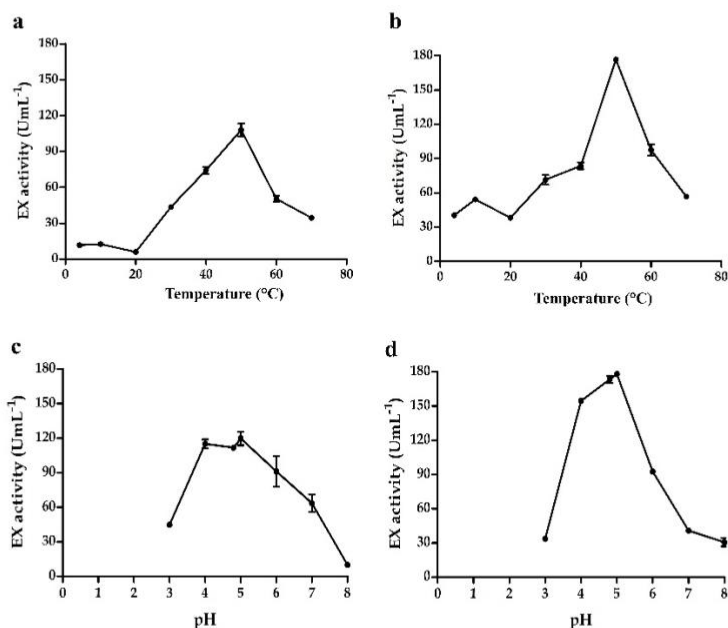

Figure 1. Effect of the temperature and pH on EX activity in supernatants from *A. niger* LBM 134 grown on SCB and CB. Optimal temperature of the EX activity in supernatants from the fungus grown on SCB (a) and CB (b). Optimal pH of the EX activity in supernatants from the fungus grown in SCB (c) and (d).

Also, pH and temperature significantly affected the enzyme stability. For that, we evaluated the effect of these physical parameters on the EX stability of the homemade enzymatic cocktails of *A. niger* LBM 134. Thermal and pH stability curves showed the effect of temperature and pH, respectively, on EX enzyme stability in the crude enzymatic extracts of *A. niger* LBM 134 grown on SCB and CB (Fig. 2). At 4 and 30 °C, the EX activity was above 50% during the studied period in both extracts. At 40 °C, the EX activity was above 50% until 12 h and 48 h in extracts from SCB (Fig. 2a) and CB (Fig. 2b), respectively; and at 50 °C, the enzyme activity decreased under 50% before the 12 h in both extracts. Respect on pH stability, the EX activity remained above 50% at pH 5, 6 and 7 until 96 h although the EX stability was higher at pH 5, remaining above 80% the enzyme activity in crude extracts of the fungus grown on SCB (Fig. 2c) and CB (Fig. 2d). Nevertheless, we evaluated the stability of the enzyme activity in the non-purified cocktails; this could lead to the action of different enzymatic inhibitors such as proteases affecting the EXs.

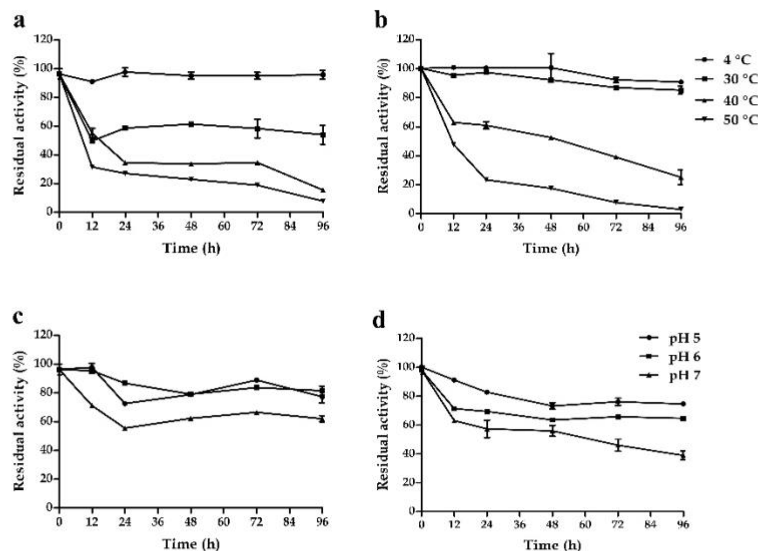

Figure 2. Thermal and pH stabilities of the EX activity in supernatants from *A. niger* LBM 134. Thermostability of EX activity in supernatants from the fungus grown on SCB (**a**) and CB (**b**). pH stability of EX activity in supernatants from the fungus grown on CB (**c**) and CB (**d**). The 100% corresponded to 108 ± 7.58 U mL^{-1} and 176 ± 1.79 U mL^{-1} in the case of the extracts from SCB and CB, respectively. EX, endoxylanase.

Figure 3. Determination of EXs profile by ND-PAGE (the gel was divided in half). **a.** a half gel stained with $AgNO_3$; molecular weight marker. **b.** the other half ND-PAGE using Congo Red staining for detecting EXs in supernatants from *A. niger* LBM 134 grown on SCB and CB and control media. Lanes correspond to culture supernatant of 1, 2 and 3 correspond to 20 µg of proteins in supernatants from the fungus grown on SCB; 4 and 5, 20 µg of proteins in supernatants from the fungus grown on the control medium of SCB; 6 and 7, 20 µg of proteins in supernatants from the fungus grown on the control medium of CB; 8, 9 and 10, 20 µg of proteins in supernatants from the fungus grown on CB.

Table 1. Apple juice and pulp clarification by enzymatic cocktails from *A. niger* LBM 134. Clarification values are expressed as percentages (%) and represent the means of the triplicates ± standard deviation.

Sample	Temperature (C°)	Time (min)	Clarification (%)	Significance
Juice + CB supernatant	45	120	22.82 ± 5.63	**b**
Pulp + CB supernatant	45	120	60.15 ± 4.74	**d**
Juice + SCB supernatant	45	120	ND	
Pulp + SCB supernatant	45	120	ND	
Control 1: juice + water	45	120	ND	
Control 2: pulp + water	45	120	ND	
Control 3: only juice	45	120	ND	
Control 4: only Pulp	45	120	ND	
Control 5: only SCB supernatant	45	120	ND	
Control 6: juice + commercial enzyme	45	120	19.67 ± 3.41	**ab**
Control 7: pulp + commercial enzyme	45	120	45.83 ± 4.88	**c**
Juice + CB supernatant	50	90	36.66 ± 4.01	**c**
Pulp + CB supernatant	50	90	11.57 ± 0.82	**a**
Juice + SCB supernatant	50	90	22.72 ± 2.80	**b**
Pulp + SCB supernatant	50	90	ND	
Control 1: juice + water	50	90	ND	
Control 2: pulp + water	50	90	ND	
Control 3: only juice	50	90	ND	
Control 4: only Pulp	50	90	ND	
Control 5: only SCB supernatant	50	90	ND	
Control 6: juice + commercial enzyme	50	90	35.95 ± 5.32	**c**
Control 7: pulp + commercial enzyme	50	90	11.83 ± 3.28	**a**

3.2. Zymogram analysis of supernatants

To verify the possible presence of EX isoforms, no denaturing-polyacrylamide gel electrophoresis (ND-PAGE) analysis were carried out. Figure 3 shows non-denaturing gel corresponding to the supernatants from *A. niger* LBM 134 grown on SCB and CB and their respective controls. Controls showed no bands on polyacrylamide gel due to the low enzymatic activity of this strain, while supernatants from the fungus grown on the bagasses showed degradation zones on the polyacrylamide gels. The EXs profile for supernatants from the fungus grown on both SCB and CB were similar showing two isoenzymes with electrophoretic mobility of approximately 25 and 35 KDa. We detected the presence of isoenzymes with less electrophoretic mobility in the cocktail from the fungus grown on SCB which can be isoforms with glycosylation events. These isoenzymes belong to EX B (family 11) and EX C (family 10), respectively (Díaz et al. 2020). These phenomena are recurring in EXs and can notably modify the molecular mass of the enzymes (Mura Escorche, 2016).

3.3. Clarification of apple juice and pulp by home-made cocktails rich in EX activity from A. niger LBM 134

The use of the homemade cocktail from *A. niger* LBM 134 grown on CB demonstrated the most clarification power on apple pulp 60.15 ± 4.74% at 45 °C and 120 min (P<0.00) (Table 1). High juice clarification, 36.66 ± 4.01% reached using the same home-made cocktail at 50 °C during 90 min (P<0.00); commercial enzyme on pulp clarification 45.83 ± 4.88% at 45 °C, 120 min and juice (P<0.00) and commercial enzyme on juice clarification 35.95 ± 5.32% at 50 °C, 90 min and juice (P<0.00). Similar observations were made by Kumar et al. (2014) and Rosmine et al. (2017) studying the clarification in other fruit juices. The clarification power is mainly due to disruption of hemicellulosic material and was higher under the action of the homemade cocktails from *A. niger* LBM 134 than the action of commercial enzymes evaluated in this study. In this context, the crude cocktails have the advantage of presenting more enzymes than purified endoxylanases leading to the degradation of other different polysaccharides instead of purified cocktails being used. This fact affects the total process of the clarification of the apple juice and pulp resulting in a visually more attractive product with better quality.

4. Conclusions

The present study features a promising approach for the production of two homemade enzymatic cocktails rich in hydrolytic enzymes, particularly in EXs, from *A. niger* LBM 134 grown on two agroindustrial wastes, SCB and CB, and the application of both cocktails on a biotechnological process, the clarification of apple pulp and juice. The fact of using agroindustrial wastes for obtaining the cocktails reduces the cost of the enzymes and for the biotechnological application.

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ADVERSARIAL AUTOENCODERS FOR AGRICULTURE YIELD FORECASTING

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ABSTRACT

For sustainable food production. In agriculture, crop yields are increasingly affected by warmer temperatures, and pest infestations caused by climate change have increased agricultural losses. Increasing local production is important to reduce our dependence on imported food and provide a buffer in case of supply disruptions such as those caused by the COVID-19 pandemic. To increase food security, it is important to optimize agricultural yields, despite the high costs associated with factors such as supplemental feeding, pest control measures, or operating costs.

We present a Machine Vision method (MV) with Adversarial Autoencoder (AAE) as an approach to crop yield optimization. Predicted leaf area is projected from initial germination to early vegetative stages. Generative machine learning models are analyzed to determine a suitable architecture for crop yield prediction. Images of romaine lettuce grown over time under different conditions (e.g., light intensity) are used as the data set. Preliminary results show that the model created is able to predict an image with sufficient accuracy based on a single condition. With our method, corrective actions can be taken early, and yields recover from initial below-average values. Further work can be done to extend the model to other conditions such as moisture, strength of available sunlight, or soil nutrient content.

1.Introduction

Climate change has adversely affected crop yields and land suitable for agriculture (Zhang & Cai, 2011). The direct effects of global warming alone are projected to lead to a 2-13% decline in yields of major crops (Wang et al., 2020) Jägermeyr et al. (2021), and yield losses could be more severe than previously thought, putting the old agricultural model under production pressure to meet the demands of the future.

This concern is driven by unsustainable agricultural practices, lower crop yields due to climate change (Schmidhuber & Tubiello, 2007), and increasing scarcity of water and arable land (Downing, 2013; Porter et al., 2017; Rosegrant & Cline, 2003). By 2050, the world's population is projected to grow to 9 billion people, leading most countries to worry about numerous food security challenges, including quality, environmental and climate impacts, and reliable access to food sources sufficient to meet growing global demand (Diouf, 2009; Porter et al., 2017; Wise, 2013). Although demand is steadily increasing, 70% more food needs to be produced by 2050, however at the same time, agriculture's share of global GDP has shrunk to just 3 percent, a third of what it was just a several decades ago.

Food security is an important global issue and will remain so for the foreseeable future, as it is more relevant than ever given the immense global impact of the COVID-19 pandemic (Zurayk, 2020). The zoonotic virus was not only introduced by a food supplier, but now reveals

significant deficiencies in the current food supply system. As a result of border and market closures, the impact of quarantines, and the disruption of trade routes, the pandemic has created an unprecedented threat to food logistics and socioeconomic systems around the world, as well as to the livelihoods of billions of people (Galanakis, 2020; Laborde et al., 2020; Zurayk, 2020). Considering the impact of climate change on pests (Skendžić et al., 2021), maintaining current crop yields could be a colossal task in the future.

Predicting crop yield is a multifaceted problem because yield is determined by a variety of factors that are not limited to factors such as genotypic variation within crops, nutrient levels in their growing media, and weather. Despite the variability in crop yield predictions, it is important that such predictions be made because all crops require time to grow and, consequently, financial and spatial resources must be allocated for their growth. Optimizing yields relative to costs is a major concern for growers.

Early crop growth models such as the Decision Support System for Agrotechnology Transfer (DSSAT) (Jones et al., 2003) and CropSyst (Stockle et al., 1994) are still used for simulating crop growth, especially for simulating the effects of climate change and/or the interactions between genetics (G), environment (E), and management (M). However, these crop growth models require extensive calibration to produce accurate results. In addition, the significant cost of runtime and maintenance limits their use to farmers who have the appropriate knowledge, equipment, and financial resources. As a result, these systems cannot be used by low-income farmers who lack the necessary resources, and the scalability of the models is also limited.

Deep Learning (DL) methods such as Convoluted Neural Networks (CNN) have been proposed as an improved method for crop prediction (Khaki et al., 2020; Sakurai et al., 2019; Sun et al., 2019) and disease and pest detection (de Ocampo & Dadios, 2018; Ferentinos, 2018; Fuentes et al., 2017; Sladojevic et al., 2016; Wallelign et al., 2018). By learning and characterizing the performance of the plants, continuous monitoring will provide new insights to optimize the growth rate of the plants (Al-Shakarji et al., 2017). The proposed solution allows finding correlations between leaf area and biomass, helping to predict plant metrics, including growth rate and leaf area. This knowledge is of particular importance for controlling plant growth parameters in response to context and feedback (Shadrin et al., 2019; Shadrin et al., 2018). In addition, DL has been shown to be able to abstract nonlinear relationships that may not be detected by traditional statistical methods.

Recent developments in DL and its application to crop yield prediction aim to improve the accuracy of current models. Numerous research papers have applied DL to crop yield prediction using remote sensing techniques for data collection and various vegetation indices for yield quantification. Chlingaryan et al. (2018) extensively analyzed the use of Machine Learning (ML) for crop yield prediction and nitrogen estimation. They identified trends in the use of vegetation indices from satellite imagery with backpropagated neural networks for more accurate crop yield prediction and predicted that future applications of ML will be more optimized and focused on specific precision agriculture applications. Kulkarni et al. (2018), attempted time series prediction using recurrent neural networks to predict yields given soil and rainfall conditions.

A neuroevolutionary algorithm based on ML was developed to predict crop yields by providing access to information about trait importance (Kanimozhi & Akila, 2020). Similarly, a genetic algorithm-based approach for crop yield prediction has been identified and shown to outperform traditional neural networks and classical statistical methods in crop yield prediction (Bi & Hu, 2021). A comprehensive literature review of crop yield prediction using DL and remote sensing was also conducted in 2022 (Muruganantham et al., 2022) noting commonalities such as the fact that the most common remote sensing technology is the Moderate-Resolution Imaging

Spectroradiometer (MODIS), common indices used to quantify performance, and ML models used in previous research.

However, this newer research focuses on predicting crop yields at the macro level using overhead imagery and data to analyze yields across different land areas. In land-poor locations, conventional farming methods are costly and inefficient due to limited land, and many local farms have switched to high-yield urban/indoor agriculture, where a greater number of crops can be grown in the same space, using supplemental light to enhance plant growth (Jones, 2018).

In urban agriculture, crop yields can still be affected by variability, such as light conditions, but conventional yield forecasts based on satellite imagery cannot be used to accurately predict growth. Identifying these regions of suboptimal yield and correcting growth rates can improve overall crop yield and optimize the return on current operations. Current methods also do not account for dynamic changes in conditions; they cannot predict a change in conditions during growth. Yields are obtained by harvesting, which would result in an interruption of the plant's growth cycle.

Research Objectives

Hence, our work seeks to:

Utilize generative ML/DL models to visualize plant growth using images captured with simple, low-cost cameras.

Identify if the generative model can forecast a visual change in yield with different growing conditions.

This paper will first introduce past work pertaining to the use of ML in crop yield prediction, before elaborating on the generation of data and introducing the architecture of the generative model used in the work. The results will then be evaluated, and the project summarized thereafter.

2. Materials and methods

2.1. Data Generation

As mentioned in the introduction, most publicly available crop yield prediction datasets were selected for analysis. For this project, these datasets were unsuitable for analysis, so a suitable image dataset had to be created. We chose to create our own imagery using a simple, commonly available camera module so that we did not need expensive hyperspectral or high-resolution camera systems that may not be available to the average farmer. However, network issues limited the establishment of affordable microcontroller-based camera modules. The images were hence instead captured using a common smartphone.

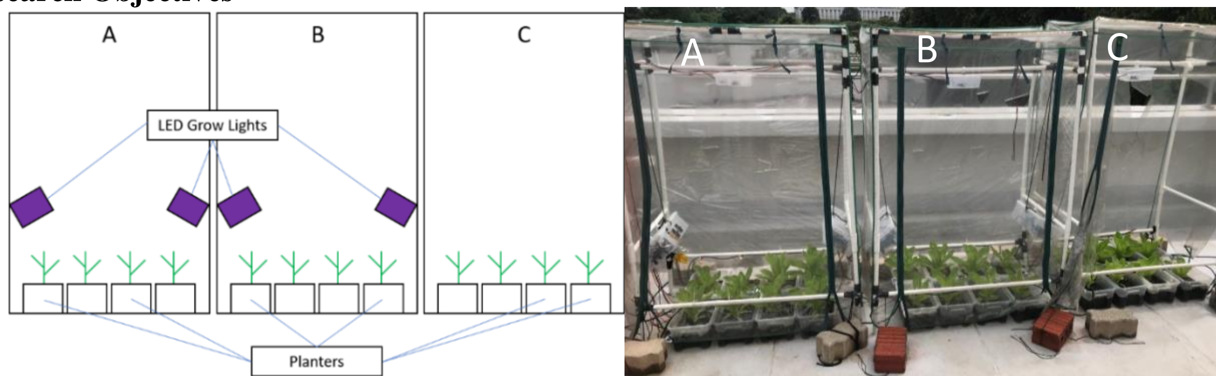

Figure 1. Schematic of experimental setup, each plant was grown in a single rectangular pot for ease of organization, 8 pots were placed in each camera perspective.

Romaine lettuce was selected as the plant for analysis because it has a short harvest period (approximately 1 month) and a large leaf area suitable for this analysis. Three experimental setups A, B, and C, as shown in Fig. 1, were created and placed outdoors under a tarpaulin to create a semi-controlled environment. Experimental setups A and B had LED grow lights attached to them, each of which was turned on 2 hours before sunrise and after sunset, providing a total of 4 hours of daylight extension. For setup C, the front of the tarpaulin was opened to create a semi-protected environment, as a fully protected tarpaulin could be too hot (in tropical regions) and humid for optimal plant growth.

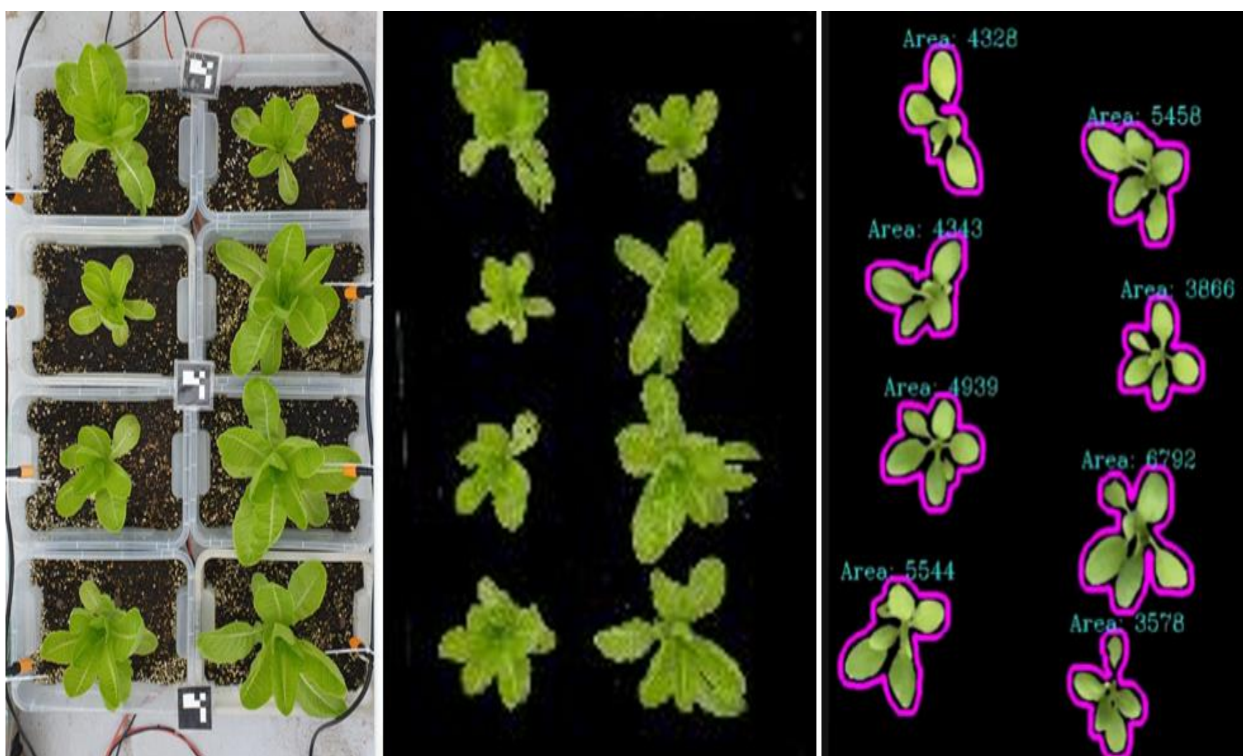

Figure 2. Comparison of raw and processed images

The images captured were then processed using the OpenCV python library (Bradski & Kaehler, 2008) to generate an image mask and resize the image. Furthermore, ArUco markers (Garrido-Jurado et al., 2014) were attempted to be utilized in this step. Due to variations in image capture, the angle, perspective, and size of the captured superstructure may change from image to image. The ArUco markers serve as image anchors, with known dimensions and orientations assigned to each marker. Accordingly, the images can be scaled and repositioned according to the known dimensions and orientations, standardizing the images and improving the accuracy of the dataset. In this step, only the information of the image relevant to the analysis is retained, and the image is converted to an appropriate size so that it can be run through the model in the next step with reasonable computational effort. Figure 2 illustrates the results of the image processing. Three batches of romaine lettuce were created over a harvest cycle of 30-40 days. For testing purposes, images of the lettuce after 34 days were used as a test data set to quantify the performance of the model.

2.2. Architecture

Generative Adversarial Networks (GANs) (Goodfellow et al., 2014) were originally considered, but the limited amount of available data and inability to account for conditions limited the application of the model in this project. Instead, the Adversarial Autoencoder

(AAE) (Makhzani et al., 2015) was identified as an appropriate architecture for the goals of this project. The AAE consists of the encoder, decoder, and discriminator and finds application in the supervised generation of data to be used for this analysis.

The combination of encoder and decoder aims to reconstruct the image and thus obtain a compressed representation of the image in terms of the hidden layer (Z), while the combination of encoder and discriminator aims to map Z to the known distribution.

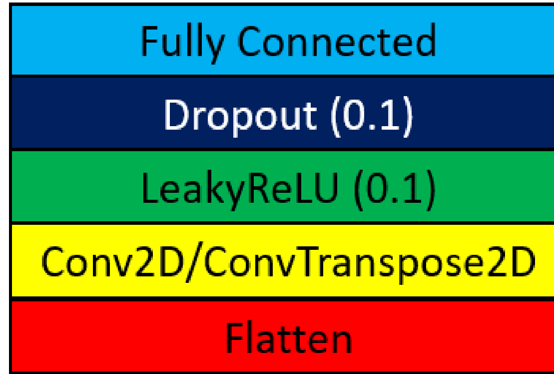

Figure 3. Legend for an Adversarial Autoencoder (AAE)

The PyTorch framework was used to code the model. For the Figures described in the following Sections 2.2.1 to 2.2.3, the layers will be identified as per the legend in Fig. 3, with the values in brackets stating the probability values as required for their respective layers.

2.2.1. Encoder

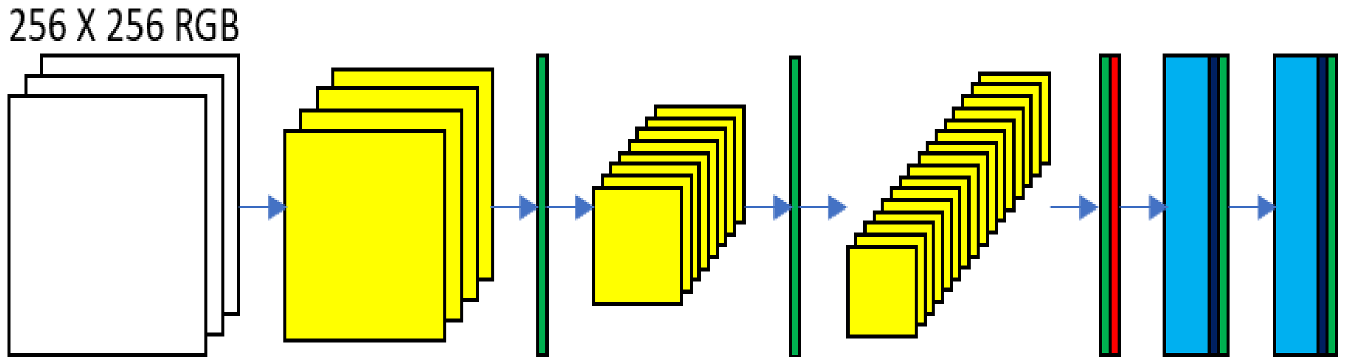

Figure 4. Encoder Architecture

Fig. 4 describes how the layers in the encoder are connected. The original encoder had only Fully Linked (FC) layers with the Rectified Linear Unit (ReLU) activation function. However, for the application in this project, convolutional layers (Lecun et al., 1998) were also used because convolutional layers act as filters to highlight areas of interest in an image. Three Conv2D layers were therefore added to the original encoder architecture before they underwent LeakyReLU activation with a probability of 0.1 and were flattened into a 1-dimensional (1D) array.

The LeakyReLU function was used because the resulting reconstruction loss was less than using ReLU and the fidelity of the generated image was slightly increased. This 1D array was then passed through 2 FC layers, with the output of the second FC layer being Z. Dropout layers with a probability of 0.1 were also added to reduce overfitting of the data set.

2.2.2. Decoder

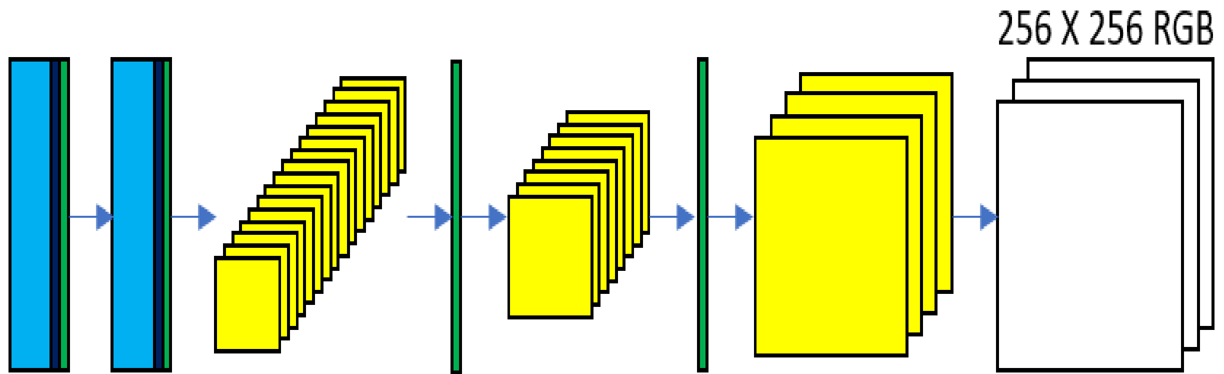

Figure 5. Decoder Architecture

Similar to 2.2.1, the original Decoder only consisted of FC layers and activation functions. The model was modified to include ConvTranspose2D layers, with inspiration from Radford et al. (2016), dropout layers, and the use of the LeakyReLU activation function for the same reasons as described in 2.2.1. The decoder takes Z and an array (Y) containing constraints such as time. Figure 5 describes how the layers are connected in the decoder. The reconstruction loss between the encoder/decoder is then calculated and tracked, and the weights of the encoder and decoder are updated.

2.2.3. Discriminator

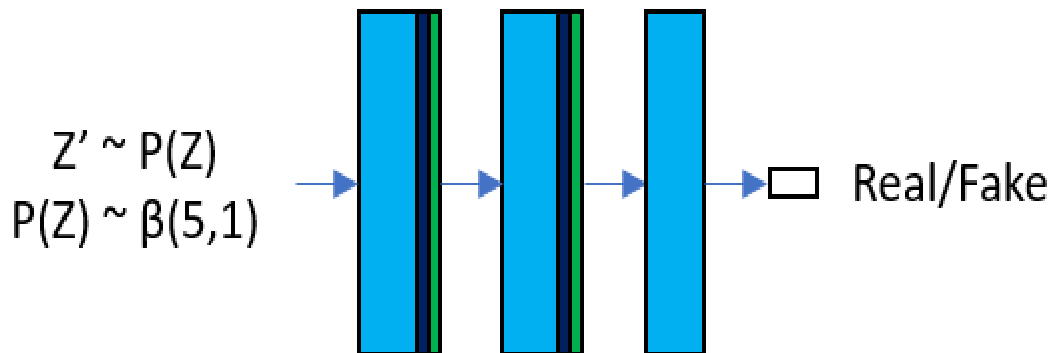

Figure 6. Discriminator Architecture

The discriminator uses 2 FC layers with the LeakyReLU activation function along with dropout layers to determine if a Z-layer sample is from the known distribution. The second FC layer is passed through another FC layer to produce a binary output indicating whether the sample is from the known distribution. The min-max GAN loss introduced by Goodfellow et al. (2014) is then calculated and then backpropagated to update the encoder and discriminator weights. For this project, a beta distribution with parameters (5,1) was used as it was found to give visually similar results to the actual image. Fig. 6 describes part of the architecture of the discriminator.

2.2.4. Combined

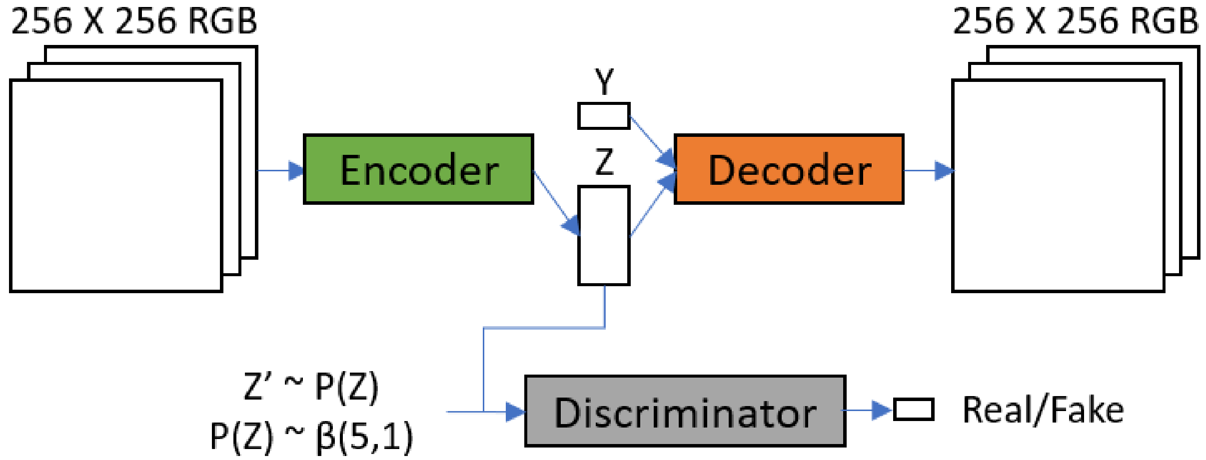

Figure 7. Combined Architecture

The encoder, decoder and discriminator are combined as shown in Fig. 7. The original image is passed through the encoder to obtain Z, an array of size 512, and Y, the array of conditions. The conditions in Y include: the time, a coded array for the degree of protection in the experimental setups, and whether additional light was present in the experimental setups. Y and Z were then input to the decoder, and an image was regenerated as described in 2.2.2.
A sample is taken from Z and Z', where Z' follows a known distribution. The two samples are passed through the discriminator in 2.2.3 and the loss is calculated. The model was run for 100 epochs using the Adam optimizer with a learning rate of $6e^{-4}$ for the generator (encoder/decoder) and a learning rate of $8e^{-4}$ for the regulator (encoder/discriminator).

2.3. Evaluation

The Mean Structural Similarity Index Measure (MSSIM) (Zhou et al., 2004) was used to quantify the fidelity of the generated image.

$$\mu_x = \frac{1}{N}\sum_{i=1}^{N} x_i \quad (1)$$

$$\sigma_x = \left(\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \mu_x)^2\right)^{0.5} \quad (2)$$

$$l(\boldsymbol{x},\boldsymbol{y}) = \frac{2\mu_x\mu_y + C_1}{\mu_x^2 + \mu_y^2 + C_1} \quad (3)$$

$$c(\boldsymbol{x},\boldsymbol{y}) = \frac{2\sigma_x\sigma_y + C_2}{\sigma_x^2 + \sigma_y^2 + C_2} \quad (4)$$

$$s(\boldsymbol{x},\boldsymbol{y}) = \frac{\sigma_{xy} + C_3}{\sigma_x\sigma_y + C_3} \quad (5)$$

$$SSIM(\boldsymbol{x},\boldsymbol{y}) = [l(\boldsymbol{x},\boldsymbol{y})]^{\alpha} * [c(\boldsymbol{x},\boldsymbol{y})]^{\beta} * [s(\boldsymbol{x},\boldsymbol{y})]^{\gamma} (6)$$

The indices x and y refer to the two images to be compared. μ refers to the arithmetic mean and σ to the standard deviation of the analyzed pixels. The luminance, contrast and structure scores are denoted to as *l*, *c* and *s* respectively.

This metric involves the extraction of three key features that contribute to the structure of the image – luminance (1), contrast (2), and structure, as described in Fig. 8. Luminance compares the average intensity of the pixel values, contrast compares the standard deviation of the signal, while structure compares the normalized signal so that the signal has a uniform standard deviation.

A series of comparison functions (3-5) are then created for luminance, contrast and structure to compare the actual and expected signals. The constants C_1, C_2, and C_3 provide numerical stability as the denominator approaches 0. The SSIM value (6) is then calculated based on the three comparison functions, with *α*, *β* and *γ* indicating the relative importance of the luminance, contrast and structure values. A value of 0 indicates complete dissimilarity (the images are completely different) and a value of 1 indicates complete similarity (the images are exactly the same).

A Gaussian weighting function of size 11x11 is introduced to compute the SSIM over localized regions of the image. The local SSIM values are then averaged to obtain the MSSIM values. The PyTorch Image Quality Assessment package (Rozet, 2022) was used to calculate this metric.

3. Results and discussions

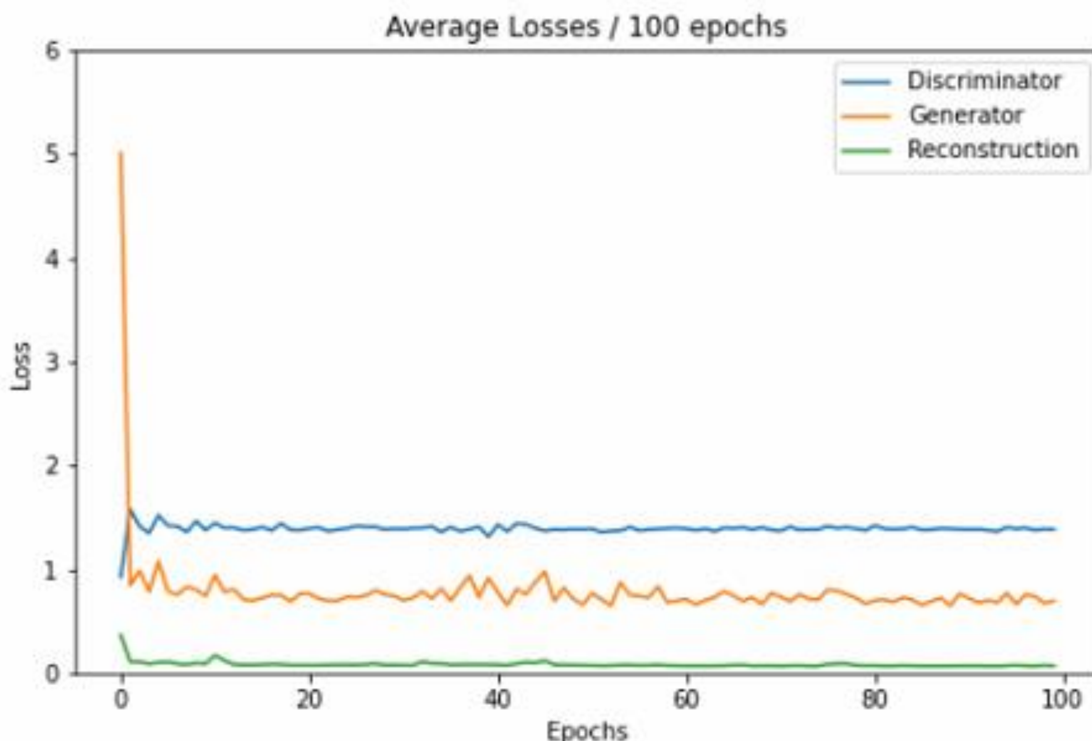

Figure 8. Loss curves for AAE

The loss curves for training the AAE model are shown in Fig. 8. Fig. 9 shows the transition from the real to the generated image for Setup A, Batch 4. It can be seen that the model is able to generate images that follow the trend of the real images.

From Fig. 10, The images produced vary over time and under different application conditions. Comparing the images from day 34, it is noticeable that the image from setting C has a larger green area than the image from setting A.

This difference becomes even more apparent when the same images are compared again on day 38. A more intense green is also observed when the Day 38 and Day 34 images are compared for both settings. The MSSIM scores for the 3 settings were then calculated using the data provided for the test. These scores are tabulated in Table 1.

Figure 9. Transition from actual to generated images, Setup A

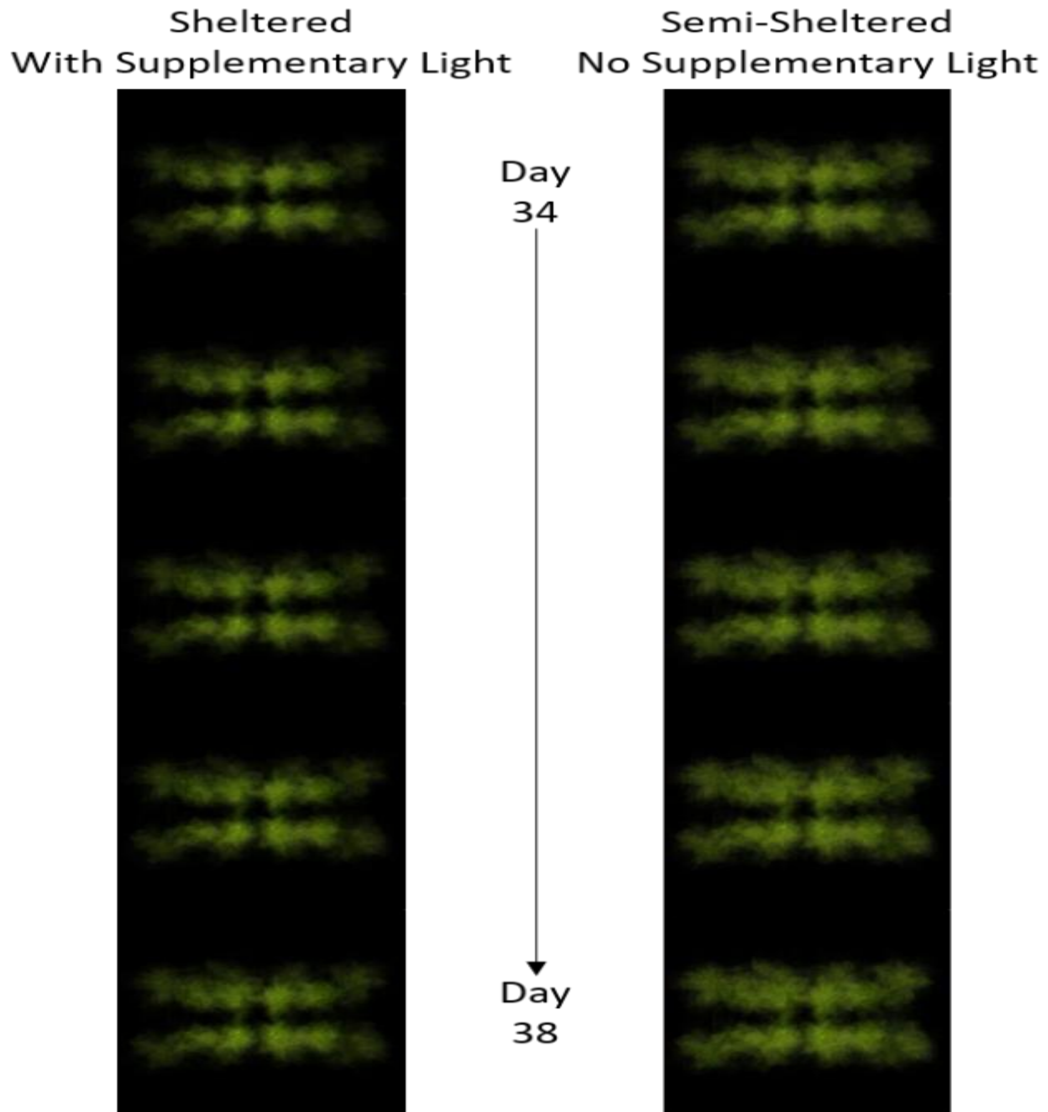

Figure 10. Comparison of generated images between Setup A (left) and C (right)

Table 1. MSSIM Scores, Setups A-C

Setup A						
Day	34	35	36	37	37	38
Score (x10)	5.76	5.76	5.81	5.82	5.73	5.70
Setup B						
Day	34	35	36	37	37	38
Score (x10)	5.83	5.76	5.76	5.67	5.70	5.69
Setup C						
Day	34	35	36	37	37	38
Score (x10)	5.26	5.09	5.24	5.01	5.12	5.16

The results from day 34 to the first instance of day 37 are from the 2nd batch, and the results from the second instance of day 37 to day 38 are from the 3rd batch. Experimental setups A and B are protected experimental setups with supplemental light, and experimental setup C is a semi-protected experimental setup without supplemental light, as described in 2.1.

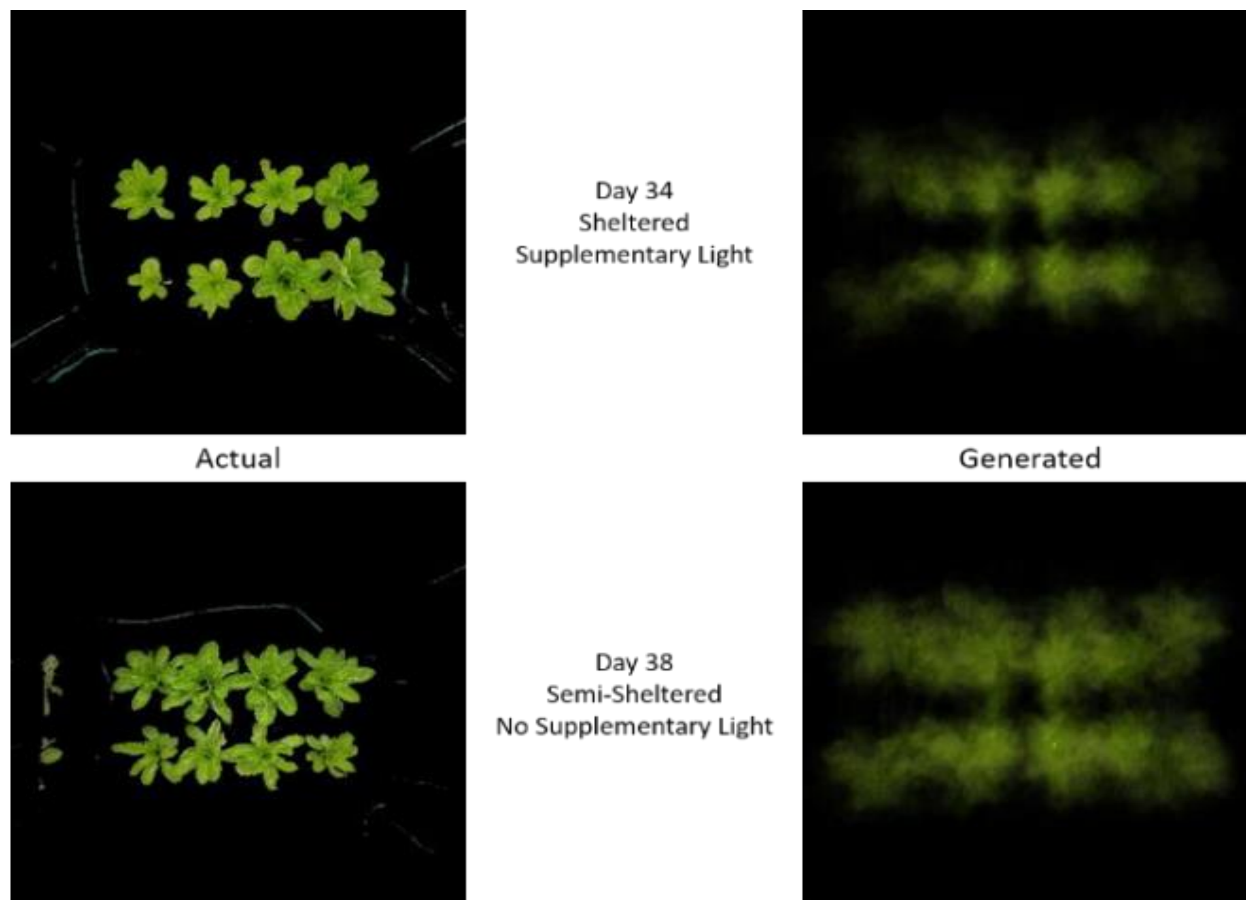

Figure 11. Sample of generated images against actual images

As can be seen from Table 1, the model did not perform poorly, but neither did it excel, with MSSIM values between 0.5 and 0.6, which is confirmed by the generated images, as shown in Fig. 11. Nevertheless, the models are able to capture the differences between configurations despite the limited amount of data. If more and more diverse data were available, the effectiveness of the model could be analyzed in more detail.

4. Conclusions

The AAE model has shown the ability to regenerate harvest images and generate images with different variables (e.g., time). This can help reduce harvest losses because the architecture can be modified to visually predict what the yield will be under changing conditions. With camera smartphones becoming more accessible, farmers can use any smartphone on hand for image capturing – avoiding the need for custom hardware deployment in a commercial setting. Regardless of the source of images (low-cost cameras or smartphones), the architecture can still be applied for crop yield prediction.

However, further work is needed. The data generated is insufficient (only 3 batches could be generated), and there are a number of external variables (e.g., outdoor temperature, solar radiation) that could not be accounted for in this project. Reference markers in the form of ArUco

markers were also used, but the OpenCV library was unable to consistently capture the markers throughout the dataset, possibly due to the reflection of sunlight on the laminated markers affecting the library's ability to read the markers. Therefore, there may be differences in the perspective and positions of the captured images, even though the authors did their best to keep them constant.

Despite the limitations mentioned above, the generation of such images is a testament to the potential of the model. If the model is developed further, the application of such an architecture in the distant future could change the definition of crop yield prediction.

In land-poor locations with the transition to high-tech, high-yield agriculture, the model can be extended with low-cost cameras to create a cost-effective image capture system, and the collected data can be applied to the model to enable a modern application of crop yield prediction given the current state of agriculture in land-poor locations. In this way, crop yields and costs could be optimized.

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KINETICS OF CHANGES IN THE GRANULOMETRIC COMPOSITION OF THERMODENATURED WHEY PROTEINS

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ABSTRACT
The study objective was to determine the optimal modes of temperature-induced denaturation of whey proteins under conditions of complete refolding to enhance the efficiency of subsequent enzymatic release of biologically active peptides. Whey samples obtained after acid, acid and rennet, and rennet coagulation were identified on the basis of physico-chemical parameters and thermal stability using multidirectional methods. The kinetics of denaturation and aggregation of particles, changes in their mean diameter depending on the physico-chemical whey composition, as well as heat treatment modes were studied. The temperature 95 ºC with the exposure time of 120 minutes should be considered as the most optimal mode in terms of maximum protein denaturation and minimum mean particle diameter. At the same time, the rate of protein denaturation and the size of aggregated particles varied depending on the deviation of pH away from pI. For sweet whey, a slightly different mechanism of temperature-induced aggregation of whey protein was noted, characterized by the predominance of hydrophobic interactions.

1. Introduction

Whey is the main secondary raw material resource of the dairy industry resulting from the use of various casein deposition technologies (Bardone *et al.*, 2018). It is internationally accepted to classify whey into two types: sweet whey (pH>5.6) obtained as a result of chymosin/pepsin-induced precipitation of casein in hard cheeses production, and acid whey (pH<5.2) formed as a result of casein isoelectric coagulation in the production of curd, fresh cheese or technical casein. The physico-chemical composition of acid whey differs from the composition of sweet one in a lower content of protein and lactose, as well as a higher level of calcium, phosphorus and lactic acid (Papademas *et al.*, 2019; Zandona *et al.*, 2021).

Today, the volume of the global whey market is about 180-190 million tons showing a constant upward trend. On average, no more than half of the produced whey is subject to industrial processing, of which 50% is processed in liquid form, 30% is dried, and the remaining share is used for protein fractionation, production of lactose and its derivatives (Paladii *et al.*, 2021; Sáenz-Hidalgo *et al.*, 2021). Unprocessed whey is often disposed with industrial wastewater at municipal wastewater treatment plants or in aeration fields. The environmental standards for the management of food waste and by-products consider whey to be harmful to the environment due to high biological oxygen demand (BOD 35-60 g/l) and chemical oxygen demand (COD 60-80 g/l), all of this combined with low pH (Das *et al.*, 2016; Bosco *et al.*, 2018; Mehri *et al.*, 2021). Therefore, tightening of environmental standards and assignment of producer personal responsibility for by-products disposal should contribute to the transition to a closed production cycle in the long run (Fermoso *et al.*, 2018). This could also be facilitated by

accessibility and intensification of technologies for deep whey processing, including those that were introduced with the advent of a new generation of dairy products with added value.

Recently, biologically active peptides obtained from secondary dairy raw materials are of considerable interest. This interest is justified by the high potential of whey proteins biological activity, especially in the manifestation of ACE-, DPP4- and renin-inhibitory effects. In the profile of potential biological activity, such functions as inhibition of alpha-glucosidase in all whey proteins and opioid-agonistic in β–lactoglobulin are also noted (Minkiewicz *et al.*, 2019; Sultan *et al.*, 2017). A wide range of positive potential effects of bioactive whey peptides encouraged the international scientific community to pay close attention to this topic (Patil *et al.*, 2022).

Bioactive peptides initiate various biological reactions in the human body along the receptor pathway and have an effect comparable to that of medicinal or hormonal drugs (Sultan *et al.*, 2017). At the same time, bioactive peptides derived from natural raw materials are known to have a number of advantages over synthetic agents in terms of therapeutic action (Kaur *et al.*, 2020). In most cases, they do not show toxic effects and do not cause other adverse effects (Zambrowicz *et al.*, 2012). Bioinformatic tools existing at the stage of planning the release of bioactive peptides allow assess the possible adverse effects, including their toxicity, and finished bioactive hydrolysis products, which, if necessary, allows changing the cleavage conditions (Kruchinin & Bolshakova, 2022). It is worth noting that biologically active peptides isolated from secondary dairy raw materials are capable of absorption with minimal degradation and entering directly into the bloodstream. This is also one of the advantages in their prospective use (Sultan *et al.*, 2017).

Commercialization of the bioactive peptides production process and the prevalence of their use in various industries, in particular in pharmaceutical and food industry, is impeded by number of reasons: insufficient clinical studies, lack of evidence base of bioactivity of isolated peptides, lack of proper systematization of their mechanisms of action and optimal methods of scaling their production (Chakrabarti *et al.*, 2018).

One of the problems when it comes to bioactive peptides extraction from secondary dairy raw materials may be the complex conformational structure of whey proteins, which, being in a globular form, are not able to undergo complete hydrolysis under the action of enzymes (Abadía-García *et al.*, 2021). It is possible to increase the availability of protein to hydrolytic enzymatic cleavage through temperature-induced protein refolding. It is noted that the complete refolding of β–lactoglobulin is achieved by heat treatment of dairy raw materials at a temperature of 95-97 °C with an exposure of more than one hour without observation of subsequent protein renaturation (Gunkova P.I. *et al.*, 2015). In studies (Halder *et al.*, 2012; Vetri & Militello, 2005) the temperature optimum of such a targeted effect is provided, which is at the level of 80 °C with an exposure time of up to 120 minutes. α-lactalbumin, being a metalloprotein, has a higher thermal stability compared to β–lactoglobulin and the ability to renature. At the same time, the irreversibility of the denaturation of α-lactoalbumin during thermal denaturation is possible under the condition of protein decalcification and destruction of all disulfide bonds (Bernal & Jelen, 1984; Salamanca & Chang, 2005).

This study objective was to determine the optimal thermal denaturation modes under conditions of complete refolding of the main protein fractions of whey, in which the protein yield from secondary dairy raw materials reaches the maximum value with the minimum possible protein aggregation, which in turn can reduce the effectiveness of controlled hydrolysis by proteolytic enzymes and hinder the release of biologically active peptides.

2. Materials and methods

2.1 Materials and Preparation of Whey

Samples of sweet whey were obtained in the industrial conditions of the company "Italian

Traditions" (Russia) from the black-and-white cow milk produced at the "Lenin State Farm" (Russia). The whey was collected at the end of the technological process of Montasio (rennet coagulation) and Mozzarella cheese production (acid-rennet coagulation).

Samples of acid whey were obtained in the production and experimental workshop of the "All-Russian Dairy Research Institute" ("VNIMI", Russia) from the black-and-white cow milk produced at the "Lenin State Farm" (Russia). The whey was collected as a result of curd production by acid coagulation of milk to a pH of 4.5-4.6 A starter culture with pure cultures of *Lactococcus lactis* strains 79 $_{5}$, 79 $_{10}$, 79 $_{13}$ (VNIMI, Russia) was used for this purpose. The acid whey was also obtained as a result of curd production by acid-rennet coagulation of milk to the final pH level of 5.0-5.2. The product was fermented using *Lactococcus lactis* strains 79 $_{5}$, 79 $_{10}$, 79 $_{13}$ (VNIMI, Russia) and the enzyme preparation Clerici 96/04 (Caglificio Clerici, Italy).

The samples obtained were delivered to the laboratory within 10 minutes, immediately heated in an incubator to a temperature of 40 ± 5 °C and separated from milk fat and casein dust on a MilkyDay FJ 90 PP separator (Austria). Purified whey samples were cooled to a temperature of 4±2°C and stored.

2.2. Experimental Design

The experiment was carried out according to the plan presented in Figure 1.

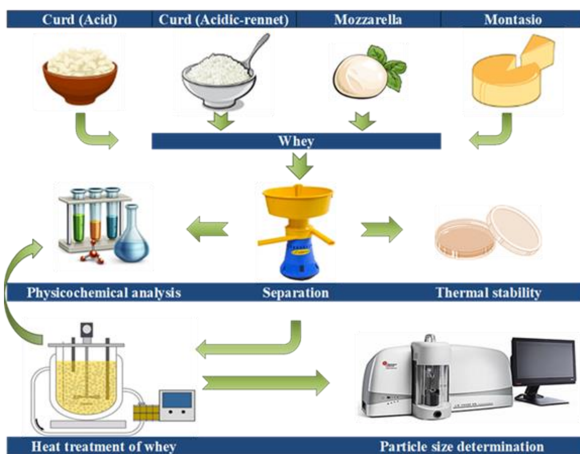

Figure 1. The experimental design

2.3. Physicochemical Analysis

The evaluation of the physicochemical parameters of sweet and acid whey types was carried out by well-known methods: humidity was determined by the thermogravimetric method according to GOST ISO 6731/IDF 21-2012; the mass fraction of fat was determined by the Gerber method GOST R ISO 2446-2011; the mass fraction of protein was determined by the total nitrogen by the Kjeldahl method in accordance with ISO 1871:2009; the mass fraction of casein and whey fractions proteins were determined according to ISO 17997-1:2004; the mass fraction of lactose was determined in accordance with ISO 26462:2010; calcium content was determined by titrimetric method according to ISO 12081:2010; active acidity (pH) was measured by the potentiometric method using an InoLab pH Level 1 high-precision pH meter equipped with a Sen Tix 61 pH. The fractional composition of proteins was determined by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate (SDS-PAGE), followed by densitometry of the intensity of staining of the tracks in accordance with the technique (Bavaro *et al.*, 2019).

2.4. Thermal Stability

The protein system resistance to denaturation during heat treatment was determined using multidirectional methods: an alcoholic sample, a chlorocalcium sample, a thermoacid sample and a thermal sample described earlier in the work (Vafin *et al.*, 2021).

2.5. Whey Heat Treatment

Whey samples were subjected to thermal denaturation in the temperature range of 75 to 135 °C with interval of 10 °C in jacket bioreactors (Alkhitekh, Russia) connected in series with a circulating glycerin bath (Alkhitekh, Russia). Heating and aging were carried out without stirring, in order to neutralize of the flocculation effect to the denaturation and aggregation of whey proteins. Protein denaturation was evaluated over a span of 120 minutes with 20 minutes interval. At the end of protein thermodenaturation the whey was

cooled to 25 °C by feeding cold water into the jacket (Figure 2).

Figure 2. Bioreactor for thermally induced denaturation of whey protein

2.6. Determination of whey proteins denaturation

The mass fraction of denatured whey proteins was determined by the method described in (Pan *et al.*, 2022). The mass fraction of the protein not denatured was determined by the Kjeldahl method in the filtrate after whey sample centrifugation at 3500×g for 10 min. The mass fraction of denatured proteins was determined as the difference between the mass fraction of protein in the initial whey and the mass fraction of protein in the fugate after centrifugation.

2.7. Particle Size Determination

The size distribution of aggregated particles was analyzed using an LS 13 320 XR laser diffraction analyzer (Beckman Coulter, USA) equipped with a universal liquid module. Distilled degassed water at room temperature was used as a dispersion medium in the module chamber. The selected averaged whey sample was introduced into the module chamber with an automatic single-channel pipette (5-10 ml) with an increased diameter of the tip inlet (5 mm). The sample was introduced until the reading signal levels of 50% were reached on the PIDS photodetectors (differential intensity of polarized light scattering). At the end of the analysis, the area of the obtained graph in the range from 0.01 to 3000 microns was evaluated. The results were calculated with refractive indices of 1.33 for water and 1.54 for the samples of different types of treated whey studied.

2.8. Statistical Analysis

Statistical data analysis was performed using the Statistica 2010 software package. All measurements were carried out in 3 independent repetitions, the results are presented as the mean (±) standard deviation (SD). Statistical analysis was performed using single-factor analysis of variance (ANOVA) at a significance value of $P < 0.05$.

3. Results and Discussions

3.1. Physicochemical characteristics of Milk Whey

The average physicochemical composition of milk whey samples as a result of curd and cheese production is presented in Table 1. The whey samples studied were characterized by significant differences in level of active acidity (pH) and content of dry substances, including total protein, lactose, minerals. This was directly related to the mechanism of coagulation of milk proteins and the technology of processing curd. Data provided in Table 1 demonstrate that the acidity of whey AW(A) (pH 4.50) obtained during acid coagulation of milk is below the isoelectric point of casein. 0.55% of proteins consisting of 0.47% whey proteins and 0.08% casein fractions are transferred to AW(A) whey. This particular content of protein in whey is associated on the one hand with the inclusion of some denatured whey proteins in the curd matrix due to complexation with k-casein, and on the other hand, the formation of casein dust due to the processing of a curd with a low density and small size of aggregated particles formed under the action of lactic acid (Dalgleish & Corredig, 2012). As a result of acid coagulation, calcium phosphate and structure-forming calcium also cleave from the casein micelle, accompanied by its transition to a soluble state, followed by partial migration to whey (93.40 mg/100 g).

The physicochemical composition of AW(A/R) whey obtained as a result of mixed milk coagulation differs from AW(A) by higher values of active acidity (pH 5.09) and lactose (3.94%). This is associated with isoelectric coagulation of milk at pH 5.20 as a result of the hydrolytic effect of pepsin on k-casein, and,

accordingly, with a lower intensity of lactose fermentation by lactic acid microorganisms. Acid-rennet isoelectric coagulation is accompanied by the transition to a soluble form of only part of the calcium-containing salts migrating into the whey (76.80 mg/100 g). The whey produced by acid-rennet isoelectric coagulation is characterized by a low content of total protein (0.39%) and, in particular, k-casein (0.02%).

Table 1. Physicochemical analysis of milk whey characteristics

Name of parameter	Curd whey		Cheese whey	
	Acid	Acidic-rennet	Acidic-rennet	Rennet
	AW(A)	AW(A/R)	SW(A/R)	SW(R)
Total solids, %	5.89±0.15	6.07±0.19	6.22±0.15	6.54±0.12
Fat, %	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Protein, %	0.55±0.11	0.39±0.07	0.72±0.04	0.89±0.10
Whey protein, %	0.47±0.04	0.37±0.07	0.69±0.04	0.82±0.08
β-lactoglobulin	0.28±0.03	0.22±0.04	0.47±0.03	0.57±0.05
α-lactoalbumin	0.14±0.01	0.12±0.02	0.17±0.01	0.19±0.03
BSA	0.046±0.003	0.047±0.003	0.043±0.002	0.05±0.004
Lactoferrin	0.005±0.001	0.006±0.001	0.005±0.001	0.008±0.001
Casein, %	0.08±0.02	0.02±0.01	0.03±0.01	0.07±0.02
αs_1-casein	0.03±0.01	0	0	0
αs_2-casein	0	0	0	0
β-casein	0.03±0.01	0	0	0
κ-casein	0.02±0.01	0.02±0.01	0.03±0.01	0.07±0.02
Lactose, %	3.55±0.14	3.94±0.11	4.46±0.09	4.62±0.13
pH	4.50±0.10	5.09±0.08	5.84±0.05	6.43±0.09
Ash,%	0.72±0.08	0.59±0.06	0.54±0.05	0.46±0.03
Calcium, mg/100 g	93.40±3.10	76.80±2.70	68.89±1.50	61.30±2.20

SW(A/R) whey with a pH of 5.84 obtained during acidification of the mixture with lactic acid formed as a result of lactic acid fermentation (residual lactose level of 4.46%) followed by rennet coagulation, is characterized by a high protein content (0.72%, including 0.03% k-casein) and low calcium content (68.89 mg/100 g).

SW(R) whey obtained during rennet coagulation of milk has a slightly acidic pH of 6.43, a high content of lactose (4.62%) and total protein (0.89%) with a minimum calcium content (61.30 mg/100 g), which is fully consistent with the theory of rennet protein coagulation (Lucey, 2017). With this mechanism of coagulation, whey proteins and lactose almost completely transfer into the whey together with an insignificant amount of the fraction of k-casein and soluble calcium.

3.2. Milk Whey thermal stability

Table 2 demonstrates assessing results of the thermal stability of whey types obtained during acidic, acidic-rennet and rennet coagulation of milk using multidirectional methods.

Table 2. Milk whey thermal stability

Name of test	AW(A)	AW(A/R)	SW(A/R)	SW(R)
Alcohol test	< 68%	< 68%	68%	85%
Calcium chloride test	±	–	–	±
Phosphate test	–	±	±	+
Acid-boiling test	1.2 mL*	0.8 mL*	0.8 mL*	0.5 mL*

– negative result; ± conditionally positive result; + positive result
*amount of 0.1N HCl withstood by whey, mL

Analysis of data obtained as a result of the study of thermal stability by alcohol test method showed high resistance to denaturation under the action of an 85% solution of SW(R) whey alcohol, while the SW(A/R) sample withstood the test with only 68% alcohol concentration. The samples of AW(A) and AW(A/R) whey types failed the alcohol test.

Thus, the concentration of the alcohol solution causing the denaturation of whey proteins correlates with a decrease in pH in the samples of milk whey types. The strong denaturing effect of alcohol solutions at low pH values is accounted for by electrostatic and hydrophobic interactions due to decrease in the negative charge of the protein (as a result of decrease in the pH of the medium) and increase in the hydrophobicity of its surface due to the contact of the solvent with nonpolar amino acid residues (Nikolaidis & Moschakis, 2018; Wagner *et al.*, 2021). The data obtained fully correlated with the results of the thermal stability of whey types determined by the phosphate test method.

The assessment of thermal stability by calcium chloride test method showed that protein precipitate in the samples of AW(A) and SW(R) whey types was poorly visualized, while in the samples of AW(A/R) and SW(A/R) there was clearly pronounced denaturation and aggregation of protein, accompanied by the separation of transparent whey. This effect is assumed to be associated with a change in the solubility of proteins as a result of increase in the concentration of calcium ions at pH 5.0 and is generally consistent with the results of studies (Dissanayake et al., 2013). The results of the acid-boiling test showed that the protein denaturation in the SW(R) whey required minimum amount of 0.1N HCl (0.5 mL), while for the AW(A) whey - maximum amount (1.2 mL). Whey samples AW(A) and AW(A/R) were denatured with the addition of 0.8 mL of 0.1N HCl. Inversely proportional dependence of the amount of 0.1N HCl required for protein denaturation on the initial acidity of the whey and the proximity of pH to pI of the main whey proteins was noted.

3.3. Thermodenaturation of Milk Whey

Differences in the physicochemical composition (Table 1) and thermal stability of milk whey samples suggest different intensity and degree of protein thermodenaturation. Figure 3 shows the kinetics of changes in the degree of denaturation of whey proteins under different heat treatment modes.

In the process of heat treatment of milk whey types with different physicochemical composition at temperature of 75 ^{o}C low percentage of protein denaturation was observed. After 120 minutes of incubation, only 15.7% of the protein from their initial whey content was denatured in the SW(R) sweet whey sample. With decrease in the pH of the studied samples, the percentage of denatured protein increased and reached 22.2% and 26.4% for SW(A/R) and AW(A/R) whey samples, respectively. The maximum percentage of denatured protein of 36.5% was observed in the AW(A) sample. Thus, the denaturation of proteins under this regime is local in nature, mainly dependent on the level of acidity of the sample.

Data analysis in the temperature range of 85-135 ^{o}C with an exposure of 120 minutes showed

a slightly different dependence of the degree of protein denaturation on pH and temperature. The sensitivity of proteins to thermal denaturation, regardless of the coefficient of thermal exposure, decreased in the following sequence AW(A)>SW(R)>AW(A/R)>SW(A/R). The maximum rate of thermally induced denaturation/aggregation of whey proteins was observed at pH equal to or close to pI (AW(A)) due to electrostatic and covalent interactions. An increase in pH (5.09 and 5.84) and its distance from the pI of the main whey proteins led to a significant slowdown in this process. However, at pH 6.43, the rate of denaturation /aggregation of sweet whey proteins, which occurs mainly due to the formation of disulfide bonds with more reactive sites (Gulzar et al., 2011), was insignificantly lower than in samples with a pH close to pI. Similar results on model whey protein systems were also obtained in the works (Nicolai et al., 2011; Nishanthi et al., 2017).

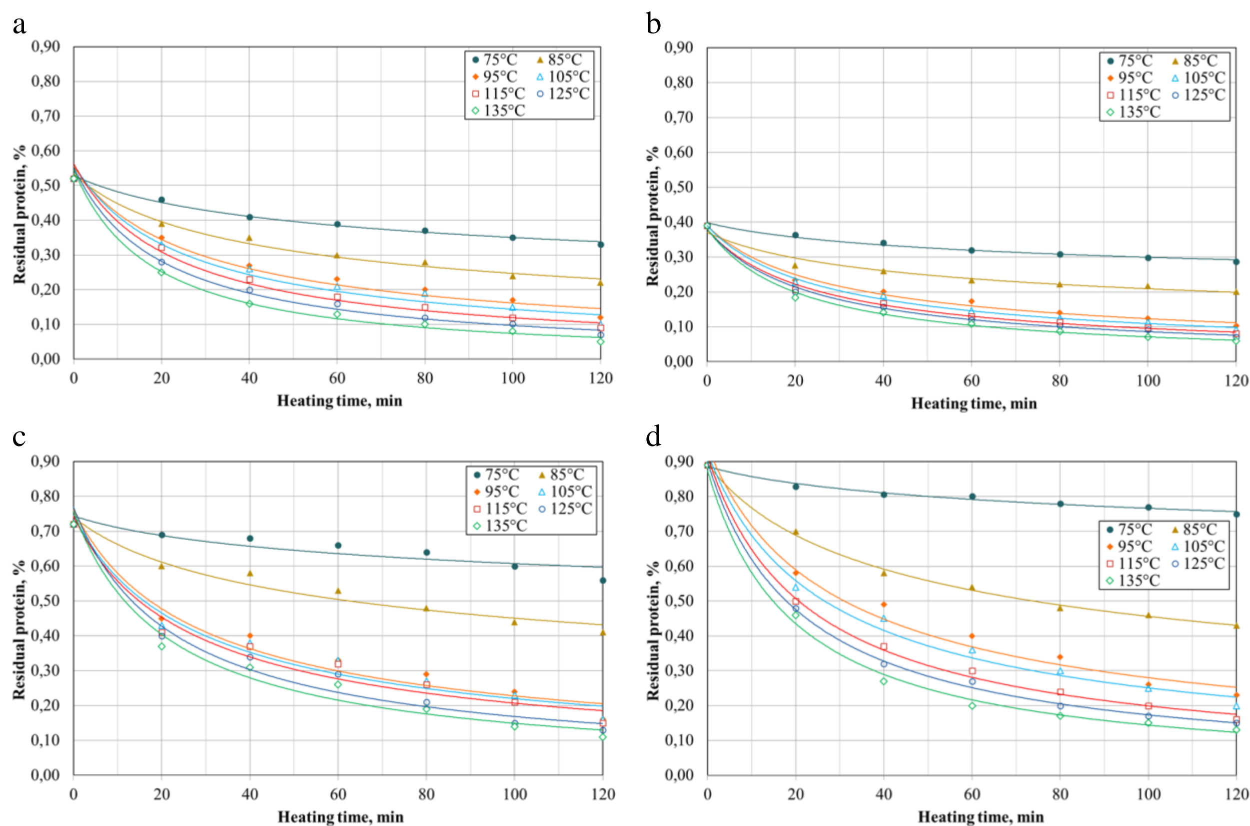

Figure 3. Changes in whey protein content at different modes of thermodenaturation process (a - AW(A); b - AW(A/R); c - SW(A/R); d - SW(R))

The degree of denaturation of whey proteins also directly depends on the temperature and duration of heat treatment. So, at a temperature of 85 °C and after 120 minutes of denaturation, 43-58% of the protein from the initial content in the whey was subjected to denaturation. Temperature rise to 95 °C and 105 °C led to an increase in the degree of denaturation to 73.6-75.0% and 76.4-80.0%, respectively. While the transition to temperatures critical for dairy raw materials led to a maximum degree of denaturation of 115 °C (79.2-82.7%), 125 °C (81.9-86.5%) and 135 °C (84.7-90.4%) with a maximum average speed of 0.072 g/min (AW(A)), 0.070 g/min (SW(R)) and 0.068 g/min (AW(A/R) and SW(A/R)). At the same time, due to exposure to high temperatures, the Maillard reaction mechanism was activated and accelerated. Aggregated proteins were characterized by a coarser structure due to the

probable formation of intermolecular isopeptide and lysinoalanine cross-links at high temperatures, which according to (Zhang *et al.*, 2021) it may limit the availability of enzymes to cleavage sites both during digestion and during directed hydrolysis. Therefore, these temperature regimes were not considered in further studies.

3.4. Particle size determination of thermodenatured whey protein

The physicochemical composition, temperature, and duration of thermally induced protein denaturation can have a direct effect on the size and structure of the resulting aggregates (Nicolai *et al.*, 2011). Therefore, the next researches were aimed at establishing the cumulative effect of these factors on the size of the protein aggregates formed under different temperature and time regimes of whey processing (Tables 3-4).

Data analysis (Tables 3 and 4) showed that at temperature of 75 °C the volume-weighted average diameter (D[4;3]) of denatured proteins and their median values (D 50) did not change significantly within120 minutes. This indicates the absence of conditions for mass aggregation of protein and fully correlates with the data presented in Figure 3. The average size of whey protein aggregates and their distribution enhanced significantly with increase in temperature and duration of thermal exposure, which is explained by a decrease in electrostatic interactions due to the predominance of hydrophobic interactions (Nicolai *et al.*, 2011). The influence of the acidity factor on the size of the denatured protein aggregates formed was observed. Whey with a pH of 4.50 (AW(A)) was characterized by the highest values of D[4;3] and D 50 aggregated proteins for all temperature-time treatment modes, as well as a high aggregation rate.

Table 3. Size distribution of aggregated protein particles (microns) in the process thermally induced denaturation of acid whey types

Parameter of heat treatment		AW(A)		AW(A/R)	
Temperature, °C	Heating time, min	$D_{[4;3]}$	D_{50}	$D_{[4;3]}$	D_{50}
0	0	1.07±0.02	0.70±0.01	0.98±0.03	0.61±0.01
75	20	1.89±0.03	1.43±0.02	1.36±0.01	0.76±0.03
75	40	3.91±0.04	2.83±0.03	1.73±0.04	0.91±0.02
75	60	4.26±0.01	3.23±0.01	1.98±0.02	1.29±0.04
75	80	5.43±0.03	4.08±0.02	3.14±0.05	2.70±0.06
75	100	6.62±0.02	4.85±0.05	4.21±0.03	3.37±0.05
75	120	7.38±0.09	5.62±0.08	5.10±0.05	4.11±0.03
85	20	3.79±0.05	2.16±0.08	2.65±0.02	2.23±0.04
85	40	4.44±0.03	3.15±0.06	3.87±0.07	2.26±0.03
85	60	14.97±0.11	9.75±0.09	9.63±0.10	7.02±0.05
85	80	23.12±0.19	11.75±0.08	16.12±0.12	9.64±0.08
85	100	29.19±0.22	15.94±0.15	23.58±0.18	11.87±0.09
85	120	42.42±0.31	26.83±0.20	34.93±0.10	21.63±0.19
95	20	6.67±0.04	4.86±0.08	4.48±0.06	3.59±0.03
95	40	10.56±0.12	7.74±0.09	7.04±0.06	5.11±0.07
95	60	30.09±0.21	23.11±0.15	23.67±0.11	11.95±0.10
95	80	53.65±0.27	27.09±0.15	39.95±0.19	24.36±0.13
95	100	87.97±0.43	32.92±0.21	57.24±0.25	26.91±0.16
95	120	94.32±0.51	40.03±0.28	66.83±0.28	32.06±0.17
105	20	9.60±0.05	7.99±0.08	8.11±0.04	7.03±0.03

105	40	19.34±0.15	13.45±0.12	15.23±0.09	11.42±0.05
105	60	35.92±0.19	20.28±0.10	29.66±0.13	18.46±0.16
105	80	60.57±0.23	33.17±0.18	48.87±0.32	32.64±0.21
105	100	91.23±0.47	45.93±0.25	63.03±0.27	37.19±0.30
105	120	112.87±0.62	65.01±0.27	74.21±0.31	42.74±0.24

The average diameter of aggregated protein particles and their median values in sera AW(A/R) and SW(A/R) decreased significantly with pH (5.09 and 5.84) moving away from pI protein. This dependence is generally consistent with the results (Buggy et al., 2018). It is worth noting that in the whey SW(R) (pH 6.43) at processing temperature of 75-95 °C values of D[4;3] and D50 were slightly higher than in the SW(A/R) sample. As far as the processing temperature increased up to 105 °C the growth of aggregates in the SW(R) whey, unlike in other types of whey, has noticeably accelerated. This is most likely related to more intensive formation of disulfide bonds alongside with the resulting flotation effect due to intense vaporization in the whey.

Table 4. Size distribution of aggregated protein particles (microns) in the process thermally induced denaturation of sweet whey types

Parameter of heat treatment		SW(A/R)		SW(R)	
Temperature, °C	Heating time, min	$D_{[4;3]}$	D_{50}	$D_{[4;3]}$	D_{50}
0	0	0.76±0.03	0.18±0.01	0.88±0.02	0.50±0.02
75	20	1.00±0.05	0.48±0.02	1.25±0.03	0.73±0.01
75	40	1.35±0.04	0.78±0.03	3.62±0.02	3.05±0.02
75	60	1.62±0.03	1.19±0.02	4.01±0.04	3.48±0.03
75	80	2.07±0.03	1.08±0.02	4.36±0.05	3.49±0.04
75	100	3.25±0.02	2.75±0.01	5.74±0.03	4.88±0.03
75	120	4.23±0.05	3.82±0.03	6.72±0.07	5.74±0.02
85	20	2.19±0.01	1.91±0.01	3.43±0.04	2.94±0.02
85	40	3.68±0.03	3.12±0.02	4.10±0.04	3.01±0.07
85	60	7.27±0.06	5.51±0.05	10.86±0.08	8.13±0.04
85	80	12.82±0.12	9.07±0.08	14.91±0.14	9.90±0.12
85	100	17.39±0.15	9.91±0.06	19.03±0.17	11.18±0.05
85	120	21.05±0.12	10.68±0.7	25.06±0.14	12.92±0.09
95	20	3.56±0.04	2.88±0.02	5.64±0.04	3.58±0.05
95	40	6.67±0.02	4.35±0.03	7.91±0.06	4.99±0.02
95	60	12.48±0.10	8.93±0.07	17.23±0.12	9.85±0.06
95	80	19.74±0.16	11.66±0.11	24.96±0.18	12.32±0.10
95	100	25.65±0.19	13.57±0.12	31.51±0.19	18.46±0.16
95	120	32.78±0.23	19.80±0.15	38.94±0.26	24.22±0.13
105	20	7.35±0.06	6.17±0.04	8.67±0.04	7.62±0.02
105	40	10.61±0.09	8.48±0.05	13.86±0.12	9.94±0.07
105	60	16.75±0.17	11.34±0.09	25.36±0.19	17.51±0.14
105	80	23.04±0.22	15.42±0.14	42.15±0.25	29.67±0.16
105	100	31.10±0.25	19.19±0.11	54.81±0.23	34.13±0.19
105	120	40.86±0.26	28.54±0.17	68.97±0.31	39.00±0.21

4. Conclusions

The high capacity of whey as a natural raw material for the production of biologically active peptides draws attention to the concern of increasing the efficiency of whey proteins hydrolysis. Deployment of globular structures along with reduced degree of protein aggregation will increase the accessibility of sites to enzymatic hydrolysis. This research shows that different combinations of temperature-time modes of heat treatment of whey types with different physicochemical composition formed protein structures with different average diameter and particle distribution, as well as the degree of denaturation. According to the results of the research, temperature of 95 °C and exposure time of 120 minutes should be considered as the optimal mode in terms of maximum protein denaturation and minimum average particle diameter. It was found that the rate of protein denaturation and the size of aggregated particles varied depending on the removal of pH from pI, in all samples except SW(R), where the mechanism of hydrophobic interactions prevailed. However, further studies in this domain are required taking into account the factors of lactose content and salt composition, as well as additional methods of whey processing: neutralization and acidification.

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INFLUENCE OF UV TREATMENT ON SOME PROPERTIES AND BIOACTIVE COMPOUNDS IN ONION BULBS (*ALLIUM CEPA L.*) DURRING STORAGE

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ABSTRACT

Due to the importance of food and health benefits, onions are used and stored in large quantities. One of the problems encountered during onion storage is besides maintaining the chemical components at optimal values, the losses recorded by sprouting and stripping the bulbs. Physical and chemical properties as well as bioactive compounds and weight loss of the onion bulbs were determined during storage under the influence of an UV-C treatment with doses of 2 $kJ \cdot m^{-2}$. The results showed that UV-C illumination treatment do not lead to significant differences concerning the size of onion during storage. Weight losses of onion were lower in onions treated by UV-C illumination during storage. Dry matter, the soluble solids and total phenolic contents remained higher in treated onions. Treatment of onions by UV-C illumination determines the suppression of bulb sprouting. These findings demonstrate that the application of UV-C illumination to onions can be a method of extending their shelf life.

1.Introduction

Preservation of vegetables is done in order to extend their shelf life by stopping the growth of microorganisms, including a series of processes that inhibit quality deterioration due to enzymatic processes (Maskepatil et al., 2019).

Onion (*Allium cepa L.),* originating in Asia Minor, is among the healthiest foods being found in the same family with garlic, shallots, Egyptian onions and leeks. The interest in onion bulbs worldwide has determined the growers to find new postharvest techniques to extend the storage life of this crop while maintaining their nutritional and organoleptic characteristics (Zudaire et al., 2017).

Due to the importance of food and health benefits, onions are stored in large quantities. Due to its characteristics of preservation and durability of transport, onions have al-ways been marketed more widely than most vegetables (Griffiths et al., 2002).

Onions have a high content of beneficial phenols with high antioxidant capacity, with an important role in preventing and reducing the progression of different types of diseases (Pérez-Gregorio et al., 2010).

Onions are also rich in vitamins, minerals and antioxidants, with a low energy value (Abdelrasheed et al., 2021). Onion wasted peels are rich in free fat (0.31%) and reducing sugars (Zhivkova, 2021).

Phenols also play an important role as a prebiotic (Yang et al., 2004), being a source of food for beneficial bacteria in the stomach, which is important for health, weight maintenance and dis-ease prevention.

Onion contains more phenolic compounds than garlic (Nuutila et al., 2003) or leeks and is one

of the best dietary sources of flavonoids, especially quercetin.

Due to its nutritional importance and health benefits, onions are stored by refrigeration in large quantities. Some of the most important conditions in terms of maintaining the quality of the onion for as long as possible are in addition to harvesting at the optimum time, the ventilation operations during storage, especially the compliance with the periods and parameters of ventilation and care for bulbs.

The biggest problem that appears during the storage of onion bulbs is due to the germination process and microbiological changes, especially their rot.

Irradiation of vegetables is an important method to reduce the load of microorganisms on their surface and extend their shelf life (Cueva et al., 2010).

UV radiation is electromagnetic radiation with a wavelength shorter than the light radiation perceived by the human eye (Zamanian and Hardiman, 2005).

The spectrum of UV radiation is between wavelengths 10 - 380 nm (1 nanometer = 10-9 m), with a frequency of 750 THz (380 nm) to 30 PHz (10 nm). UV radiation with a maximum germicidal effect is UV-C in the wavelength spectrum of 254 nm (the point at which the nucleic acids of microorganisms have the maximum absorption) (Tran et al., 2022).

These radiations are surface sterilizers with an effect on microorganisms, especially bacteria and viruses. UV-C radiation has a disinfectant effect, as it affects the DNA structures of microorganisms, causing a photochemical effect on thymine (Kalisvaart, 2004). They dimerize, which means that two adjacent information carriers are improperly linked (Schreier et al., 2007).

This molecular change means that DNA cannot be used for the essential process of transcription (metabolism) and replication (cell division). As a result, the microorganism is inactivated and dies.

Exposure to UV-C radiation has been used as a post-harvest treatment of fruits and vegetables and is useful in delaying fruit senescence and reducing fruit decay (Wang, Chen and Wang, 2009). These, modifies the expression of genes involved in cell wall degradation delaying the softening of fruits and vegetables (Pombo et al., 2011).

Studies on the use of UV-C illumination on fresh horticultural produce have focused on the selection of appropriate doses for different species, but less attention has been paid to the effect of radiation intensity (Lin et al., 2017).

Previous studies have shown that the use of UV-C illumination of plant products combined with fresh storage has led to an extension of their shelf life (Cote et al., 2013). The duration of UV-C illumination varies, previous research indicating 30 minutes (to obtain a dose of 2.0 kJ·m-2) (Nour, Plesoianu and Ionica, 2021).

The present study was conducted to investigate the effect of UV-C illumination on some properties and bioactive compounds in onion during storage. Weight and sprouting losses during storage as a result of treatment were also investigated.

2. Materials and methods

2.1. Plant material

Daytona F1 hybrid onion bulbs purchased from local producers in Dolj County, Romania were used as plant material. Daytona F1 is a semi-early hybrid of onion, a standard variety in onion culture that offers stable and quality yields year after year. The bulb has a golden color, an attractive shape and a good storage capacity even in unarranged spaces. It has a very well-developed root system, which gives it resistance to drought-induced stress. Daytona F1 has medium resistance to *Fusarium oxysporum* and pink root rot *(Pyrenocheta terrestris*), yielding 55-60 tons/hectare.

2.1.1. Sampling

The effect of UV-C illumination treatments on the physicochemical characteristics of onion, as well as weight loss during their long-term storage, was studied.

The onion was sorted and packed in plastic net bags with a capacity of 15 kg. Two working options have been established, namely:

- Control variant (C) was to store the onion bulbs in refrigerated conditions: 4°C temperature and 75% relative air humidity
- the bulbs of the second variant (V1) were subjected to a treatment with UV illumination using a UV LED lamp for 30 minutes, equivalent to a dose of 2.0 $kJ \cdot m^{-2}$ measured by a UVC portable digital radiometer (TN-2254, Taine Co., Ltd., Taiwan, China). The UV lamp was placed 30 cm away from the onion.

The onion bulbs were placed in an insulated enclosure on a single layer and after 15 minutes, they were turned over and the treatment was continued for another 15 minutes. After that, they were packed and stored under the same conditions of temperature (4°C) and relative humidity (75%).

For each variant, the amount of onion studied was 75 kg (5 bags). During storage, onion samples were taken in order to highlight the changes that occurred as well as the effect of UV-C illumination. The onion was stored for 4 months, experiments were executed in five repetitions, and the results were expressed as average ± standard error of average repetitions.

2.2. Analytical methods

Dimensions, average bulb weight, volume, size index, shape index, total dry matter, soluble solids content, titratable acidity, total phenolic content as well as antioxidant capacity and weight loss of the onion bulbs were determined during storage.

Onion linear dimensions (length, L; width, W; thickness, T) were determined with a Luthier digital caliper manufactured by Stewart MacDonald (USA) and the results were expressed as mm.

Average onion weight (g) was determined by individual weighing on an analytical scale model ABT-320-4M manufactured by Kern (Balingen, Germany. The volume of the fruit was determined by a cylinder of volume on the principle of Archimedes, the results being expressed in cm3. Size index was calculated using the formula: (L+T+W)/3. Weight loss during storage was measured by monthly weighing of a predetermined lot of onion the results being expressed as a percentage.

Dry matter (%) was determined by drying 5 g of fresh onion sample to a constant weight at 105°C and soluble solids content (TSS%) was determined of onion juice was measured with a digital refractometer (Hanna Instruments, Woonsocket, USA).

The titratable acidity was determined by titration of a known amount of water extract of onion with 0.1N NaOH using phenolphthalein as indicator and is expressed as g malic acid/ 100 g fresh weight (fw).

2.2.1. Total phenolic content

The total phenolic content was assessed according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Briefly, a 100 µl aliquot of the extract was mixed with 5 ml of distilled water and 500 µL of Folin-Ciocalteu reagent. After 3 min, 1.5 mL (20% w / v) sodium carbonate solution was added and the reaction mixture was diluted with distilled water to a final volume of 10 mL.

After stirring vigorously and incubating in the dark at 40 ° C for 30 minutes, the absorbance was measured at 765 nm on a Varian Cary 50 UV spectrophotometer (Varian Co., USA).

A calibration curve was prepared using standard gallic acid solutions. The results were expressed in milligrams of gallic acid equivalents (GAE)/ 100 g fresh weight.

2.2.2. Antioxidant activity

Antioxidant activity was measured in methanolic extract using the DPPH test (2,2-diphenyl-1-picrylhydrazyl).

Sampling was performed according to the same protocol described for the total phenolic content. The free radical scavenging capacity of DPPH free radical extracts was evaluated as described by Oliveira et al. (2008), with some modifications.

Each ethanolic onion extract (50 µl) was mixed with 3 mL of 0.004% (v / v) DPPH methanolic solution. The mixture was incubated for 30 minutes at room temperature

in the dark and the absorbance was measured at 517 nm on a Varian Cary 50 UV-VIS spectrophotometer. DPPH free radical scavenging capacity was calculated with reference to Trolox (6-hydroxy2,3,7,8-tetramethylchroman-2-carboxylic acid), which was used as a standard reference.

A methanol/water control was used in each analysis. All tests were performed in triplicate and the results were expressed in mmol Trolox / 100 g fresh weight (fw).

2.2.3. Statistical analysis

Results were expressed as means ± standard deviation. Effect of storage time was analyzed using the least significant difference (LSD) test and differences at $p < 0.05$ were considered to be significant. The statistical analysis was carried out using Statgraphics Centurion XVI software (StatPoint Technologies, VA, USA).

3. Results and discussions

3.1. Physical properties and weight loss of onions during storage

Table 1. The variation of the dimensions, size index and shape index of the onion

Variant/Storage period (months)	**Control (C)**	**UV-C treatment (V1)**
Length (L) (mm)		
Beginning of storage	63.05±5.66	63.05±5.66
1	60.88±7.67	58.89±8.79
2	60.49±8.11	61.14±8.78
3	60.08±8.28	60.96±8.40
4	60.08±7.90	50.20±8.50
Width, (W) **(mm)**		
Beginning of storage	60.52±6.41	60.52±6.41
1	60.35±7.45	57.87±9.41
2	59.33±8.00	58.42±8.76
3	59.33±8.30	59.14±9.10
4	58.87±8.21	46.50±8.74
Thickness, (T) (mm)		
Beginning of storage	62.17±6.50	62.17±6.50
1	63.16±7.27	59.97±10.16
2	60.81±7.23	62.47±8.25
3	61.46±7.52	61.75±7.29
4	61.24±7.45	53.10±7.85
Size index		
Beginning of storage	61.88±5.02	61.88±5.02
1	61.46±7.39	59.24±8.62
2	60.21±7.49	60.67±7.91
3	60.27±7.64	60.61±7.56
4	60.06±7.51	60.09±7.71
Shape index		
Beginning of storage	0.99±0.11	0.99±0.11
1	1.04±0.04	1.00±0.14
2	1.01±0.08	1.02±0.12
3	1.02±0.09	1.02±0.12
4	1.01±0.08	1.02±0.12

*Means followed by different superscript letter in a column are significantly different (LSD test, P<0.05).

Size and shape are needed to describe a product defined with some dimensional parameters (Devojee et al., 2021). The size of the onion decreased constantly during storage (Table 1). It is also seen that the largest decrease in size is recorded in onion bulbs treated by UV-C illumination.

However, no significant differences were recorded between the two variants in terms of dimensions, size index and shape index of the onion.

Another trend is observed in terms of the weight and the volume of the onion (Table 2). The weight and volume of onion bulbs are a characteristic of the variety and there is a correlation between these and some chemical components such as flavonoids (Pérez-Gregorio et al., 2014; Riggi et al., 2019). Although these characteristics evolved negatively during storage, with weight and volume losses, it was found that they were higher in control (C) variant. No significant differences were found in terms of variations in onion bulb volume.

Storage of onions involves many pre-harvest and postharvest factors which determine weight losses (Petropoulos, Ntatsi and Ferreira, 2017).

The weight loss of onions (table 3) has steadily increased during storage, which has been more pronounced in the last two months, being higher in the variant treated by UV-C illumination compared to the control (C).

Table 2. The variation of the dimensions, size index and shape index of the onion during the storage

Variant/Storage period (months)	Control (C)	UV-C treatment (V1)
Weight (w) (g)		
Beginning of storage	121.1±5.57^{c}	121.1±5.57^{b}
1	115±5.60^{bc}	105.3±5.16^{a}
2	110.6±5.49^{b}	110.7±5.42^{a}
3	107.7±5.36^{b}	108.7±5.39^{a}
4	97.38±4.81^{a}	107.4±5.22^{a}
Volume (V) (cm^3)		
Beginning of storage	126.1±6.19	126.1±6.19
1	124.8±6.09	116.5±6.10
2	121.5±5.97	120.5±5.69
3	117.4±5.49	124.3±6.11
4	116.8±5.85	118.6±5.82

*Means followed by different superscript letter in a column are significantly different (LSD test, P<0.05).

Table 3. Weight loss of onion during storage

Variant/Storage period (months)	Control (C)	UV-C treatment (V1)
Weight loss (%)		
Beginning of storage	-	-
1	1.52±0.06^{a}	2.17±0.99^{a}
2	2.67±0.13^{b}	3.71±0,19^{b}
3	4.08±0.20^{c}	5.46±0.26^{c}
4	5.47±0.27^{d}	7.00±0.32^{d}

*Means followed by different superscript letter in a column are significantly different (LSD test, P<0.05).

3.2. Variation of the chemical properties of onions during storage

The dry matter in onions consists mainly of fiber, starch and sugars; these include non-structural carbohydrates such as fructose and glucose, non-reducing sugars such as glucose, and fructans (Ríos-González et al., 2018). The dry matter content in onion decreased steadily in the first two months of storage and then increased in the third month of storage, returning to a downward trend in the last month of storage (Table 4).

The soluble solids content is an important quality parameter and is related to the degree of preservation of the onion (Mallor et al., 2011). Onion at harvest maturity (Table 4) had a soluble solids content of 7.75%, which decreased steadily during their storage, which is consistent with data obtained by Saeed, and Mohsen (2015) and Chávez-Mendoza et al. (2016). The largest decrease in the soluble solids content is observed in the control variant (6.98%).

In both variants, in the second and third months of storage is a sharp increase followed by a sharp decrease in the last month of storage. Soluble solids content reduction during storage is due to the increase of the carbohydrate metabolism through respiration (Mota et al., 2019).

The titratable acidity values increased continuously until the third month of storage, when, in the illuminated variant, there was a decrease of it. However, the increases in titratable acidity did not have a specific trend in both variants studied, which is consistent with the data presented by Zudaire et al. (2020).

This can be attributed to the fact that during storage at low temperatures, respiratory intensity decreases as well as metabolic activities (do Nascimento Nunes and Emond, 2002).

Table 4. The dry matter content (DW %), soluble solids content (TSS%) and titratable acidity in the onion during storage

Variant/Storage period (months)	Control (C)	UV-C treatment (V1)
Dry matter (DW%)		
Beginning of storage	9.52±0.63^{c}	9.52±0.63^{b}
1	8.51±0.12^{b}	8.17±0.16^{a}
2	8.35±0.30^{b}	7.24±0.48^{a}
3	8.82±0.21^{b}	7.77±0.38^{a}
4	7.01±0.18^{a}	7.45±0.46^{a}
Soluble solids content (TSS%)		
Beginning of storage	7.80±1.18^{ab}	7.80±1.18
1	7.47±0.69^{ab}	7.20±0.91
2	7.86±0.65^{ab}	7.64±0.76
3	8.03±0.70^{b}	7.27±0.47
4	7.02±0.59^{a}	7.12±0.35
Titratable acidity (g malic acid/100 g fw)		
Beginning of storage	1.68±0.08^{a}	1.68±0.08^{b}
1	2.01±0.13^{b}	1.34±0.06^{a}
2	1.67±0.09^{a}	1.34±0.07^{a}
3	2.01±0.09^{b}	2.01±0.09^{c}
4	2.01±0.10^{b}	1.67±0.09^{b}

*Means followed by different superscript letter in a column are significantly different (LSD test, $P<0.05$).

The phenols, have an important role from a nutritional point of view (Liguori et al., 2017). Postharvest processing and storage affect the content of phenolic acids and flavonoids in foods (Tomás-Barberán, Ferreres and Gil, 2000).

In our study onion has a high total phenolic content (Table 5) with higher values than those reported by Zudaire et al. (2019).

It steadily increased over two months of storage and then fell sharply in the third, resuming its upward trend in the last month of storage. This upward trend has also been mentioned in the literature (Zudaire et al., 2020; Juániz et al., 2016).

Table 5. Variation in total phenolic content and antioxidant activity of onion during storage

Variant/Storage period (months)	Control (C)	UV-C treatment (V1)
Total phenolic content (milliequivalent gram of gallic acid (GAE)/100 g fw)		
Beginning of storage	76.25±2.76[a]	76.25±2.76[b]
1	76.06±2.85[a]	76.88±3.11[b]
2	82.19±3.08[b]	76.25±2.80[b]
3	70.93±3.21[a]	66.87±2.81[a]
4	76.56±3.60[ab]	80.93±2.98[b]
Antioxidant activity (AOA) (mmol Trolox / 100 g fw)		
Beginning of storage	0.74±0.04[b]	0.74±0.04[b]
1	1.34±0.07[c]	0.77±0.03[b]
2	0.60±0.03[a]	0.57±0.02[a]
3	1.55±0.07[d]	0.76±0.04[b]
4	1.49±0.06[d]	1.14±0.05[c]

*Means followed by different superscript letter in a column are significantly different (LSD test, $P<0.05$).

Regarding the effect of UV-C illumination on the total phenolic content, there is initially a slower increase in the first 2 months of storage and a sudden increase in the last month of storage of the onion. At the end of the storage, the UV-C illuminated onion had a higher phenolic content than the control (80.93 milliequivalent gram of gallic acid /100 g fw compared to 76.56 milliequivalent gram of gallic acid /100 g fw in the control).

Juániz et al. (2016) reported that the increase in phenolic compounds in the stored onion corresponded to an increase in antioxidant activity determined by the DPPH method (Table 5), which is also confirmed by the data found in this study. Previous reserch has shown a strong correlation between antioxidant activity and total phenolic content (Shim, Yi, and Kim, 2011).

Sprouting can be prevented by different chemical treatements or an appropiate storage temperature (Suojala, 2001). It was found that the bulbs in the version treated by UV-C illumination did not show swelling and sprouting processes comparing with control (C) that registered 4% sprouted bulbs, a slight dehydration and stripping.

4. Conclusions

Storage conditions did not affect the morphological parameters of the onion and have helped to maintain a low respiration rate, weight and chemical compounds loss being relatively small.

Onions belonging to the cultivar Daytona F1 have highly characteristics in terms of natural antioxidant compounds. UV-C illumination treatments maintain at a higher level the content of onions in phenolic compounds as well as their antioxidant activity.

UV-C illumination leads to a more pronounced decrease in onion size during storage. The individual weight and volume of the onion evolves negatively during storage, the total weight loss being higher in the UV-C treated variant.

UV-C illumination treatment with doses of 2 $kJ\cdot m^{-2}$ resulted in a higher maintenance of dry matter, total soluble solids and total phenol content.

The antioxidant activity of onion evolves in accordance with the content in total phenols, still, a decrease being observed under the influence of UV-C treatment during storage.

Treatment of onions by UV-C illumination leads to the suppression of bulb dehydration and stripping. UV-C illumination treatment of onion significantly suppressed water loss, thus delaying their degradation during storage. Treating onion bulbs by UV-C illumination prevents them from sprouting.

The results suggest that UV-C illumination with doses of 2 $kJ\cdot m^{-2}$ can be a method of maintaining onion bulbs quality and extending their self-life.

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EFFECT FERMENTATION ON VOLATILE COMPOUNDS OF PACKAGED CASTOR OIL -MORINGA SEEDS CONDIMENT (*OGIRI*)

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ABSTRACT
The effect of fermentation on volatile compounds of packaged castor-moringa seeds condiment (*ogiri*) was investigated. Blends of castor oil seeds and moringa seeds were used to produce fermented condiment (*Ogiri'*). A 100% castor *ogiri* (MC_{100}) and 100% moringa *ogiri* ($MM_{100)}$) that served as control were packed in *uma l*eaves and fermented for 48 h. Castor and moringa *ogiri* were blended in ratio 86. 21:13.29, fermented in different fermentation times (46.81 h, 51.99 h, 51.64h and 47.52 h) and packaged in *Uma* leaves, aluminum foil, aluminum foil and plastic containers designated as MMC, FMC, FMC_{64} and Plastic container. The volatile compound of packaged fermented castor- moringa seeds (*ogiri)* were evaluated using Gas –Chromatography / Mass Spectrometry (GC-MS). The result of amino acids showed that only eighteen amino- acids were detected. A total of 162 volatile compounds were identified in packaged castor – moringa *ogiri.* The compound identified were various types of acids, ester, hydrocarbons, aromatic compounds, alcohol, ketones, aldehydes, sterols, among others. The predominant volatile compound found in packaged fermented castor- moringa seeds *were* acids followed by esters. Samples FMC_{64} (86.21: 13. 29 fermented for 51. 64 h) had higher concentration in hydrocarbon (11.52%), ketones (2.40 %), sterols (15.31%) and vitamins (7.48 %.) than other samples..

1. Introduction

Fermented condiments are consumable substances added in small quantities to add flavor, taste, some nutrients and relish to the main food produced from leguminous plant or oil seeds. These fermented condiments include "ogiri" from castor-oil seeds (*Ricinus commumis*), "dawadawa" from African yam beans (*Parkia biglobosa*) and "Ugba" African oil bean (*Pentaclethra macrophyllia*). According to Kalopo *et al.* (2009*)* fermented condiment provided dietary fibre, energy, mineral and vitamins (vitamin A and vitamin B_2). It also has potential uses as protein substitutes and functional ingredients (Achi, 2005). Nigerians spent about $12 million on food flavoring imports between 1983 and 1998, with a projected 15 % annual increase in the future (Essien, 1983; Odunfa and Oyewole, 1998). This emphasizes the importance of fermented vegetable proteins, which have a lot of potential as key protein and micronutrient sources, as well as basic ingredients for dietary supplementation. Most of indigenous condiments are produced through the process of fermentation. Fermentation actually hold a promise as a food processing methods that can be used to diversify the food uses of some under exploit plant food like moringa *olerferia,* among other locally available oil seeds.

Castor oil bean (*Ricinus communis*) is a member of the spurge family, *Euphorbiaceae*. The protein of castor oil seed contains ricin and ricinoleic which are toxic substances; nevertheless, the level is reduced or eliminated during fermentation (Odunfa, 1985). According

to Annongu and Joseph, (2008), castor seed is deficient in some indispensable essential amino acids such as lysine, iso-leucine and tryptophan is absent.

Moringa Oleifera Lam belongs to the *Moringaceae genus*, which has fourteen species (Morton, 1991). It is native to the Indian subcontinent, but it has spread throughout the world, including to Nigeria. Ram (1994) and Anhwange *et al.* (2004) indicated that moringa seeds contain all the essential amino acids "lysine, cystine, valine, methionine, isoleucine, leucine, phenylalanine and threonine" in appreciable quantities. These essential amino acids are often in short supply in most legumes and oil seeds. There is need to supplementation of fermented condiment (*ogiri*) with oil seeds that rich in sulphur containing amino acid like moringa seed (Ram, 1994, Gueguen *et al.,* 2016).

Ogiri and other fermented condiment have a very short shelf life, ineffective and unattractive packaging materials that resulted in stickiness and the characteristic putrid odour have caused instability in the commercial status of this traditional condiment (Arogba *et al.,* 1995), thus the need to research how to find good packaging materials that will protect, maintain it in good condition, and preserve the flavor until it reaches consumers in perfect freshness, flavour, appeal, and appearance.

Volatile profile of fermented castor oil bean was investigated by Ojinnaka and Ojimelukwe, (2013), the compounds identified were ester, alcohol, ketones, furan acids and others. Chukeatirote *et al.,* (2017*)* studies the volatile compounds in fermented soybeans prepared by a co-culture of *bacillus substilis and rhizopus oligosporus,* a total of 165 volatile compounds were identified including "alcohol, aldehydes aromatic, ketones, acids, ester, pyrazine, sulphur- containing and miscellaneous". There is little or no information on study of amino acids and volatile compounds of packaged castor – moringa *ogiri.* This present study aims at evaluating effect of fermentation on amino acids and volatile profile of packaged castor moringa *ogiri*

2. Materials and Methods

2.1. Materials

Castor oil seeds were procured from Nwko-- Igboukwu market in Aguata L.G.A in Anambra state and Moringa seeds were purchased from central market Kaduna in Kaduna State. The aluminium foil and plastic container were purchased from Eke –Ekwulobia. An *uma* leaves was also procured from "Amudo" village in Ekwulobia.

2.2. Methods

2.2.1. Traditional processing of fermented castor oil seed "ogiri"

The castor oil seed ogiri was produced according to method described by (Odunfa 1985). The castor oil seeds (1.5 kg) were dehullled mechanically, washed and boiled for 8 h in 9 litres of water. The boiled castor oil seed was fermented for 96 h by natural fermentation. Fermented castor oil seeds were mashed into paste. Subsequently, the paste was distributed into the various packaging materials which comprises of *Uma* leaves, aluminum foil, and plastic containers and was allowed to stand for secondary fermentation and flavor maturation for 48 h.

2.2.2. Traditional processing of fermented moringa seed "ogiri"

The method of Onyekwelu (2016) was adapted to production of fermented moringa seed *ogiri.* Moringa seeds (1.5 kg) were dehullled manually, boiled for 3 h after being washed. The boiled moringa seeds were fermented for 96 h by natural fermentation. Fermented moringa seeds were mashed into paste. Subsequently, the paste was distributed into the various packaging materials which comprises of *uma* leaves, aluminum foil and plastic container and was allowed to stand for secondary fermentation and flavor maturation for 48 h.

2.2.3. Formulation

Castor oil seeds and moringa seeds *ogiri were* blended in ratio, packaging and fermented in different fermentation time in as shown in Table 1 below.

Table 1. Formulation of castor *ogiri* and moringa *ogiri*

Castor seeds *ogiri*	Moringa seed *ogiri*	Packaging	Fermentation time (h)
100		*Uma leaf*	48
	100	*Uma leaf*	48
86.21	13.29	*Uma leaf*	46.8
86.21	13.29	*Foil*	51.99
86.21	13.29	*Foil*	51.64
86,21	13.29	*Plastic*	47.53

2.2.4. Packaging of castor- moringa seeds ogiri

After the primary fermentation, each design points of castor- moringa *ogiri* were distributed in packaging materials comprises *uma* leaves, Aluminum foils and plastic container and kept for further analysis.

2.2.5. Determination of volatile compound profiles

The volatile compounds of Castor – moringa ogiri were determined using gas chromatography/mass spectrometry (GC-MS) analysis. On a Perkin Elmer GC Clarus 500 system with an AOC-20i auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) apparatus, the following settings were employed to carry out GC-MS analysis, "column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 μM df, composed of 100 % Dimethylpolysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 μI was employed (split ratio of 10,1) injector temperature 250 ºC; ion-source temperature 280 ºC". The oven temperature was set to 110 °C (isothermal for 2 minutes), then increased at a rate of 10°C/min to 200 °C, then 5ıC/min to 280 °C, with a final time of 9 minutes (isothermal at 280 °C). Mass spectra was taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time of 36min.

3. Results and discussion

3.1. Percentage composition of volatile compounds of packaged castor moringa *ogiri*

Percentage composition of volatile compounds of packaged castor moringa *ogiri is shown in T*able 2. These volatile compounds are of various type such as acids, ester, hydrocarbon, aromatic compounds, alcohol, indole, ketones, aldehydes, sterols, vitamin and others were identified in packaged castor - moringa *ogiri* acids. Sample MM_{100} had the highest percent **of** acids (91.16%) while sample FMC_{64} had the lowest percentage (1.42 %) of acids. Acids, hydrocarbon, vitamins and Miscellaneous compounds were found present in packaged fermented castor moringa seeds while hydrocarbon and miscellaneous compounds were absent in 100% fermented castor and moringa seeds packed in *uma* leaves

3.2.Identified volatile compounds in packaged fermented castor- moringa seeds (*ogiri*)

The list of the identified compounds and their molecular formula, molecular mass, retention time and percentage concentration of fermented castor- moringa seeds (*ogiri)* packed in different packaging materials are shown in Table 3. The spectrum reveals that 22, 29,38,26,28 and 19 volatile compound were identified in sample MC_{100}, MM_{100}, FMC, MMC FMC_{64} and PMC respectively. Sample PMC had the highest concentration of oleic acid (60.87%) followed by sample MC_{100} (53.32%) while samples MM_{100} and FMC had the lowest concentration of oleic acids 2.70% and 0.70%. Oleic acids were the most abundant compound in samples PMC (60.87%), MC_{100} (53.37%) and FMC (49.92%). In this study acids were most dominant compounds. This was in agreement with Ojinnaka and Ojimelukwe (2013) who found acids to be most dominant volatile compound in fermented castor oil seed. Azokpota *et al.* (2008) found pyrazines to be the major constituent in sonru, aftin, and iru, which contradicts this report. Aldehydes in locust beans *daddawa*, soyabean and melon seeds o*giri*

(Onyenekwe *et al.,* 2012). Adebiyi *et al* (2021) found esters to major constituent in *daddawa* bambara groundnut. According Larcoche *et al* (1999) some acids were synthesis through leucine and isoleucine. Although acids give fermented foods some acidic, fruity, and sour tastes, (Park *et al.*,2013), they have been reported to be undesirable compounds that give unpleasant characteristics such as rancid, sweaty, and pungent flavor (Frauendorfer and Schieberle, 2008).

Table 2. Percentage Composition of Volatile Compounds of Packaged Castor- Moring *ogiri*

Volatile Compound group	MC_{100} (%)	MM_{100} (%)	FMC (%)	MMC (%)	FMC_{64} (%)	PMC (%)
Acids	70.65	91.16	67.23	51.77	1.42	77.26
Esters	3.45	4.21	4.86	0.62	3.20	-
Hydrocarbon	4.67	0.10	3.06	3.71	11.52	5.57
Aromatic	-	0.10	-	4.41	0.41	-
Alcohol	-	0.52	-	-	-	0.36
Indole	-	-	5.29	6.89	-	-
Ketones	-	0.25	1.03	1.12	2.40	1.10
Aldehydes	-	0.65	4.56	1.05	1.01	1.80
Sterols	-	1.0	4.07	2.31	15.31	1.10
Vitamins	2.98	0.28	2.84	2.60	7.48	4.04
Others (Miscellaneous compounds)	10.86	2.41	13.60	25.89	22.22	9.06

Key :
MC100= 100% castor *ogiri* package with Uma leaves fermented for 48 h, MM100= Moringa *ogiri* packaged with Uma leaves fermented for 48 h, MMC = 86.21% castorogiri, 13.29 % moringa *ogiri* packed in uma leaves fermented for 46.81 h, FMC = 86.21% castor *ogiri*, 13.29 % moringa *ogiri* packed in aluminum foil fermented for 51.99 h. FMC64 = 86.21% castor *ogiri*, 13.29 % moringa *ogiri* packed in aluminum foil fermented for 51.64h, PMC = 86.21% castor *ogiri,* 13.29 % moringa ogiri packed in plastic container fermented for 47.52 h.

Ester was another major abundant compound in castor – moringa *ogiri* packed in different packaging materials. The most abundant esters were found to be ricinoleic acids methyl ester in sample MC_{100} with concentration of 3.45 %, other esters were octadecenoic acids, ethyl ester, 9, 12 - octadecadienoic acids, ethyl ester and oleyl oleate detected in samples MM_{100}, FMC_{64} and MM_{100}. During fermentation, esters are mostly formed when alcohol is esterified with fatty acids (Sluis *et al.*, 2011). Esters are also produced from chemical reaction between microbial acids and alcohol metabolites (Wittanalai *et al.,*2011), this reaction is catalysed by microbial esterase during fermentation (Eskin, 1990). When compared to their alcohol precursors, esters with few carbon atoms are extremely volatile at room temperature and have a lower perception threshold (Izco and Torre, 2000; Nogueira *et al.*, 2005). It has a fruity floral flavor to it. The sharpness of unpleasant free fatty acids can be reduced or marked by ester. Therefore, esters must have contributed to the flavour of the product.

Table 3. Identified Volatile Compounds in Packaged Fermented Castor- Moringa Seeds (*ogiri*)

Peak	RT (min)	Compound Names	Formula	Molecular MASS	MC_{100}	MM_{100}	FMC	MMC	FMC_{64}	PMC
		Acids								
1	18.792	Oleic acid	$C_{18}H_{34}O_2$	282	53.37	2.70	49.92	39.97	0.70	60.87
2	19.171	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.76	10.95	5.29	4.97	0.72	5.46
3	21.225	9, 12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	-	-	-	-		O.37
4	21.615	Octadecanoic acid	$C_{18}H_{36}O_2$	284	6.68	10.95	5.60	4.65	-	7.14
5	22.675	trans-delta-9 octadecenoic acid	$C_{18}H_{34}O_2$	282	-	63.96	1.52	-	-	0.80
6	23.391	Ricinoleic acid	$C_{18}H_{34}O_3$	298	5.84	0.14	4.90	2.18	-	2.62
7	23.730	Arachic acid	$C_{20}H_{40}O_2$	312	-	2.56	-	-	-	-
		Esters								
1	20.780	Hexadecanoic acid, propyl ester	$C_{19}H_{38}O_2$	298	-	-	0.12	-	-	-
2	20.858	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	-	0.10	0.13	-	-	-
3	21.566	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	-	-	0.35	-	-	-
4	21.710	oleic acid, ethyl ester	$C_{20}H_{36}O_2$	3.10	-	-	0.85			
5	21.956	Octadecanonic acid ethyl ester	$C_{20}H_{40}O_2$	312		1.41	0.25			
6	22.674	Glyceryl Monooleate	$C_{21}H_{40}O_4$	356				0.62		
7	22.955	Ricinoleic acid, methyl ester	$C_{19}H_{36}O_3$	312	3.45	-	0.56			
8	23.581	9,12-Octadecadienoic acid , methyl ester	$C_{19}H_{34}O_2$	294	-	-	-	-	2.99	-
9	24.485	10-Undecenoic acid, butyl ester	$C_{15}H_{28}O_2$	240	-	-	-	-	0.21	-
10	24.820	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	328		0.10				
11	25.280	Oleyl oleate	$C_{36}H_{68}O_2$	532	-	1.38	-	-	-	-
12	26.134	Cinnamic acid, 2-bornyl ester	$C_{19}H_{24}O_2$	684	-	1.22	-	-	-	-
13	26.321	Heptanoic acid, octyl ester	$C_{15}H_{30}O_2$	242						

		Hydrocarbon								
1	3.595	2-pentene, 2-4 dimethyl	C_7H_{14}	96					0.43	
2	10.075	Tridecane	$C_{13}H_{28}$	184					1.20	
3	27.370	Squalene	$C_{30}H_{50}$	410	4.67	0.10	3.06	3.71	9.89	5.57
		Aromatic compounds								
1	3.795	Phenol	C_6H_6O	94				4.41	0.41	
2	27.754	Benzene, (1-methylnonadecyl)- (2-Phenyleicosane)	$C_{26}H_{46}$	358		0.10				
		Alcohol								
1	15.215	11-Tetradecen-1-ol, acetate,	$C_{16}H_{30}O_2$	254						0.36
2	24.186	Phytol	$C_{20}H_{40}O$	296		0.52				
		Indole								
1	9.985+	Indole	C_8H_7N	117			5.29	6.89		
		Ketones								
1	20.465	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280		0.22	1.03	1.12	2.40	1.10
2	26.192	1-Penten-3-one, 4,4-dimethyl-1-phenyl-	$C_{13}H_{16}O$	188		0.03				
		Aldehydes								
1	23.455	Cis-9-Hexadelenal	$C_{16}H_{30}O$	238			1.28			1.22
2	23.606	9-Tetradecenal, (Z)	$C_{14}H_{26}O$	210		0.65	3.13			
3	24.675	Cis- 13-Octadecenal	$C_{18}H_{34}O$	266			0.15	1.05	1.01	0.58
		Sterol								
1	25.071	Stigmasterol	$C_{29}H_{48}O$	412		0.23	0.82	0.89	4.38	
2	26.590	Gamma-sitosterol	$C_{29}H_{50}O$	414		0.77	3.25	1.42	10.35	1.10
3	26.904	26-Hydroxycholesterol	$C_{18}H_{46}O_2$	402					0.58	

		Vitamins								
1	28.334	delta Tocopherol	$C_{27}H_{46}O_2$	402	1.11		1.12	1.07	3.01	1.54
2	29.167	Gamma Tocopherol	$C_{28}H_{48}O_2$	416	1.87		1.72	1.53	4.47	2.50
3		Vitamin E (2H-1-Benzopyran-6-ol, 3,4-dihydro-)	$C_{29}H_{50}O_2$	430		0.28				
		Others (miscellaneous compound)								
1	7.692	2-piperidinone	C_5H_9NO	99			1.13			
2	9.870	Benzeneethanamine, N-(1-methylethylidene)-	$C_{11}H_{15}N$	161			3.06	10.56		2.50
3	20.461	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_9$	596	0.49					
4	21.767	Octadecanamide	$C_{18}H_{34}NO$	283					1.61	
5	21.801	Silane, (cyclohexyloxy) trimethyl-	$C_9H_{20}OSi$	172	3.31		3.23	2.80		3.43
6	22.049	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{34}O_9Si_9$	666	4.23		0.30		3.19	
7	23.005	2-Palmitoylglycerol,2trimetlylsilyl ether	$C_{25}H_{54}O_4Si_2$	474			0.14			
8	23.630	Palmitoyl chloride	$C_{16}H_{31}ClO$	274		0.32				
9	23.659	9-Octadecenamide	$C_{18}H_{35}NO$	281			5.32	6.44	15.73	3.13
10	24.670	1,3-Dipalmitin trimethylsilyl ether	$C_{38}H_{76}O_5S_i$	640		0.14				
11	25.170	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	290			0.10		0.83	
12	25.521	Dodecanamide, N,N-diethyl-	$C_{16}H_{33}NO$	255			0.73	3.84		
13	26.260	Octadec-9Z-enol trimethylsilyl ether	$C_{21}H_{44}OS_1$	340		0.82				
14	26.385	Acetamide,N,N-dioctyl-	$C_{18}H_{37}NO$	283			0.19			
15	27.821	N-Hexadecylacetamide	$C_{18}H_{37}O$	283			0.51	2.25	0.86	

Key: MC100= 100% castor ogiri package with *Uma* leaves .fermented for 48 h, MM100= Moringa ogiri packaged with *Uma* leaves fermented for 48 h, MMC = 86.21% castor ogiri, 13.29 % moringa ogiri packed in *uma* leaves fermented for 46.81 h, FMC = 86.21% casto rogiri, 13.29 % moringa ogiri packed in aluminum foil fermented for 51.99 h. FMC64 = 86.21% castor ogiri, 13.29 % moringa ogiri packed in aluminum foil fermented for 51.64h, PMC = 86.21% castor ogiri, 13.29 % moringa ogiri packed in plastic container fermented for 47.52 h

Two volatile compounds were detected as ketones in castor – moringa *ogiri* packed in different packaging materials, which are 13-Hexyloxacyclotridec-10-en-2-one and 1-Penten-3-one, 4,4-dimethyl-1-phenyl-. Sample FMC_{64} had highest concentration of 13-Hexyloxacyclotridec-10-en-2-one (2.40%). This ketone is found present in sample MM_{100}, FMC, MMC and PMC. Ketone was not detected in sample MC_{100} (100% castor *ogiri* packed in *uma* leaves). 1-Penten-3-one, 4,4-dimethyl-1-phenyl- was found present in sample MM_{100} This may be as a result of the longer fermentation and effect of packaging materials. "The high concentration of ketone in sample FMC_{64} might be due to partial oxidation of the alcohols as well as synthesis through several metabolic pathways, especially reduction of methyl ketone" (Curioni and Bosset, 2002; Akkad *et al.,* 2019). This is contradicting the report of Ojinnaka and Ojimelukwe (2013) that identified oxacyclotetradecan-2-one in fermented castor seed with 3 % lime. Ketones are derived from the breakdown of amino acids and lipids, with the presence of compounds that affect food flavor (Adebo *et al*., 2018). Ketones contribute to flavour of castor- moringa *ogiri.*

Alcohol identified in castor –moringa *ogiri* were 11-Tetradecen-1-ol, acetate, and phytol in samples PMC and MM_{100} but absent in other samples. Reduction of corresponding aldehydes and oxidation of acids were used to produce alcohols (Pham *et al*.,2008). Aldehydes and ketones are relatively unstable intermediate compounds that can be easily reduced to alcohol, according to Estrella *et al*., (2004). Low concentration of alcohol in fermented condiments possibly as a result of the thermal treatment used during processing (Cho *et al.,*2017; Wang *et al.,* 2019) alcohol present in fermented condiment help to prevent this condiment from spoilage, this is known to acts as antifungal and prevent food spoilage (Onyenekwe *et al.,* 2012). Alcohol related compound could be contributed to the aroma of castor – moringa *ogiri.*

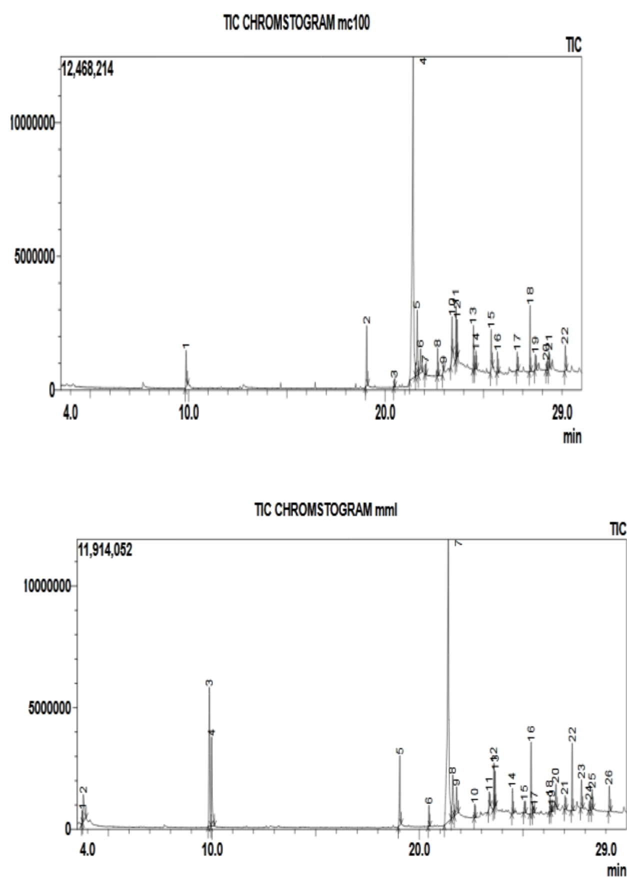
The aldehydes detected in castor- moringa *ogiri* packed in different packaging materials were cis-9-hexadecenal, 9-Tetradecenal and cis-13-Octadecenal found present in almost all samples but absent in sample MC_{100} (100% castor *ogiri* packed in *uma* leaves). Similar trends were reported by Ojinnaka and Ojimelukwe (2013) that aldehdyes was not detected in castor seed ogiri. The presence of aldehydes may be due to presence of moringa *ogiri* present in samples.

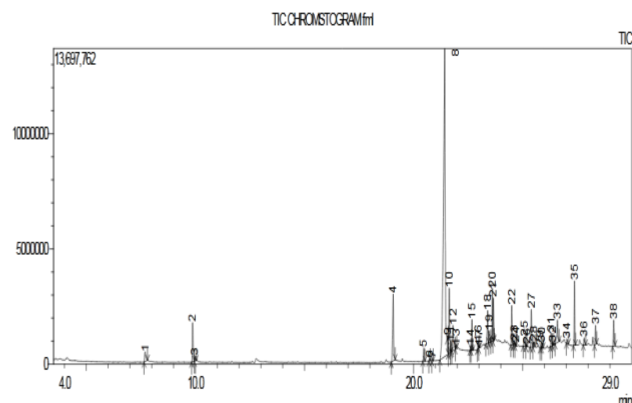
The oxidation of fatty acids, as well as oxidation catalyzed by lipoxygenases and hypo-oxidase enzymes, produces aldehydes, which are important flavor compounds (Nzigamasabo, 2012). Aldehydes are also known as a key component in the formation of heterocyclic compounds (Ziegleder, 2009). The presence of aldehydes in the fermented condiments is probable associated with variable factors such as Millard reaction, streckers degradation or microbial metabolism occurs during the fermentation.

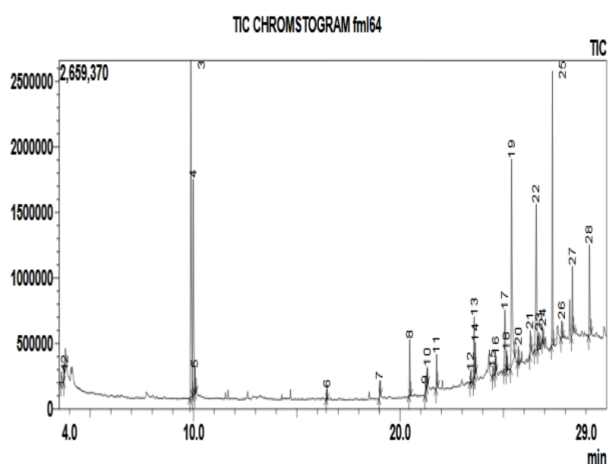
Sterols are a group of compounds found in abundance in Sample FMC64.with highest concentration of stigmasterol (4.38 %) and gamma sitesterol (10.35 %). The 26-hydroxycholestrol was identified only in sample FMC_{64}. None of these Sterols was detected in sample MC_{100}. Sterols were detected in sample contain proportion of moringa ogiri. This is in agreement with Gupta *et al.* (2014) that found stigmasterol and β- sitosterol present in moringa seed, these sterol possess medicinal properties. Stigamsterol and β- sitosterol function as antioxidant, hypoglycemic, hyroid inhibiting properties and effective against cardiovascular disease anti-inflammatory and anti –diabetic properties among others (Kris *et al*., 2002,

Singh, 2006; Jamal *et al.,*2008; Gupta *et al.,* 2014). Natural occurring plant cholesterol, according to Ogbe *et al.* (2015), may benefit animal and human health when taken as food supplements or organically in food on a regular basis for a suitable amount of time.

Vitamin –related compound was detected in all the samples of castor- moringa *ogiri.* delta tcopherol and gamma tocopherol were identified in samples MC_{100}, FMC, MMC, FMC_{64} and PMC but found absent in sample MM_{100} (100 % moringa *ogiri* packed in *uma* leaves). Vitamin E (2H-1-Benzopyran-6-ol) was detected only in sample MM_{100}.Sample FMC_{64} had highest concentration in delta tocopherol (3.01 %) and gamma tocopherol (4.47 %). The highest concentration of vitamin in aluminum foil may be as result of aluminum is a good moisture and oxygen barrier therefore retard oxygen in the products (Ima *et al.,* 2016; Kubik and Zeman, 2014). This vitamin related compounds are nutritional and dietary important. It also acts as an antioxidant, preventing lipid peroxidation from spreading (Frei, 2004).







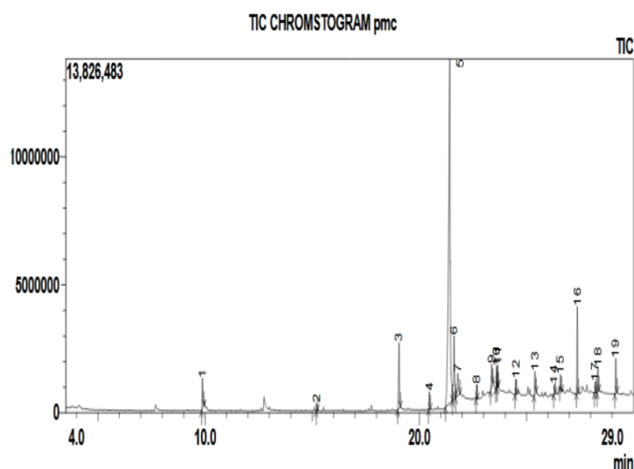


Figure 1. Chromatograms of the volatile compounds of packaged feremtned castor moringa seeds

Aromatic, hydrocarbon, and N- containing compounds were identified which found to be minor groups. Phenol was detected in sample

MMC and FMC_{64}. Adebiyi *et al.* (2021) identified phenol in *dawadawa* bambara groundut while Ojinnaka and Ojimelukwe (2013) found no phenol in fermented castor seed. Phenol is contected to benzene ring of phenol moiety. According to Koleva *et al.* (2018), phenols are a major class of antioxidants with significant biological and free radical scavenging properties.

Other volatile compounds identified were Benzeneethanamine, N-(1-methylethylidene)- and Silane, (cyclohexyloxy)trimethyl- found in presence in samples MC_{100}, FMC, MMC and PMC while Palmitoyl chloride, 1,3-Dipalmitin trimethylsilyl ether and Octadec-9Z-enol trimethylsilyl ether were identified in sample MM_{100}. This result agrees with report of many researchers that diversity of aroma volatile compounds in fermented food condiments such as Burkinaso, *soumbala,* Ghanaian soy-*dawadawa,* Beninese *afitin, iru* and *sonru,* fermented castor oil seed, *dawadawa* bambara groundut (Tanaka *et al*., 1998; Leejeerajumnean *et al.*, 2001, Azokpota *et al*., 2008, Ojinnaka and Ojimelukwe,2013, Adebiyi *et al.,* 2021).

4. Conclusions

Fermented condiment of strong aroma and flavor can produced from castor and moringa seeds based on evaluation of volatile compounds. Packaging material and fermentation time had an influence on volatile compound. Castor-moringa *ogiri* packed in aluminum foil had highest concentration in ester, hydrocarbon, ketones, aldehydes and sterol and vitamins. The volatile compound of various types such as acids, esters, aldehydes, flavour - related compound and constituent could be responsible for organoleptic properties and nutritional quality.

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QUALITY ASSESSMENT OF *AFRAMOMUM DANIELLI* SPICED FRUIT LEATHER FROM AFRICAN STAR APPLE FRUIT

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ABSTRACT

Quality assessment of *Aframomum danielli* spiced fruit leather from African Star Apple was investigated. The sugar sweetened fruit leather samples were spiced with 0.2g to1.0g *A. danielli* powdered extract, while the control had no *A. danielli.* The quality of the processed fruit leather was assessed over a 12-week storage period, with changes noticed in the colour of the control sample. Losses in ascorbic acid, β-carotene and lycopene followed similar trend. The percentage loss in ascorbic acid for the control and the treated samples were 23.78% and 8.18% respectively. For lycopene, the control lost 44.7%, while the treated samples, 39.2% maximum, over same storage period. Sample F had the highest mean score for β-carotene (0.117mg/100g) after 12 weeks, while the control had the least, 0.046mg/100g. Titratable acidity of the samples increased with storage, as the pH values decreased. Acidity reduced the loss rate of ascorbic acid, β-carotene and lycopene contents. Microbial load of the samples reduced with increasing quantity of the spice. Sample spiced with 1.0g *A. danielli* had no growth during the storage period, and retained nutrients better. The control sample had a better rating in all the parameters measured alongside the sample spiced with 0.2g of the spice.

1. Introduction

Nutritious, flavourful and fruit-based fruit leather is a confectionery product usually eaten as a snack or dessert (Blessing *et al*., 2015). Fruit leather could also be defined as products made from fruit purees (sometimes sweetened with sugar or honey), that are spread thinly and dried. The dried sheets are cut into desired strips or rolled into cylindrical shapes or a pureed fruit poured thinly on a flat surface and then dehydrated to yield a dry thin, pliable food product that resembles a sheet of leather (Eze *et al.,* 2014). They can have a shelf life of up to a year when stored at a favourable room temperature. Fruit leathers, loved by both adults and children, can be made at home as they are very easy to make and do not require expensive equipment or difficult techniques (Ragga, 2017). They could be made from various fruits such as apple, mango, apricot, peaches, plums, berries, pears, grapes etc. (Kumar *et al*., 2013), with high fibre fruits being of choice (Adepoju & Adeniji, 2012). African Star Apple fruit is rich in fibre (Adepoju & Adeniji, 2012) and high in pectin (Nwosu *et al*., 2014), making it an excellent choice for fruit leather production.

African star apple (*Chrysophyllum albidum*) is a tropical fruit, belonging to the family *sapotaceae* tagged star apple or gold leaf tree and native across tropical Africa especially in southern Nigeria and other areas of West Africa countries like Uganda, Niger Republic, Cameroon and Cote d'ivore (Adewusi & Bada, 1997). Harvesting is done by shaking of the tree or with forked sticks for very ripe ones, as they are attacked by biological organisms (Adepoju & Adeniji, 2012). They are high in

vitamin C (more than orange or guava), minerals and fibre, and these high nutritional contents made it an excellent nutritional fruit with quality attributes and flavour (Blessing *et al*., 2015; Adisa, 2000). The fruit serves as a cheap source of nutrients, and as a result, increasing production and consumption will significantly improve consumer's nutrition, as they are equally of great economic value to industrial, medicinal and food uses.

The fruit is nearly spherical and slightly pointed at the tip. When ripe, it is orange-red, yellow, or yellow-brown in colour, sometimes with brownish speckles (Adepoju & Adeniji, 2012). Within the fruit is a yellowish pulp surrounding five brown seeds arranged in regular star shape. The problems associated with African Star Apple fruit include that of seasonality and perishability. As a result, the fruit could be processed to other valuable products to meet the demand of consumers and solve seasonal glut problem, rapid spoilage, and unavailability in regions of poor and unfavourable condition for growth, storage and transportation (Nwosu *et al*., 2014).

Antioxidant properties of spices have been recognised years back. It has been reported that spices effectively increase the antioxidant capacity of foods, with effects depending more on food matrices (Ndukwu & Ben-Nwadiba, 2005). The use of local spices to control the activities of micro-organisms in foods has been reported by some authors such as (Adedeji & Ade-Omowaye, 2013). *Aframomum danielli* is one of the various spices that have been used and reported, and common in West Africa. It was reported to impact pungency, spicy aroma and inhibits the growth of micro-organisms like *salmonella enteriditis, pseudomonas fragi and fluorescens, proteus vulgaris, streotococcus pyrogenes, staphylococcus aureus* and some *aspergillus species* (Adegoke & Skura, 1994).

Post-harvest losses, which are common in third world countries, can be reduced through the production of more stable, economic and convenient value added products like fruit leather. Since the fruit is seasonal and unavailable during off season; processing into fruit leather could be one way of curbing or reducing the shortage. Producing fruit leather from the fruit will also help to increase farmers' income by utilization of available indigenous raw material; it will also help in giving added value to indigenous crops, as well as increase the volume and quality of agricultural output. The addition of *A. danielli* is believed to improve the flavour of the fruit leather, as well as inhibit the growth of micro-organism and probably extend the shelf life of the fruit leather.

This research was designed to evaluate the quality attributes and storage stability of *Aframomum danielli* spiced fruit leather made from African Star Apple.

2. Materials and methods

2.1. Raw materials sourcing

Fresh African Star Apple fruits used for the work were sourced from a farm in Ilorin, Kwara State; dried pods of *A. danielli* from a local market, while sucrose used was purchased from a confectionery store in Ilorin town in Kwara State.

2.2. Method and production of fruit leather

The seeds of the *A. danielli* were removed from the pods, cleaned and milled into powder and then sieved with a wired mesh to obtain fine powder. The African Star Apple fruits sourced were washed and then blanched at 70ºC for 5 minutes to ensure easy peeling and also to inactivate enzymes, after which they were peeled and de-seeded. They were cut into smaller sizes and blended at different ratios (Table 1). Properly homogenised samples were then poured onto a drying trays previously oiled with glycerol to prevent sticking, dried in a dehydrator at 60ºC for 8 hours, cooled and packaged in Ziploc bag for further processing.

2.3. Analysis of colour

The colour attributes (Hunter L, a and b values) of the raw fruit and fruit leather samples was obtained with a Rapid Visco Analyser of a Minolta portable chroma-meter. It records L*, a* and b* values and calculate

the brown index. Each sample was measured at four spots using standard L*= 53.44, a*= -24.94, b* = 12.94 values. Whiteness index (WI) was calculated according to Hsu *et al*., (*11*).

$$WI = 100[(100-L^*)^2 + (a^*)^2 + (b^*)2 \;]^{0.5} \quad (1)$$

Table 1. Blending Ratios of the Fruit Leather Samples

Samples	African Star Apple (g)	*A.Danielli* (g)	Sugar (g)
A	100	-	20
B	100	0.2	20
C	100	0.4	20
D	100	0.6	20
E	100	0.8	20
F	100	1.0	20

2.4. Lycopene

Lycopene was extracted using a mixture of hexane, ethanol and acetone in the ratio 2,1,1 (v/v) by the method of Suwanaruang, 2016. The lycopene levels were calculated thus,

Lycopene (mg/kg) = Abs (503nm) x 537 x 8 x 0.55/0.1 x172 OR Abs (503nm) x 137.4. (2)

2.5. β-Carotene estimation

β-carotene was determined by the method of Kumari *et al*., 2011, and estimated thus,

β-carotene (mg/100ml) = 0.216 x A663-0.304 x A505 + 0.452 x A453. (3)

2.6. Ascorbic Acid Determination

This was done by the method of Novozamski *et al*., 1983. It was calculated using calibrated curve of L-ascorbic acid (0.020-0.12mg/ml; Y = 3.4127X-0.0072; R2 = 0.9905). The results were expressed in terms of mg of ascorbic acid/100g of extract.

2.7. Titratable acidity (TTA)

This was determined using the method of AOAC 2000. It was calculated thus,

TTA= N (NaOH) x Titre value x Citric acid value x DF x 100/10 (4)

Where, N= normality of NaOH, which is 0.01; Citric acid value= 0.0064; DF= Dilution factor.

2.8. pH determination

The pH of the samples was determined using the method of AOAC, 2000. The pH meter was calibrated using buffer solutions of standard pH of 7.01 and 4.0.

2.9. Microbiological Analysis

Microbial load of the samples were determined by the method of Adegoke, 2000. One gramme each of the sample was dissolved in 1 ml of 2% sterile sodium citrate solution in order to prepare a suspension. 1 ml of the suspension was then used for the serial dilution. 1 ml of diluted sample was placed in sterile disposable petri-dishes (sterilin) in triplicates. At 44ºC to 50ºC, the media were poured on the samples in the petridishes and allowed to set, inverted and incubated for 48 hours.

2.10. Sensory Evaluation of the Fruit Leathers

The fruit leathers produced were judged for aroma, appearance, texture, taste and overall acceptability by 35 panel of members who are untrained, but randomly selected. A 9-point hedonic scale of the modified method of Stone & Sidel, 1992 was used for scoring with 1 corresponding to disliked extremely, while 9 was liked extremely.

2.11. Statistical analysis

The mean and standard deviation of the data obtained were calculated. The data were

evaluated for significant differences in their means with analysis of variance (ANOVA) ($p<0.05$). Differences between the means were separated using turkey test as packaged by SPSS software (version 20.0).

3. Results and discussions

For the raw African star apple fruit used, the β-carotene content was found to be 0.017mg/100g, which was lower than 340.19µg/100g (0.340mg/100g) reported by Adepoju & Adeniji, 2012. The ascorbic acid content was 10.7mg/100g, which was slightly lower than 12mg/100g reported by Dauda, 2014. The variation noticed in the reported values could be attributed to varietal differences, weather, location, soil composition, method of analysis, pre-treatments etc. (Adepoju & Adeniji, 2012; Ureigho & Ekeke, 2010). Lycopene content was 0.93mg/kg and the pH, 3.7 (similar to 3.5 reported by Dauda, 2014). Low pH value may cause carbonyl group not to dissociate and participate in hydrogen bonds that supports gel structure. The brix was 18, and TTA, 4.56g/L, reflected in the acidity level of the fruit and preservation of the fruit leather (Uzma *et al.*, 2014).

The fruit yield and pulp to peel ratio were 53.57% and 2.61 respectively, and were higher than 47.74% and 2.53 reported by Dauda, 2014. The result, however, revealed that the fruit can be a good raw material for fruit leather production, as suggested by the yield.

3.1. Result of colour determination.

The sample colour after twelve weeks of storage is as shown in Tables 2. The raw African star apple fruit had initial L*, a* and b* values of 38.72, 9.30 and 12.96 respectively, but exhibited significant differences at $p<0.05$ between the treated samples and the control. The colour of the treated samples with higher quantities of the spice were brighter and better retained than those with little or no added *A. danielli*. After twelve weeks, the colour of the samples ranged from (34.05-36.63) for L*, (8.05-10.20) for a* and (3.13-7.46) for b* values. Samples A, B, E and F were not significantly different for L* values which represents whiteness. As for a* that represents redness; no significant difference in the samples with 0.6 to 1.0g of *A. danielli*. From the results however, it could be said that *A. danielli* spice had effect on the colour parameter of the fruit leather samples.

The slight changes in lycopene and β-carotene contents during storage might have contributed to the changes noticed in the colour parameters, with the intense red colour indicative of higher lycopene content.

Lycopene belongs to the group of anti-oxidants called carotenoids, an anti-oxidant responsible for the red pigmentation in fruits such as tomato and water melon (Christian *et al.*, 2008). The lycopene content of the fruit leather samples appeared to increase with up to 2.91 mg/kg compared to the value recorded for the raw fruit used. The recorded increase in lycopene may probably be due to concentration. It could also be due to gradual microbial degradation of the samples. Perhaps, longer drying time, as reported, could have led to the loss of lycopene through isomerization and degradation (Okilya *et al.*, 2010). Drastic reduction in the lycopene content was recorded within the first four weeks, and as storage progresses, reduction rate fell drastically (Table 3). Significant differences were noticed among the samples after week four (Table 3). The initial drastic reduction could be attributed to oxidation or degradation of the lycopene, and as the fruit leather became acidic and total titratable acidity increased, the reduction rate equally reduced, probably due to the impact of *A. danielli.*

Table 4 showed the ascorbic acid content of the samples stored for twelve weeks. The ascorbic acid of the raw fruit used was 10.70 mg/100g, while that of the samples ranged from 10.69 to 10.75 mg/100g. The values, however, reduced by 8% to 24% over the storage period, with the control sample losing about 24% (the highest). The reduction recorded could be attributed to oxidation of the ascorbic acid to dehydro-ascorbic acid, and

down to 2,3-diketogulonic acid and finally to furfural complexes, which was similar to browning reactions or consumption as a reagent in maillard reaction. Loss of ascorbic acid in fruits, juices, fruit leathers etc. had been reported severally. For example, Jain & Nema, 2007 reported 176.27 to 104.87 mg/g loss of ascorbic acid in guava leather; Rao & Roy, 1980 reported losses in mango leather stored for 3 months, while Sreemathi *et al*., 2008 equally reported losses in sapota-papaya fruit leather etc. Ascorbic acid losses in samples treated with *A. danielli* were far less than those of control with significant differences noticed (Table 4).

Table 2. Colour of the Fruit Leather Samples after 12 Weeks.

Sample	L*	A*	B*
A	34.98±0.52a	8.05±0.16c	3.79±1.52d
B	34.81± 0.28a	8.90±1.11b	3.13±0.08e
C	34.34±1.17b	8.44±0.88c	5.10±1.23c
D	34.05±0.93b	9.96±0.78a	5.54±1.32b
E	35.91±0.84a	10.14±0.33a	6.31±1.32b
F	36.63±3.23a	10.20±1.31a	7.46±0.14a

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

Table 3. Lycopene Content (mg/Kg) of Fruit Leather Samples

Samples	Initial	Week 4	Week 8	Week 12
A	3.78±0.01a	2.51±0.00c	2.30±0.01b	2.09±0.01ab
B	3.74±0.06a	2.50±0.04c	2.31±0.00b	2.32±0.11a
C	3.78±0.01a	2.59±0.01c	2.42±1.00a	2.37±0.01a
D	3.82±0.00a	3.00±0.06a	2.44±0.04a	2.32±0.11a
E	3.79±0.02a	3.04±0.01a	2.51±1.01a	2.45±0.03a
F	3.77±0.01a	2.73±0.38b	2.56±0.00a	2.47±0.01a

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

The initial β-carotene contents ranged from 0.174 to 0.180mg/100g with no significant differences, but reduced by 35-56% over twelve weeks. The control sample deteriorated the most, with 56.25% loss, while samples with higher contents of *A. danielli* lost minute quantities of β-carotene (Table 5).

Table 4. Ascorbic Acid Content (mg/100g) of Fruit Leather Samples

Samples	Initial	Week 4	Week 8	Week 12
A	10.72±0.22a	9.95±0.10c	9.81±0.00c	8.17±0.02c
B	10.70±0.10a	10.30±0.00b	10.09±0.00b	9.87±0.01b
C	10.75±0.03a	10.34±0.21b	10.18±1.01b	9.99±0.11a
D	10.73±0.02a	10.43±1.12a	10.30±1.13a	9.98±0.01a
E	10.69±0.00ab	10.51±0.00a	10.32±0.11a	10.14±1.02a
F	10.71±0.21a	10.52±0.22a	10.40±0.00a	10.19±0.22a

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

Table 5. β-Carotene Content (mg/100g) of Fruit Leather Samples

Samples	Initial	Week 4	Week 8	Week 12
A	0.174±0.00a	0.095±0.02c	0.081±0.04c	0.046±0.05b
B	0.176±0.00a	0.110±0.04b	0.089±0.01c	0.077±0.00b
C	0.175±0.01a	.120±0.00ab	0.108±0.00ab	0.097±0.01ab
D	0.180±0.02a	0.130±0.00a	0.123±0.03a	0.099±0.00ab
E	0.180±0.02a	0.140±0.01a	0.125±0.01a	0.110±0.01a
F	0.180±0.02a	0.150±0.02a	0.130±0.12a	0.117±0.02a

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

The titratable acidity (TTA) of a solution is an approximation of the solution's total acidity. The TTA ranged from 1.28-1.52 g\L over the period (Table 6). The increase in the value of TTA was due to the reduction of the pH values or development of acidic substances by the degradation of pectic bodies or hydrolysis of polysaccharides and non-reducing sugars through acid utilization to give hexose sugar (Ugwu & Umeh, 2015). This acid, from report, improves palatability, nutritive value, influence flavour, brightness, colour, stability, consistency and keeping quality of the product (Adisa, 2000; Dauda, 2014; Jain & Nema, 2007).

No growth was recorded until the fourth week (Table 7). Samples with *A. danielli* did not record growth until the eighth week in samples with 0.2 and 0.4 grammes of *A. danielli*. However, samples treated with 0.6 and 0.8g of *A. danielli* did not record growth until week twelve, but sample with 1.0 gramme of *A. danielli* had no growth throughout the period of storage (Table 7). The results confirmed the preservative potentials of *A. danielli* against bacterial growth and agree with the reports of Adedeji & Ade-Omowaye, 2013 and Adegoke & Skura, 1994 on the anti-microbial activities of *A. danielli*.

The inhibitory property of *A. danielli* was revealed though sugars had been reported to inhibit microbial growth through osmotic pressure. A combination of sugar or sweeteners and low pH values could decrease, but may not terminate the growth rate of spoilage yeast. It was seen that the antifungal properties of *A.*

danielli were overwhelming, as it had great effect.

Table 6. Titratable Acidity (g/L) of Fruit Leather Samples

Samples	Initial	Week 4	Week 8	Week 12
A	1.28±0.01a	1.41±0.01a	1.45±0.00ab	1.52±0.01a
B	1.31±0.01a	1.40±0.01a	1.44±0.01ab	1.49±0.00b
C	1.31±0.01a	1.40±0.01a	1.43±0.00ab	1.47±0.01b
D	1.30±0.01a	1.38±0.01b	1.42±0.00ab	1.45±0.00b
E	1.32±0.02a	1.36±0.02b	1.40±0.01ab	1.43±0.01b
F	1.31±0.01a	1.34±0.00b	1.37±0.01ab	1.40±0.00b

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

Table 7. Mould Count (CFU/g) of Fruit Leather Samples

Samples	Initial	Week 4	Week 8	Week 12
A	-	3±1.00a	5±1.00a	8±0.00a
B	-	-	3±0.00b	6±0.00b
C	-	-	1±0.10c	2±0.11c
D	-	-	-	1±1.00d
E	-	-	-	1±1.00d
F	-	-	-	-

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

Table 8. Sensory Attributes of the Fruit Leather Samples

Sample	**AROMA**	**APPEARANCE**	**TASTE**	**TEXTURE**	**OVERALL ACCEPTABILITY**
A	8.00±0.81a	7.25±0.96a	7.75±0.50a	6.50±0.58a	8.50±0.58a
B	6.50±0.58b	7.50±0.51a	7.75±0.50a	6.75±0.50a	8.50±0.58a
C	6.50±0.58b	6.25±0.50b	5.50±1.29b	4.75±0.43c	6.50±0.53b
D	6.00±0.82b	6.25±0.50b	6.25±0.50b	5.25±0.96b	6.75±0.51b
E	6.75±0.50b	6.75±0.47a	7.50±0.58a	5.25±0.96b	8.00±0.81a
F	6.00±0.82b	6.00±0.82b	6.50±1.25b	5.50±0.52b	6.35±0.50b

Values are means of 3 determinations. Means with the same letter down the column are not significantly different ($p<0.05$).
A= 100g of African Star Apple with 20g sugar;
B= 100g of African Star Apple, 20g sugar and *0.2g A. danielli*
C= 100g of African Star Apple fruit, 20g sugar and *0.4g of A. danielli*
D= 100g of African Star Apple fruit, 20g sugar and 0.6g of *A.danielli*
E= 100g of African Star Apple fruit, 20g of sugar and 0.8g of *A.danielli*
F= 100g of African Star Apple fruit, 20g of sugar and 1.0g of *A. danielli*

Table 8 shows the sensory evaluation carried out on the samples, and it shows that samples A, B and E were better accepted in terms of appearance, taste and overall acceptability, while samples A and B were better in texture. On the overall, samples A (control), B and E (0.2 and 0.8 grammes of *A. danielli*) were preferred than others. Although the sample with 1g of A. *danielli* exerted the greatest effect in terms of preservation, but was not the most preferred, most probably due to its high pungency.

4. Conclusions

It could be concluded that value can be added to African star apple fruit by processing them into various other products like fruit leather. The addition of *A. danielli* spice improved flavour and aroma and equally extend shelf life of the samples. Combination of sugar and spice increased sensory and keeping quality of samples, with higher quantities of the spice conferring longer shelf life, though with less acceptability, probably due to their pungency.

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DEVELOPMENT POTENTIAL OF *ERAGROSTIS TEF* AS A FLOUR ALTERNTIVE

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ABSTRACT

Teff (*Eragrostis tef*) *is* known as a health functional food and has been gaining its popularity across the world. Teff is a staple crop and has been cultivated for thousands of years in Ethiopia and Eritrea, currently producing 90% of the world's teff. As the number of consumers are increasing, who look for an alternative to wheat flour especially for people with celiac disease, more countries are increasing teff production. People at all ages can benefit from consuming teff as it contains dietary fiber, iron, potassium, and *etc*. It is also helpful for people with obesity and diabetes due to its low glycemic index. Therefore, modern society pursues health-oriented thinking, and teff is expected to be in the spotlight as a good food for all ages to consume. However, information on the nutritional benefits of teff and research on food development using teff are insufficient. This review paper is a literature review, and the method is a narrative review. In addition, it is suggested that the frequency of Teff intake can be increased by presenting the introduction of foods with high nutritional value using Teff by providing basic data on nutrition and efficacy through a close investigation of Teff.

1. Introduction

Teff (*Eragrostis tef*) is an annual plant in the rice family (D'Andrea, 2008) and is a kind of grain. Chloridoideae and teff's Eragrostoidae are synonymous and similar (Costanza *et al*., 1999). The word teff is derived from the Amharic word teffa, which means "lost", is elliptical and has a particle size of 1 mm or less within 2 mm (Belay *et al*., 2009; Bultosa, 2007). Teff is a tropical cereal originating in the highlands of northern Ethiopia (Tadesse, 1993). Since the beginning of Ethiopia, it has been cultivated for thousands of years. In Ethiopia, Eritrea, South Africa, the United States, Australia, India, Canada, Switzerland, the Netherlands, Europe and North America, 200 million people around the world are cultivated and produced (Costanza *et al*., 1979; Zhu, 2018; Assefa *et al*., 2015; Shumoy and Raes, 2016). There are white, red, and brown types of teff, and foods made with white teff are preferred, but health-conscious people prefer brown teff and the consumption is increasing (Gebremariam *et al*., 2012; Cherie *et al*., 2018). Especially in Ethiopia, more than 30 million people consume teff every day (Nascimento *et al*., 2018), and in Europe and North America, it is steadily consumed (Shumoy and Raes, 2016). In addition, teff is an important nutrient source for people as a food with the potential to grow anywhere in the world and a good source of thiamine. Gluten-free foods include rice, soybeans, corn, millet, buckwheat, tapioca, amaranth and cassava (Awulachew, 2020; Niro *et al*., 2019). Teff is an important nutrient source for people. Teff is a health food containing protein, fiber, crude fiber, polyphenols, and unsaturated fatty acids. It has higher amino acid content than other grains such

as wheat, rice, and corn (Spaenij-Dekking *et al.*, 2005; Gebru *et al.*, 2019; Hager *et al.*, 2012; Abebe *et al.*, 2015). It is richer in dietary fiber, minerals, magnesium iron, folic acid, zinc, manganese, phosphorus, calcium, and copper than wheat, barley, and sorghum (Zhu, 2018; Mengesha, 1966; Abebe *et al.*, 2007; Post *et al.*, 2012; Campo *et al.*, 2016). It also contains 8 essential amino acids (leucine, lysine, methionine, valine, isoleucine, threonine, tryptophan, phenylalanine) and (Hager *et al.*, 2012) tannin and other polyphenols, which are derivatives of flavonoids, proven to be an important source of physiological activity (Shumoy and Raes, 2016; Urga *et al.*, 1997; Ravisankar *et al.*, 2018). According to a recent study, it is said that K, P, Ca, Mg, Na, B, AI, and Fe are the most abundant, and Er, Eu, and Sb are also detected in trace amounts (Dame, 2020). Because of its low GI index, Teff can be preferred as an diabetic diet as a non-pharmacological treatment for patients with diabetes to manage their blood sugar level (Gebru *et al.*, 2020). In particular, teff contains a significant amount of phenolic, which is beneficial for cardiovascular disease and cancer prevention due to its antioxidant activity (Dykes and Rooney, 2007; Awika and Ronney, 2004), and can reduce the digestibility of food by inhibiting digestive enzymes while acting as a natural antioxidant (Maheshu *et al.*, 2013; Qiang *et al.*, 2006). Iron can be taken as a supplement or fortifying agent, but certain side effects can cause nausea, constipation, and diarrhea, so it is safer to consume it as a natural grain (Kassebaum *et al.*, 2014; McLean *et al.*, 2009). In progress, research is being conducted on various foods such as making food by using teff with fenugreek, oats, okara, and rice, or adding kimchi and lupine to Injera dough. In addition, teff can be considered to be excellent as a feed source for animals due to its good health function and quality (Legessea *et al.*, 2020; Leykun *et al.*, 2020; Minarovičová *et al.*, 2019; Gebru and Sbhatu, 2020, Yegrem *et al.*, 2021; Kakabouki *et al.*, 2020; Hawa *et al.*, 2018).

As the world enters an aging society, the health care industry is now thriving, and the importance of healthy food has become the center of interest among people today. Therefore, in this study, teff, which is excellent in terms of its physiological benefits to individual's health, is considered an alternative to wheat flour, and it is believed that it can successfully meet consumers' needs. This study will provide basic data of the nutrition and efficacy of teff, and consequently discuss the importance of teff consumption for individuals.

2. Ethiopia with teff as a staple

Anemia refers to a condition in which the ability to transport oxygen in the body is reduced due to a decrease in the number of red blood cells in the blood and an insufficient content of hemoglobin. A number of people in developing countries are diagnosed with anemia, accounting for 25% of the world's population (McLean *et al.*, 2009). Iron deficiency is a common problem not only in low-income countries, but also in developed countries, and it can cause developmental problems for babies and children. Thus, it is especially important for growing children and premenopausal women to consume iron.

One study found that the iron content of wh eat bread containing 30% teff increased twice ir on intake. As a result, the basic iron content that pregnant women need to consume can be maintained with bread containing 30% teff (Kassebaum *et al.*, 2014). In another study found that Ethiopian sportsmen around the world are known to have good physical strength. Ethiopia produced a number of marathon winners, because their traditional food called injera is rich in iron, which help to deliver more oxygen by increasing hemoglobin levels (Andrews *et al.*, 1990). Teff powder is the main ingredient of injera and is fermented with water to make it round and thin to form a pancake-like shape (Fischer et al., 2014). It is eaten a lot as a staple food and served with most meals in Ethiopia (Urga *et al.*, 1997).

3. Nutritional problems of gluten

Teff was previously a kind of grain that was not widely known, but as its nutrition and efficacy are gradually known, it is in the limelight by consumers and is establishing itself as a superfood (Spaenij-Dekking *et al*., 2005, Zhang *et al*., 2016). Teff is a healthy alternative for people with gluten intolerance. Gluten, a protein present in wheat, barley, and rye, is a problem of permanent intolerance causing inflammation to the microvilli in the lining of the small intestine, mucous membranes and gastrointestinal tract, which results in limiting the absorption of nutrients. Accordingly, the lack of nutrients affects skin diseases, anemia, osteoporosis, and growth inhibition, and is linked to diseases such as infertility and small intestine cancer. This is called celiac disease and is a disease of the digestive system and allergies (Green and Cellier, 2007; Green *et al*., 2005; Gil-Humanes *et al*., 2014; Laureati *et al*., 2012; Susanna and Prabhasankar, 2013; Heo *et al*., 2013; Lebwohl *et al*., 2018). About 1% of the world's population, including both young children and adults, complained of difficulty with tolerating diet with gluten (Gujral *et al*., 2012; Aguilar *et al*., 2016; Blanco *et al*., 2011; Bourekoua *et al*., 2018; Turkut *et al*., 2016) and only about 1-2% of the population in Europe was found to have gluten intolerance (Allen and Orfila, 2018). People with celiac disease must adhere to a strict diet with gluten-free products and flour substitutes for effective treatment. In particular, the Western-style diet has a lot of flour-based food and bread is a staple food, so it is difficult to choose food because there are many restrictions. In addition, People with celiac disease are deficient in vitamin B12, iron, zinc, copper, and minerals, so food choices are important, and the choices are limited (Bascuñan *et al*., 2017; Barera *et al*., 2004). In order to solve this problem, it is considered that more attention and research on food such as teff and food development research using it are needed.

4. Gluten-free food research using teff

In the study for patients with celiac disease, it was found that a diet using Teff was effective. Researches using teff are being conducted, including bread, cookies, soup, raw noodles, pasta, beer etc (Bultosa, 2007; Gebremariam *et al*., 2012; Zhang *et al*., 2016; Green *et al*., 2005, Hopman *et al*., 2008; Ronda *et al*., 2015; Hager *et al*., 2012).

A study by Blanco *et al*. (2011) found that gluten-free bread with teff added was increased in carbohydrates, fiber, essential amino acids, iron, calcium, copper and zinc. In another study, teff is considered to have a slow aging rate, and in Korea, where rice is consumed as a staple food, it is considered to be good for use in porridge, rice cake, and rice (Campo *et al*., 2016; Bultosa *et al*., 2002; Joung *et al*., 2017). Therefore, since there are various foods made using rice, it is considered good to make *Gangjeong* and *Dasik*, including rice cake, a traditional Korean food. In addition, it is considered to be good to add to side dishes or use as a garnish instead of sesame seeds. As the number of consumers seeking health orientation increases every year, the need for research on the development of food containing teff, a flour substitute, becomes important, and it is judged as a material that has the potential to help many people. In particular, gluten intolerance and health-conscious people, including celiac disease patients who have to consume gluten-free food and have to follow a diet, are encouraged to actively use Teff for their health. Consumption of health foods, superfoods, wellness foods, and gluten-free foods is rapidly increasing because people's interest and desire for a healthy life are increasing day by day as the lifespan of humans is prolonged. Accordingly, it is necessary to study the development of products with improved functionality and taste, and it is considered that cooks, nutritionists, and researchers must continuously conduct research to contribute to a healthy human life.

In 1941, in a report by pediatrician and scientist Willem Karel Dicke, the onset of a gluten-free diet became increasingly known to consumers over time (van Berge-Henegouwen and Mulder, 1993). In the U.S., sales of gluten-free products rose by 6% in 2015-2016, and the global market is projected to increase

significantly from \$5.6 billion to \$8.3 billion in 2020-2025 (Statista). As a result of these statistics, the number of consumers choosing gluten-free foods is increasing, and it can be seen that not only patients with celiac disease but also those with poor digestibility for flour foods are increasing. Among the gluten-free products, it was found that the demand for bread was higher than that of other products (Encina-Zelada *et al.*, 2018), and the researchers studied gluten-free breads and muffins using various grains including teff, such as buckwheat, sorghum, rice, and amaranth. Bread research is more active than other products, and studies on gluten-free foods using amaranth, quinoa, buckwheat, potato starch, sorghum, rice corn flour, green corn, green banana flour, legumes, and lupines are in progress. It was thought that the improved gluten-free foods and flour-based foods on the market should also be continuously developed and researched (Palavecino *et al.*, 2014; de la Barca *et al.*, 2010; Ferreira *et al.*, 2016; Camelo-Méndez *et al.*, 2018; Foschia *et al.*, 2017; Zandonadi *et al.*, 2012; Gambuś *et al.*, 2009; Altındağ *et al.*, 2015; Rodrigues Ferreira *et al.*, 2009; Foschia *et al.*, 2017; Alvarez *et al.*, 2017; Lamacchia *et al.*, 2014; Collar *et al.*, 2014).

5. Problems with side effects and complications from celiac disease

Celiac disease is also said to be a genetic and non-hereditary disease, but it is closely related to wheat. In Korea, rice is a staple food, but the intake of wheat flour is increasing every year due to the prevalence of Western-style diet, and as a result, the possibility of celiac disease, gluten allergy, and skin diseases are increasing (Ministry of Food and Drug Safety 2011). Celiac disease has symptoms such as diarrhea, recurrent abdominal pain, fatty stool, chronic fatigue, weight loss and nutrient absorption disorders, gastrointestinal symptoms, aphthous stomatitis, decreased bone density, and short stature (Kelly *et al.*, 2015; Reilly *et al* ., 2011). Complications of celiac disease include hyposplenic syndrome, RCD, intestinal lymphoma, small intestine adenocarcinoma, and jejunoileitis. In addition, celiac disease is associated with intestinal-brain axis and inflammatory states and migraines, and can cause a variety of neurological conditions; seizures including epilepsy, cerebellar ataxia and chronic neuropathy (peripheral neuropathy), myoclonic ataxia, progressive leukemia and dementia. With this complication, neurological symptoms can occur both in children and adults with celiac disease (Cooke and Smith, 1966, Hadjivassiliou *et al.*, 2002; Zelnik *et al.*, 2004; Arzani *et al.*, 2020). Diseases related to celiac disease include autoimmune type 1 diabetes, Hashimoto's thyroiditis, Graves' disease, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, herpes dermatitis, vitiligo, Addison's disease, alopecia, psoriasis, IgA deficiency. And Autoimmune atrophic gastritis, autoimmune hemolytic anemia, Sjogren's syndrome, scleroderma, systemic lupus erythematosus, polymyositis, rheumatoid arthritis, myasthenia gravis, and IgA nephropathy (Burger's disease). Idiopathies include dilated cardiomyopathy, epilepsy with or without laryngeal calcification, cerebellar ataxia, peripheral neurosis, multiple myoclonic seizures, multiple sclerosis, cerebral atrophy, chronic inflammatory bowel disease, sarcoidosis, and atopy. The chromosomes include Down syndrome, Turner syndrome, and Walliam syndrome. If you continue to have complain of symptoms of abdominal pain, diarrhea, intestinal obstruction, fever, weight loss, and severe asthenia even after eating a gluten-free diet, you should be suspected of complications. After age 50, the late diagnosis of celiac disease and failure to follow a strict gluten-free diet will result in mortality. It can be high (Caio *et al.*, 2019; Al-Toma *et al.*, 2006; Rubio-Tapia *et al.*, 2016). A recent study found a high prevalence of celiac disease in patients with Wilson's disease (Drastich *et al.*, 2012), and it was also found to be associated with type 1 diabetes (Marchese *et al.*, 2013). In addition, studies have shown that the more gluten-containing flour foods consumed during the first five years of life, the higher the risk of immune and celiac disease in children with genetic celiac

disease (Aronsson *et al.*, 2019). When celiac disease cannot absorb various nutrients including calcium, it is directly related to growth problems in children and adolescents, so care should be taken. In the elderly, it can be said to be a serious problem due to a decrease in bone density and an increase in the risk of fracture (Mautalen *et al.*, 1997; Motta *et al.*, 2009; Vasquez *et al.*, 2000). Therefore, celiac disease is a problem of malabsorption, which is a major problem of nutritional deficiency and calcium deficiency. Since this factor affects later bone changes, the association between bone mineral density, osteoporosis and fracture should always be considered when diagnosing celiac disease. In celiac disease, calcium intake is a very important nutrient for treatment, so it is recommended to consume it on a gluten-free diet that is high in calcium, phosphorus, and magnesium (Sdepanian *et al.*, 2003). A gluten-free diet could have clinical benefits for women with autoimmune thyroid disease (Krysiak *et al.*, 2018; Lundin and Wijmenga, 2015), and pain was improved after a gluten-free diet for 6 months and 12 months in patients with endometriosis and chronic pelvic pain (Marziali *et al.*, 2012; Marziali and Capozzolo; 2015). It is emphasized that there is a need for continuous research on gluten-free foods, and efforts to improve foods with enhanced nutrients and quality through scrutiny of existing gluten-free products (Kulai and Rashid, 2014; Berti *et al.*, 2004).

6. Health benefits of teff as a flour alternative

The biggest advantage of teff is that it is gluten-free, so it can be used as a flour substitute for people especially for those who suffering from celiac disease, and its high iron content is a good ingredient for children and pregnant women (Hopman *et al.*, 2008; Cerami, 2017).

The number of diabetes patients around the world has been significantly increasing every year (Danaei *et al.*, 2011). As a result of a survey of people aged 20 to 79 in 2021, the global prevalence of diabetes was 10.5% (536.6 million), and the global diabetes-related medical expenditure was $966 billion in 2021. It is expected to increase by 12.2% (783.2 million people) in 2045, and is estimated to be $1.54 trillion in 2045 (Sun *et al.*, 2022). It is also estimated that 1 in 9 people between the ages of 20 and 79 with diabetes will die, with those under 60 having the highest number of deaths, with an estimated 4.2 million deaths (Saeedi *et al.*, 2020).

Diabetes is a metabolic disorder in which blood sugar levels increase whereas the body does not produce enough insulin to use glucose from blood for energy. Causes include high fat diet leading to obesity, sedentary lifestyle, and decreased exercise capacity. In addition, it was found that obesity and diabetes incidence were increasing due to excessive consumption of fat and sugar in diet and lifestyle-related diseases (Guariguata *et al.*, 2014). Therefore, for diabetics, the amount and quality of carbohydrates, the level of glucose, and the response of cells to insulin are the main points, which is a part that requires a lot of attention when ingesting carbohydrates (Wolever, 2000).

One study found that low-glycemic foods could reduce the risk of type 2 diabetes, and that, unlike other grains, teff with a low GI index could play a major role in diabetes prevention (Augustin *et al.*, 2015; Wolter *et al.*, 2013). Compared to wheat, it has a lower glycemic index and higher fiber content, which helps control blood sugar, and its relatively high dietary fiber can lower fasting blood sugar levels (Post *et al.*, 2012). Taking teff lowers Cholesterol levels and blood pressure, improving blood sugar and insulin sensitivity in diabetics, and can help with gastroesophageal reflux disease, duodenal ulcer diverticulitis, constipation and several gastrointestinal disorders, including hemorrhoids (Wolter *et al.*, 2013; Anderson *et al.*, 2009) And if the person is overweight and obese, supplementing fiber has a great effect on weight loss, so it can be said that it is a grain that has the potential as an excellent food for people who are on a diet.

7. Conclusions

It should be understood that all populations and age groups in the world should be aware of

the risk of side effects and diseases that come from excessive consumption of flour. Therefore, in order to promote teff, it is necessary to develop various foods using teff and to pay attention to marketing to inform various media. It is expected that more research on teff will come out, and it is expected that gluten-free food will gradually develop.

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ASSESSMENT OF BUTTON AND OYSTER MUSHROOM NUTRITIONAL QUALITY USING VARIOUS DRYING METHODS

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ABSTRACT

Edible mushrooms are in high demand due to their flavor and nutritional benefits. Mushrooms are a rich source of carbohydrates, with high fibre content and modest protein content containing the majority of the amino acids, and vitamins. This study was conducted to evaluate the effects of different pretreatment drying methods on the nutritional quality of dried mushrooms (Button and Oyster mushroom) and also to estimate the Vitamin D_2 content. The experiment was carried out based on the three pretreatment techniques: SD, HD and UV + HD. Significant differences in proximate composition were observed between the fresh and dried mushroom samples. The average mean value of crude protein, crude fat, crude fiber, ash, and carbohydrates of dried mushroom samples were 33.9, 19, 5.7, 10.8 and 98.1% respectively and found to be statistically significant too. Sundried oyster mushrooms had their vitamin D_2 level increased by two folds in comparison with sundried button mushrooms. Finally, the intake of these mushrooms should be encouraged as a complement to impoverished people's main foods. As a result, addressing nutritional issues in children, pregnant women, and immune-compromised individuals is a necessity.

1. Introduction

Vitamin D, often known as the "Sun Vitamin," is a fat-soluble vitamin that aids in bone metabolism and has immune-modulating effects. Ergocalciferol (D_2) and Cholecalciferol (D_3) are the two major forms of Vitamin D required for humans. The current Adequate Intake (AI) for Vitamin D for most adults is 800 IU/day whereas for infants is 600 IU/day (Phillips et al, 2013). Vitamin D is found naturally in a few foods, and it may also be synthesized endogenously when UV rays from the sun impact the skin and cause vitamin D production. Vitamin D insufficiency, on the other hand, is the most under diagnosed and undertreated nutritional condition around the world. Especially in tropical countries like India, Vitamin D deficiency prevails in epidemic proportions of 70% to 100% in the general population (Sanwalka et al, 2016). Vitamin D deficiency is believed to have a predominant role in India's high rates of infections like arthritis, rickets, osteoporosis, cardiovascular disease, diabetes, cancer, and TB (Tuberculosis). (Ritu et al, 2014).In order to overcome Vitamin D deficiency in humans, Vitamin D has to be supplemented in diet. Vegetable sources like yeast, mushroom, cheese are rich in Precursor of Vitamin D_2 whereas animal sources such as fish, egg, beef liver, seafoods etc were precursors of vitamin D_3 (O'Mahony et al, 2011). Ergosterols, a precursor of vitamin D_2, are found in mushrooms and other plant foods. The lack of natural plant-based vitamin D_3 sources has

necessitated the development of alternatives to increase vitamin D_3 consumption. Vitamin D_3 is added to most meals, while vitamin D_2 (ergocalciferol) is commonly found in nutritional supplements and vegetarian goods such as soy milk (Phillips et al, 2013). Hence, supplementation of Vitamin D_2 in foods gains attention.

Commercially, Vitamin D_2 is made by exposing yeast to ultraviolet light (UV), which transforms the fungal sterol ergosterol to ergocalciferol. Ergosterol is found in all mushrooms, although the amount of vitamin D_2 varies greatly. Among them, oyster mushrooms reported to have higher Ergosterol content than others (Philips et al, 2011). Higher Ergosterol content and larger surface area makes mushroom more preferable for Vitamin D fortification. The oldest method used for food preservation is the drying method. The drying process reduces the moisture content of food, which inhibits the microbial development. The drying process is a good way to keep solid meals safe for a long period on a large scale (Muhamad et al, 2019). Pre-treatments are used to speed up the drying process, improve food quality, and increase food safety (Longvah et al, 1998). This drying operation is carried out for the following reasons: to extend the shelf life, to improve the quality and to reduce the volume which results in reduced packaging and transportation cost (Vijayan et al, 2017).

The objective of this work is to investigate the effect of direct sunlight, UV treatment and Hot air UV treatment on the macronutrient contents of *Pleurotus ostreatus* and *Agaricus bisporus* and also to examine the level of Vitamin D_2 content.

2. Materials and methods

2.1. Materials

2.1.1. Collection of Fresh Mushroom

All mushrooms were selected according to the size of the cap (d = 50 mm) and it was cleaned thoroughly to remove adhering matter. After washing with tap water, it is dried so that the moisture absorbed by the mushrooms does not influence the analysis (Balan, V et al., 2021). Then, the samples were stored at 4 °C in a refrigerator and processed within 24 h after harvest.

2.2. Methods

2.2.1. Pre-treatment Processes

Pre-treatment is required for foods to be treated before the canning process; pre-treatment varies per food. The pre-treatment process aids in the preservation of color, nutrition, taste, and overall quality.

2.2.2. Drying Process

The whole mushroom was washed thoroughly under running tap water to eliminate any adherent extraneous debris. The brown and damaged portions of the mushroom were removed. Washed and cleaned mushrooms were taken for the experimental studies. The mushroom was dried in three various ways: sun-drying (SD), Hot air oven drying (HD) and Ultraviolet radiation + Hot air oven drying (UV + HD).

2.2.3. Sun Drying (SD)

Around 200 g of button and oyster mushrooms were taken for sun drying after washing. Then the button and oyster mushrooms were chopped into small pieces. After chopping, the mushrooms were placed in separate plastic trays and dried in the open sun rays from morning to evening at an ambient temperature of 32±5 ℃ for 2 days. The mushrooms were ground dried in a mixer and made as a fine powder. Then, the finely powdered mushroom was stored in a separate plastic container at room temperature (Maray et al., 2018).

2.2.4. Hot Air Oven Drying (HD)

Hot air oven drying was done by taking 200 g of a button and oyster mushrooms were chopped into small pieces. After chopping, it was placed in petri dishes and kept in the oven. The temperature was kept constant at 62 ℃ respectively for 2 days. Once the mushroom turned into a brown color due to the removal of moisture content, it was made as a fine powder and used for analysis (Maray et al., 2018).

2.2.5. Ultraviolet + Hot Air Oven Drying (UV+HD)

The final pre-treatment process was performed using ultraviolet radiation followed by hot air oven exposure of chopped mushrooms. Initially, the laminar hood was sterilized using ethanol, and then chopped mushroom was made to treat with UV rays for 5 mins with intensity 0.6–1 W/m^2. Being treated with UV rays, button and oyster mushroom was reduced in a hot air oven by keeping the temperature at 62 °C for 2 days. Then the dried mushroom was grinded and stored at room temperature (Francesca Gallotti et al., 2020).

2.2.6. Determination of Carbohydrate

The mushroom sample of about 100mg was taken and it was hydrolyzed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl. From the hydrolyzed sample, 0.5 ml was taken for test analysis. Consequently, the standard solution was prepared by making the different concentrations as a working standard. Each test tube was made up to the volume of 1ml in all test tubes by adding distilled water. Then, 4 ml of anthrone reagent was added. Thereafter, it was heated for about 8 mins in a boiling water bath till a bluish green color appears. Then cooled rapidly and the absorbance was taken at 630nm (Sadasivam 1996).

2.2.7. Determination of Crude Protein

Different dilutions of BSA solutions were prepared by mixing protein standard solution and water in a test tube. The unknown samples were taken in another test tube. All the test tubes were made up to the volume of 3 ml by using distilled water. The alkaline copper reagent of 4.5 ml was added to each test tube and incubated for 10 mins at room temperature. After that, 0.5 ml of folin – ciocalteu reagent was added to all test tubes and heated till the formation of bluish - green color. Then, the absorbance was measured at 630 nm (Nielson 2017).

2.2.8. Determination of Lipid content

The sample (80 g) was taken in a round bottomed flask; it was extracted using 200 ml of acetone of boiling point (56 °C). The extraction process continues for about 2 hours till most of the solvents are distilled from the flask into the extractor. The amount of lipid was calculated from the residue remaining after evaporation of solvents (Min et al., 2010).

$$\%\text{Acetone extract} = \frac{(\text{Weight of flask} + \text{Weight of extract}) - (\text{Weight. of flask}) \times 100.}{\text{Weight of sample}} \quad (1)$$

2.2.9. Determination of Ash

The sample (2 g) was ignited at 400 °C for 4 hours or until whitish-grey ash was formed in a muffle furnace. The crucible was then weighed after being inserted in the desiccator (Ismail et al., 2017).

$$\%\text{Ash} = \frac{(\text{Weight of crucible} + \text{ash}) - (\text{Weight of crucible})}{\text{Weight of sample}} \quad (2)$$

2.2.10. Determination of Moisture

The sample (2 g) was taken in a petridish was dried in an oven to remove moisture content for 60 °C for about 36 hours. Then, it was cooled using a desiccator and weighed. The drying and weighing process continues until a constant value is achieved (Nielsen 2010).

$$\%\text{ Moisture} = \frac{(\text{Weight of sample} + \text{dish before drying}) - (\text{Weight of sample} + \text{dish after drying}) \times 100}{\text{Weight of sample taken}} \quad (3)$$

2.2.11. Determination of Fiber

The sample (5 g) was taken in a beaker. In which, 50 ml of sulphuric acid (H_2SO_4) and potassium hydroxide (KOH) solution was added and boiled for 30 mins. After that, the samples were washed using distilled water after that sieve. Then, 20ml acetone was added and it was left undisturbed for 20 mins. And then, the samples were filtered and dried for 30 mins in a hot air oven (Gul et al., 2009).

$$\%\text{ Fiber} = \frac{(\text{Weight of crucible with dry residue}) - (\text{Weight of crucible with ash}) \times 100}{\text{Weight of sample taken}} \quad (4)$$

2.2.12. Estimation of Energy value

Food's energy content is an important factor to consider. The calorie content per weight of food is referred to as the energy density of the food. Carbohydrates are the most essential of the three primary nutrients needed for energy. The body can use protein and fats for energy when carbohydrate has been depleted. Our body breaks down nutrients into smaller components and absorbs them to use as energy. The energy available in a mushroom powder was calculated by multiplying the number of grams of carbohydrate and protein was multiplied by 4 and fat was multiplied by 9 respectively. Then add the results together (Schakel et al., 1997).

Energy (in Kcal) = 4× (Carbohydrates and Proteins in grams) + 9 × (Fat in grams)

2.2.13. Quantification of Vitamin D_2

The Vitamin D_2 content was analyzed by high performance liquid chromatography (HPLC) using the methodology previously reported by (Philips et al, 2011). Briefly, mushroom samples with [2 h]-vitamin D_2 added as an internal standard which has been saponified in methanolic KOH and it was purified by solid-phase extraction. HPLC was performed to isolate the vitamin D fraction, and vitamin D_2 was then separated by reverse-phase HPLC with UV detection at 265 nm and quantified based on the ratio of sample peak area to [2 H]-vitamin D_2 ratio relative to an external standard curve from analysis of vitamin D_2 standards spiked with an equivalent amount of internal standard.

2.2.14. Statistical Analysis

The statistical analysis was done using one-way ANOVA. The P value and F value were calculated to measure the significance of the results obtained.

3.Results and discussions

3.1. Effect of Pre-treatments methods for Mushroom

Three different drying methods were performed in this study: Sun drying at ambient temperature 32.5±5 °C, Hot air oven drying at 62 °C and finally UV+ HD drying at 62 °C were carried out on button and oyster mushroom (Figure 1 to 6).

Figure 1. Pre-treatment of Button mushroom (BM) by Sundrying

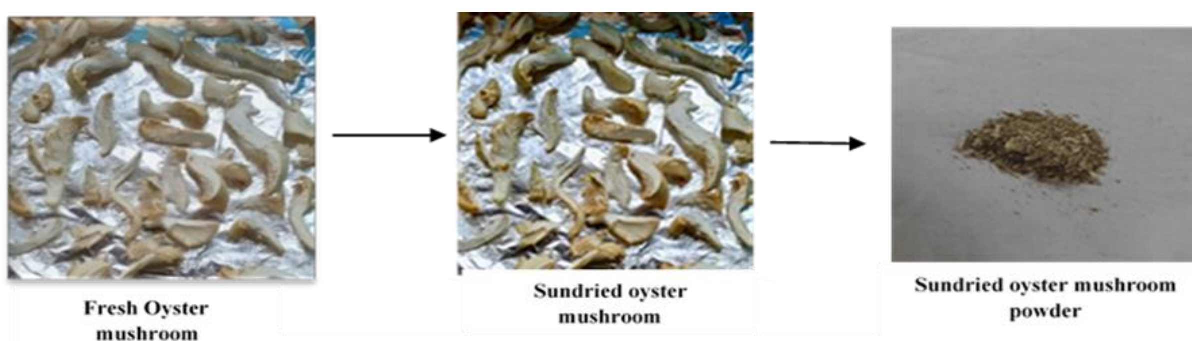

Figure 2. Pre-treatment of Oyster mushroom (OM) by Sundrying

Figure 3. Hot air oven drying (HD) treatment for Button mushroom (BM)

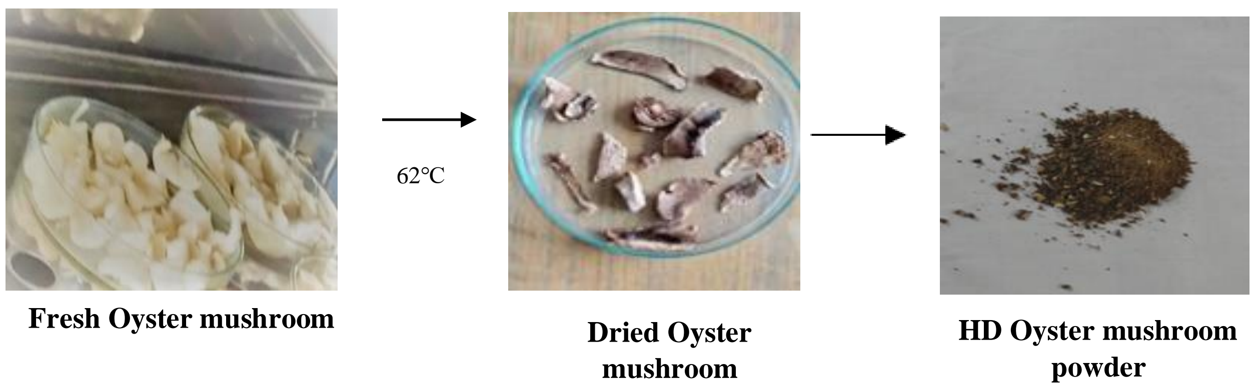

Figure 4. Hot air oven drying (HD) treatment for Oyster mushroom (OM)

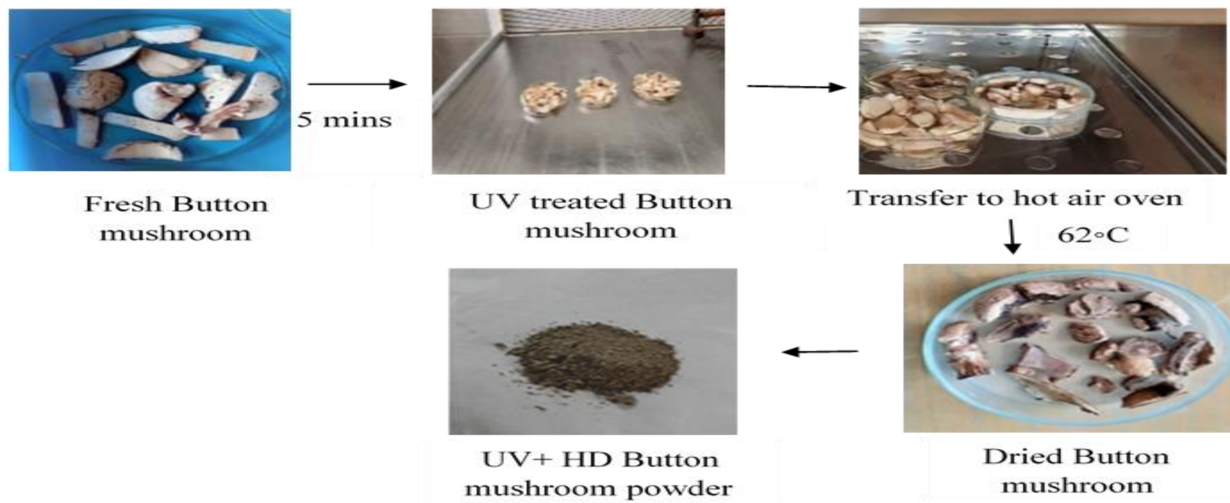

Figure 5. Pre-treated Button mushroom (BM) under UV followed by Hot air oven drying (HD) treatment

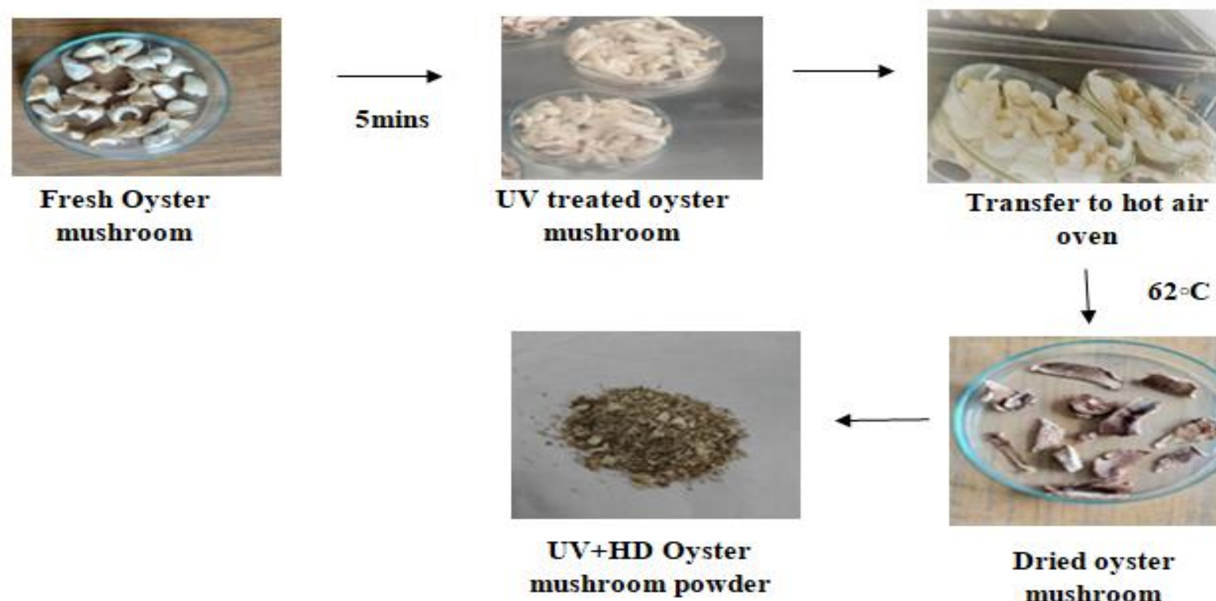

Figure 6. Pre-treated Oyster mushroom (OM) under UV followed by Hot air oven (HD) treatment

3.2. Proximate composition of dried button and oyster mushroom

Proximate analysis was carried out on two edible mushroom species: *Pleurotus ostreatus* and *Agaricus bisporus* for both pretreated and fresh mushrooms. Proximate analysis of the mushrooms including moisture, fat, fiber, ash, protein and carbohydrate were determined. The fresh mushroom proximate analysis values are represented in Table 1.

Table 1. Composition of fresh mushroom

Analysis	Fresh BM*	Fresh OM*
Carbohydrate	46.17	48.16
Protein	33.48	28.85
Fat	3.36	2.47
Ash	5.7	9.76
Fiber	20.9	12.87
Moisture	92.45	88.75

*BM – Button mushroom, OM – Oyster Mushroom

3.2.1. Carbohydrate content

Carbohydrate plays a significant role in food since it provides energy for the human being. Polysaccharide is the major carbohydrate present in mushrooms and also it's determined to have immunomodulation and antitumor properties (Chang et al, 1982). The present study revealed that *Agaricus bisporus* (98.1g) determined to have significantly higher carbohydrate content compared to *Pleurotus ostreatus* (89.9g) under sun drying treatment whereas the lowest value (16.2g) was recorded for the samples dried in HD button mushroom (16.2g) and UV + HD oyster mushroom (15.4g). The values were represented in figure 7. The significant amount of carbohydrate is found in the dried mushroom which is in correlation with the values reported by (Chandravadana et al, 2005).Hence the sun dried mushroom serves as good dietary fiber with calorific value. Because of the concentration of nutrients, the carbohydrate percentage increases as the moisture content decreases during drying. The increase in protein in dried samples is caused by the dehydration of the water that exists between proteins. Carbohydrates, like the majority of heat-sensitive nutrients in food, tend to lose their function. This explains why the total carbohydrate content of vacuum and oven-dried samples was lower than that of free samples (Sim et al., 2017).

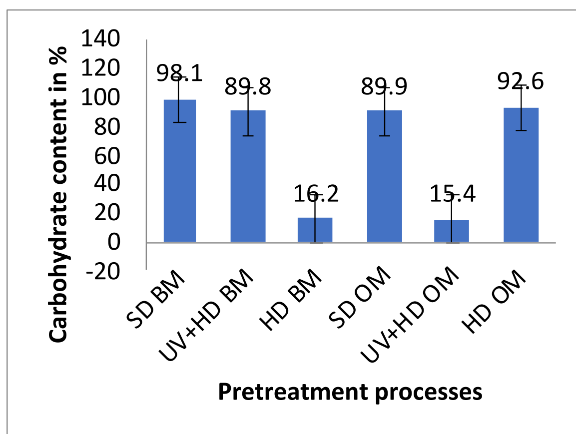

Figure 7. Analysis of Carbohydrate content in dried mushroom

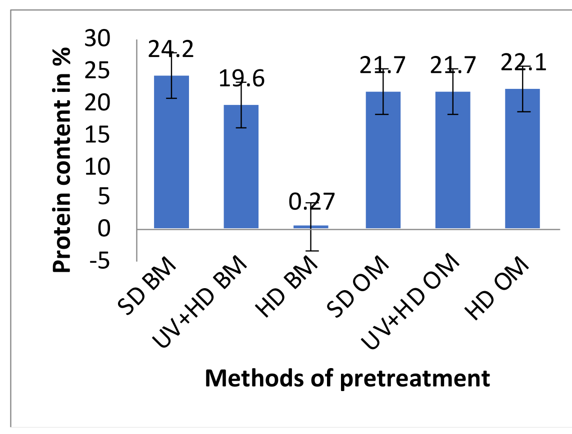

Figure 8. Analysis of protein content in dried mushroom

3.2.2. Protein Content

Protein accounts for more than half of the total nitrogen in mushrooms, and its quantity varies depending on the species, substrate composition, and size of pileus and the harvest season. Mushrooms have 19 to 35 percent protein by dry weight, compared to 7.2 percent for rice, 13.2 percent for wheat, and 25.2 percent for milk. In this study, the protein content ranged from 19.6 g to 33.9 g. Protein content varies widely across mushroom species, ranging from 11 to 42 g/100 g dry fruit bodies.

Except for hot air oven drying of the button mushroom, other pretreated processes identified to show similar protein content (Figure 8). According to a prior study, the water solubility index rises as particle size decreases, owing to greater surface area and enhanced protein solubilization. This means that superfine mushroom powder has a higher concentration of nutrients soluble in water than coarser powders, making it easier to utilize as a food ingredient in recipes (Wu et al., 2012). Mushrooms have a high protein content that rivals that of animal protein sources. The protein content of mushroom mycelia was higher than that of the available fruiting bodies. Nonetheless, the protein content of mushrooms can be influenced by a variety of factors such as their developmental stage, mushroom type, cultivation location, and post-harvest treatments (Sim et al., 2017).

3.2.3. Fat content

Generally, mushrooms tend to have lower fat content but they are found to have polyunsaturated fatty acids which are essential for human health and also it contributes to the reduction of serum cholesterol level. Especially, the higher content of linoleic acid is one of the reasons for considering mushrooms as a healthy food (Goyal *et al*, 2015). In general, the fat content of cultivated mushrooms ranges between 0.6 – 3.1% as dry weight and also the fat content widely varies depending on the mushroom type and cultivation method. The crude fat contained in this study ranged from 1.1g to 1.9g. The fat content was higher in mushrooms treated with SD BM, UV+HD BM and SD OM with values of 19 g, 19 g and 17 g respectively (Figure 9 and 10). Lower fat content was observed in UV+ HD OM (11.9 g). The estimated fat content values in this study were reported to be the same by (Singh et al, 2016). While comparing the *Agaricus bisporus* and *Pleurotus ostreatus*, the lower fat contents were found in various pretreatments of *Pleurotus ostreatus*. It emphasizes the role of substrate on the fat content used for the cultivation of oyster mushrooms (McGrath et al, 1982).

3.2.4. Fiber Content

Edible mushrooms showed a hypocholesterolemic impact in the diet, possibly due to dietary fibers such as beta-glucans. This may promote intestinal motility

while lowering bile and adsorption of cholesterol levels. The percentage ranges from 4% to 13% of total dietary fiber consumption. The sum of the nondigestible carbohydrates (chitin) and lignin (plants) makes up the total dietary fiber (TDF). Usually, the chitin content of the mushroom was greater in *A. bisporus* fungus that causes *Pleurotus* (Manzi et al, 2004). While comparing dried mushrooms to fresh or frozen mushrooms, fresh mushrooms have higher beta-glucan content than dried ones. It denotes that the heat treatment affects the beta-glucan content. Afiukwa et al. found that *Plerotus ostreatus* had a fiber content of 29.00%, which is much greater than the values reported in this investigation. Sun dried *Pleurotus spp* has 12.59 percent crude fibre compared to 12.58 percent for oven dried *Pleurotus spp* mushrooms, according to (Dunkwal et al, 2007).

3.2.5. Ash content

Ash is defined as fully non-burnable inorganic salts. Potassium and phosphorus are the principal ash elements in mushrooms. According to studies, mushrooms are high in minerals and can also be a superior source of minerals than vegetables. Ash content data of the dried mushrooms are represented in (Figure 9, 10). HD and UV + HD dried button mushroom samples show the ash content of 10.2 g and 8.6 g respectively and also the content is statistically lower than that of SD BM (10.8 g). The study by (Muyanja et al, 2014), sun-dried and oven-dried treated powders had ash levels ranging from 0.44 to 0.54 g/g dry matter. Pretreatment oyster mushrooms showed the ash content value of 10.7g and 10.2 g in SD and HD dried samples which is statistically higher than the contents of UV + HD dried samples (4.6 g). These findings exactly matched with (Singh et al, 2016) study. The sun dried pretreatment is more efficient for both button and oyster mushroom as per ash contents.

3.2.6. Moisture content

The elimination of moisture content during processing may boost nutrient concentration in the mushroom by extending its shelf life. The weight of the mushroom fell fast as the temperature increased; the drying temperature had a significant impact on the moisture removal of the mushroom. The moisture content was almost remaining the same for the mushroom treated in all conditions in this study. The current study's findings revealed that the moisture content is low for both oyster and button mushroom (Figure 9 and 10) as compared to (Sunday et al, 2016 and Tolera et al, 2017). While using drying methods, case hardening can occur, resulting in greater moisture content in samples than when utilizing traditional drying methods under identical treatment circumstances.

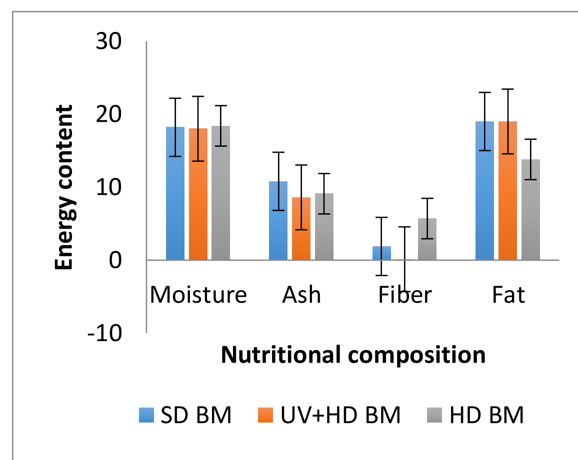

Figure 9. Graphical representation of proximate analysis of dried button mushroom

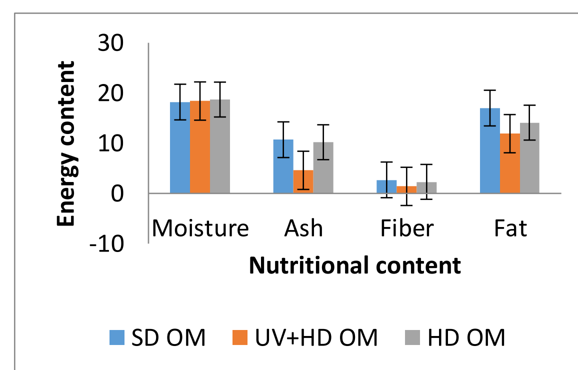

Figure 10. Graphical representation of proximate analysis of dried oyster mushroom

3.2.7. Estimation of energy content in dried mushroom

A characteristic of food is energy density or the amount of energy (in kilocalories) per unit of food (in gram). As a result, it may be determined by dividing total kilocalories by total gram for meals, and the entire diet. Various dietary components, such as macronutrient and water content, have an impact on energy content. Water has the most impact on a food's energy content since it adds significant weight without providing energy. Fat is the most important macronutrient due to its high energy level when compared to protein or carbohydrate. The sundried mushrooms determined to have significant energy content compared to other pretreatment methods in both the mushroom varieties in this study (Figure 11). The higher energy content in sundried mushroom is due to the presence of higher protein content (Refer figure 8).

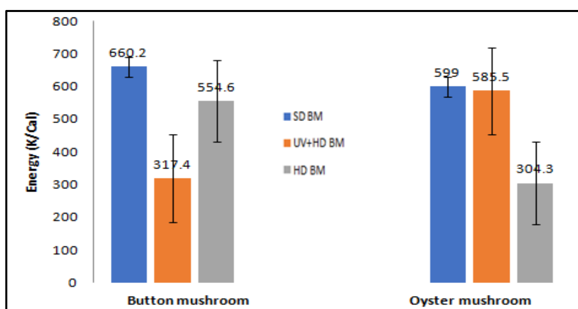

Figure 11. Overall energy calculation for drying processes

3.2.8. Quantification of Vitamin- D_2 content

The Vitamin D_2 content was assessed for sun-treated button and oyster mushroom, since the proximate composition values found to be appropriate for sun dried mushroom rather than other drying methods.

The vitamin D_2 concentration was found to be 83.3 µg/100 g and 45 µg/100 g for sun dried oyster and button mushroom in the present study. The quantity of ergosterol present in dry matter of sun dried mushroom was summarized in Table 3. The study by (Nolle et al, 2017) showed that sun-dried mushrooms contained 36 µg/g dry matter vitamin D_2; whereas the (Urbain et al, 2015) study experimentally proved that the vitamin D_2 content increased to 17.6 µg per 100 g of fresh mushroom.

Table 2. Quantity of ergosterol present in sun dried mushroom

Sample	Sundried Button mushroom	Sundried Oyster mushroom
Ergosterol(µg/100g)	45	83.3
SD	34.87312	31.67832
RSD(%)	1.2153	1.1864

The reason behind the result discrepancy can be correlated with the mushroom variety, ergosterol content, exposure dose and time as well as temperature. The quantity and quality of solar energy reaching the earth's surface is affected by latitude and season, which has an impact on vitamin D synthesis (Webb et al, 1988). Sun drying is a realistic approach for the natural creation of ergocalciferol in mushrooms and a great vitamin D source for vegans, even if commercial UVB radiation boosts vitamin D_2 synthesis in mushrooms.

3.3. Statistical analysis

The statistical analysis was performed for the proximate analysis values using single factor ANOVA. The values were found to be statistically significant with $p < 0.05$.

Table 3: Statistical analysis performed for the proximate values

Source variation	SS	df	MS	F value	P-value	Fcrit value
Between groups	333.21	1	333.2	7.97	0.02	5.32
Within groups	334.35	8	41.79	-	-	-
Total	667.57	9	-	-	-	-

4. Conclusions

The nutritional quality of the dried mushroom was statistically significant when varied pretreatments and drying techniques were used. Among them, the sun drying method was effective in maintaining and increasing the amount of vitamin D_2 in the dried mushroom. The sun dried mushrooms represent a prominent source in providing dietary vitamin D_2. This is especially important because just a few foods provide naturally high levels of vitamin D_2 content, such as salmon (12.4 mg/g), herring (15.4 mg/g), and egg yolk (7.8 mg/g). As a result, a little quantity of sun dried mushrooms can provide the necessary daily consumption of vitamin D_2 (600-800 IU) for humans. Drying mushrooms in low-cost, locally available sun drying methods presents a viable alternative to UV treatment method for natural vitamin- D_2 enrichment, which might lead to the production of safe vitamin D_2 enriched foods. As a possible future work, the other edible mushroom species must be evaluated in order to optimize processing and preservation methods for the distribution of high-quality dried mushrooms in order to promote mushroom production, preservation, and consumption in developing nations.

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PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING ACTIVITY OF THE AQUEOUS EXTRACT OF *JUGLANS REGIA* (WALNUTS)

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ABSTRACT
The world of plants is full of resources and virtues from which people take not only his food but also active substances that often provide a benefit to his body. This research aimed to the valorization of a medicinal plant known by their traditional use; *Juglans regia* L (*Juglandaceae*) by phytochemical screening of bioactive compounds and evaluation of the antioxidant activity of leaves and bark aqueous extracts. The extraction yield indicated that the aqueous extract decocted from the leaves presented high yield (28.23 ± 0.63%) compared to the other extracts. Qualitative phytochemical tests demonstrated a richness of extracts in bioactive compounds by the presence of total and Gallic tannins, saponosids and coumarins in all extracts. Quantitative determination revealed that total phenolic content in the aqueous leaf decoction extract (553 mg GAE / g) was higher than in the other extracts. For the determination of flavonoids, the aqueous decoction extract of the leaves and even the bark was found to be the richest (254 and 226 mg QE/g respectively). The results of the antioxidant activity showed that the totality of the extracts possess advantageous antiradical properties, in particular the aqueous extract of the bark which has an IC_{50} value (0.02 mg / ml) close to that of ascorbic acid (0.019 mg / ml). The study of the antioxidant activity of extracts of Juglans regia L. suggests that this plant represents a natural source of chemical molecules that has very important biological activities.

1.Introduction

Medicinal plants are considered as a source of essential material for the discovery of new molecules necessary for the development of future drugs (Maurice, 1997). The therapeutic use of the extraordinary virtues of plants for the treatment of all diseases of the human is very old and evolves with the history of humanity (Gurib-Fakim, 2006). These natural products contain a large number of molecules that have multiple interests put to use in the food industry, cosmetology and pharmacy; these compounds include coumarins, alkaloids, phenolic acids, tannins, terpenes and flavonoids (Bahorun *et al.,* 1997). Oxidative stress is involved in many diseases as a triggering factor or associated with complications. Most oxidative stress-induced diseases appear with age as aging decreases antioxidant defenses, increasing mitochondrial multiplication of radicals (Girodon *et al.,* 2010). Among the biological activities of medicinal plants, in recent years attention has been focused on antioxidant activity because of the role it plays in the prevention of chronic diseases such as heart disease, cancer, diabetes, hypertension, and Alzheimer's disease by combating oxidative stress (Meddour *et al.,* 2013). The evaluation of phytopharmaceutical and antioxidant properties remains a very interesting and useful task, especially for plants that are less frequently used or are not known in traditional medicine

(Teixeira Da Silva, 2004).

Juglans regia (walnut) is a species that belongs to the Juglandaceae family. It is rich in vitamins (B1, B2, B3, B5, B6) and minerals (selenium and manganese). It also provides an excellent intake of omega 3 fatty acids (Weir *et al.,* 2004). Green leaves and nut husks contain oxygenated aromatic compounds (naphthoquinones), the most specific of which are juglone, flavonols (hyperoside, juglanoside) and gallic and ellagic tannins. *Juglans regia* has been used extensively in medicine; the leaves have been used traditionally in the treatment of cutaneous inflammations, ulcerations. They have an anti-diarrheal, antiseptic and astringent effect (Almeida, 2008). Walnut leaves are also used against rickets, anemia because they have fortifying and toning actions (Ait Youssef, 2006).

To this end, our work aims at contributing by a phytochemical study, as well as the evaluation of the antioxidant activity of the aqueous extracts of the leaves and the bark of *Juglans regia* L. (walnut).

2.Materials and methods

2.1. Plant material

It consists of two parts (leaves and bark) of the species *Juglans Regia.* They were collected during July 2017. Collection and identification of the bark and leaves was carried out in the El Qoll area (Skikda), Algeria. The different parts (leaves, bark) were dried in a dry place at room temperature for a few days. Once dried, the parts of the plant were reduced to powder and subjected to extraction.

2.2. Preparation of aqueous extract of *Juglans regia*

The extraction was carried out according to the methods of Romani et al (2006). For maceration, 10 g of powder of the leaves (or bark) are macerated in a volume of 100 ml of the solvent (distilled water) at room temperature, protected from light and with magnetic stirring for 48 hours. After filtration, the extracts obtained were then placed in a desiccator to completely remove the solvent.

In other hand, for decoction, 10 g of leaf powder and bark was added to 100 ml of the solvent (distilled water) in a reflux system. After extraction, the mixture was filtered to obtain the decocted. The concentrated filtrate was dried in the oven and was collected in sterile flasks.

The yield of the various extracts obtained was defined as being the ratio between the mass of the dry extract obtained and the mass of the plant material used (Harborne, 1980). This yield was calculated by the equation:

$$R\ (\%) = (Me / Mp) \times 100. \qquad (1)$$

R (%): Yield in%.
Me: Mass of the extract.
Mp: Mass of the plant.

2.3. Qualitative analysis

This was a qualitative study aimed at the search for the main chemical groups (alkaloids, flavonoids, tannins, saponosides, coumarins ...). The characterization tests were based on precipitation and complexation reactions with formation of insoluble and colored complexes. The observed staining was caused by the use of a suitable reagent and is generally due to the formation of a conjugation into a molecule (Harborne *et al.,* 1980, Rai and Carpinella, 2006).

2.4. Quantitative analysis

Colorimetric methods based on the use of the UV-visible spectrophotometer were used to evaluate the amount of polyphenolic compounds in the plant material.

2.4.1. Total Phenol Content

The total polyphenols were assayed according to the method of Folin-Ciocalteu (FC) (Singleton and Ross 1965, Benhammou 2009). The FC reagent consisted of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMO12O40). The oxidation in an alkaline medium of this reagent by the oxidable groups of the phenolic compounds led to the formation of a mixture of blue oxide. The intensity of the coloration produced was proportional to the amount of polyphenols present in the analyzed extract. 1.25 ml of Folin-Ciocalteu was added to 1 ml of

sodium carbonate Na_2CO_3 at 2% and 0.25 ml of diluted extract (or standard substance of gallic acid and its dilutions), incubated at room temperature for 90 min. Absorbance was measured at 765 nm. The results were expressed in mg gallic acid equivalent / g dry plant (mg GAE / g dry) by referring to the gallic acid calibration curve.

2.4.2. Total Flavonoid Content

The determination of total flavonoids was carried out by the aluminum trichloride ($AlCl_3$) method according to the protocol of Dewanto et al. (2002). The formation of a covalent bond between the aluminum trichloride and the hydroxyl (OH) groups of the flavonoids produced a yellow complex. 1 ml of diluted extract (or quercetin solution and its dilution) was added to 0.3 ml of sodium nitrite (5% $NaNO_2$). After 5 min, 0.3 ml of aluminum trichloride (10% $AlCl_3$) and 2 ml of sodium hydroxide (4% NaOH) were added to the mixture. The volume was completed to 10 ml. Absorbance was measured at 510 nm. The results were expressed in mg quercetin equivalent / g dry plant by referring to the quercetin calibration curve.

2.4.3. DPPH Radical Scavenging Assay

To evaluate the antioxidant activity, the DPPH method was used according to the protocol described by (Sanchez-Moreno *et al.,* 2002). 50 µl of different concentration or standard (ascorbic acid) were added to 1.95 ml of the methanolic solution of DPPH (0.0025 g / l). In parallel, a negative control was prepared by mixing 50 µl of methanol with 1.95 ml of the methanolic solution of DPPH. The absorbance was read against a control prepared for each concentration at 517 nm after 30 minutes of incubation in the dark and at room temperature. The results were expressed as anti-free radical activity or the inhibition of free radicals in percentages (I %) using the following formula:

$$I\% = [1 - (\text{Abs Sample} - \text{Abs Control})] \times 100$$

I %: Percentage of antiradical activity.
Abs Sample: Absorbance of the sample.
Abs control: Absorbance of the negative control.

2.4.5. Calculation of IC_{50}

IC50 or 50% inhibitory concentration (also called EC50 for Efficient Concentration 50) was the concentration of the tested sample required to reduce 50% of DPPH radical. IC50 was calculated graphically by linear regression of the graphs, inhibition percentages as a function of different concentrations (Benhammou *et al.,* 2008). For the entire experiment, each test was performed in triplicate and the results were calculated by the average of three trials.

3.Results and discussion

3.1. Extraction yield

Based on these results, it was found that the aqueous leaf decoction and maceration extract (28.23 ± 0.63, 22.13 ± 0.43% respectively) showed higher yields than the bark (Figure 1).

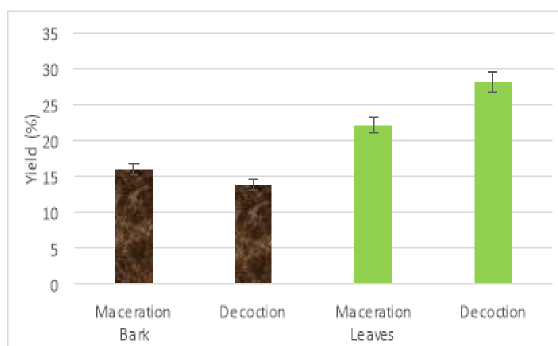

Figure 1. Extraction Yields of aqueous extracts of *J. regia.*

The contents of extracts vary not only from one plant to another of the same family but also according to the parameters of the solid-liquid extraction of the polyphenols, the temperature, the extraction solvent, the particle size and the coefficient of diffusion. Extraction with polar solvents and at elevated temperatures has been shown to provide higher yields of extract (Majhenic *et al.,* 2007). In general, plant diversity is responsible for the wide variability in the physicochemical properties that influence polyphenol extraction (Koffi *et al.,* 2010, Mahmoudi *et al.,* 2013).

3.2. Qualitative analysis

The qualitative analysis of plant extracts was based on color reactions or precipitation by specific chemical reagents, performed on extracts reconstituted from a plant sample.

The analysis of these experimental results leads us to the presence of total and Gallic tannins, coumarins and saponosids in all extracts of leaves and bark of *J. regia*. Anthocyanins were present in small amounts in the aqueous extract decocted and macerated of leaves and absent in aqueous extracts of bark. It was noticed that Irridoids were present in the aqueous extracts of leaves (Table 1). Flavonoids were present only in the aqueous extract macerated of leaves. Nirmala *et al.* (2011) reported the presence of the same classes of chemical families found in the leaves and barks of *J. regia.* This difference in the results would be due to the choice of the part to extract and the extraction method, as stated by Mamyrbekova - Bekro *et al.,* (2013).

3.3. Quantitative analysis

3.3.1. Total phenolic content

The total phenolic content was determined according to the calibration curve of gallic acid. The results were expressed in mg gallic acid equivalent / g of dry plant (mg GAE / g) (Figure 2).

The results obtained revealed that the level of these phenolic compounds in the various extracts was interesting. It was noted that the level of total phenolic content in the aqueous leaf decoction extract (553 mg GAE / g) was higher than in the other extracts. Even the aqueous extract of maceration bark showed considerable levels of total polyphenols (330.5 mg GAE / g). Like our results, Wu (2004) reported high levels of total phenolic content ranging from 680 to 2016 mg GAE / g for 10 varieties of Walnut. Kornsteiner *et al.,* (2006) also reported higher values for walnut (from 1020 to 2052 mg GAE / g). Ogunmoyole *et al.,* (2011) estimated a total polyphenol level equal to 200.2 mg GAE / g for the aqueous extract of Walnut, which confirmed the richness of the bark and the leaves of *J. regia* in phenolic compounds. Levels of phenolic compounds in Walnut were influenced by environmental factors, soil composition, and level of ripening (Wakeling, 2001).

Table 1. Phytochemical tests of the aqueous extracts of *J. regia.*

	Phytochemical tests						
Extracts	Total tannins	Irridiods	Gallic tannins	Saponosides	Flavonoids	Coumarins	Anthocyans
B-maceration	+++	-	+++	++	-	+	-
B-decoction	+++	-	+++	++	-	++	-
L-maceration	+++	+	+++	+++	+	++	+
L-decoction	+++	+	+++	++	-	++	+

B : Bark, L : Leaves

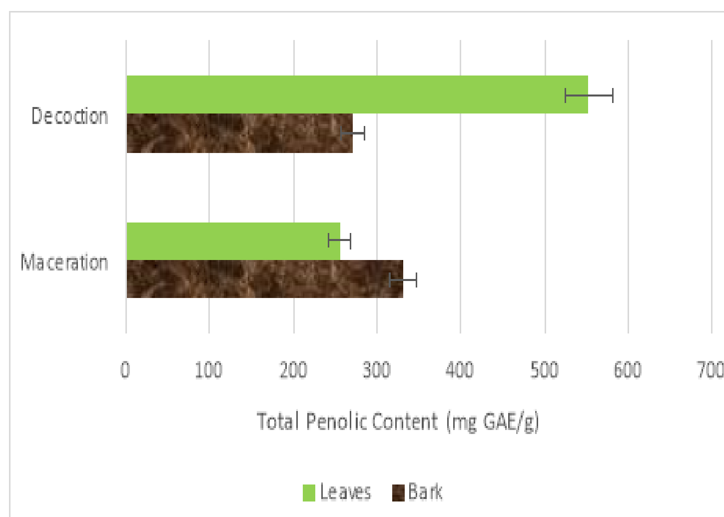

Figure 2. The total phenolic content of *Juglans regia* extracts.

3.3.2. Total flavonoid content

In return, the flavonoid content was calculated in mg quercetin equivalent / g dry plant (mg QE / g dry) while referring to the calibration curve of quercetin.

For the determination of flavonoids, the aqueous decoction extract of the leaves and even the bark was found to be the richest (254 and 226 mg QE/g respectively) followed by the aqueous extract macerated leaves (127 mg QE/g). On the other hand, the aqueous extract macerated from the bark had minimal flavonoid contents (91 mg QE/g dry) (Figure 3). Our results appeared in agreement with other studies. Referring to the results of Ogunmoyole *et al.,* (2011) who estimated a total flavonoid level equal to 148.2 mg QE/g for the aqueous extract of Walnut.

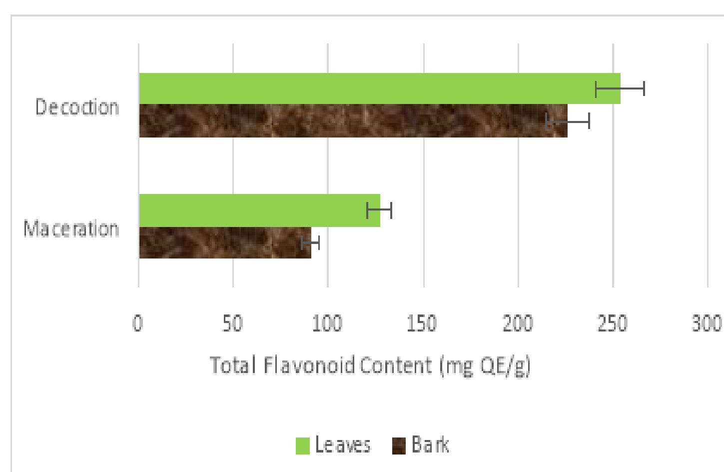

Figure 3. The total flavonoids content of *Juglans regia* extracts.

3.3.3. DPPH Radical Scavenging Assay

Different concentrations ranging from 0 to 1000 µg / ml of *J. regia* extracts were tested for their antioxidant activity *in vitro*. In order to compare their antioxidant activity with that of ascorbic acid, a calibration curve carried out by ascorbic acid was plotted (Figure 4).

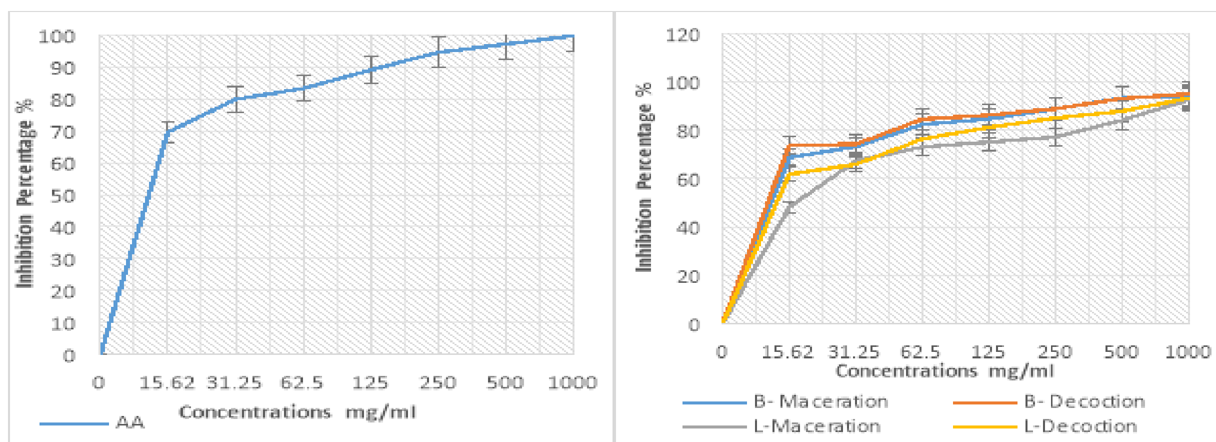

Figure 4. Inhibition Percentage (%) according to different concentrations (AA: Ascorbic Acid, B: Bark, L: Leaves).

In general, all four extracts tested caused a greater decrease in absorbance at 515 nm depending on their concentration (Figure 4). For walnut bark extracts, the DPPH free radical scavenging capacities by the macerated and decocted aqueous extract are 93.4 and 93.2% respectively in the concentration of 500 mg / ml. Whereas for extracts of walnut leaves, DPPH free radical scavenging capacities by the macerated and decocted aqueous extract are 84.2 and 87.9% respectively in the concentration of 500 mg / ml.

To better characterize the antioxidant power, it was necessary to introduce the IC_{50} parameter. It was defined by the effective concentration of substance that causes the reduction of 50% of the DPPH radical in solution. Therefore, the lower value of IC_{50} indicated a higher antiradical activity of the extract (Pokorny and Korczac, 2001).

The comparative study showed that the IC_{50} value of the macerated aqueous leaf extract has an IC_{50} value (0.03 mg / ml) greater than the ascorbic acid (0.019 mg / ml). While, the aqueous extract of the bark has an IC_{50} value close to that of ascorbic acid (0.02 mg / ml) (Figure 5). This indicated that this extract has a high antioxidant capacity because the very low IC_{50} value.

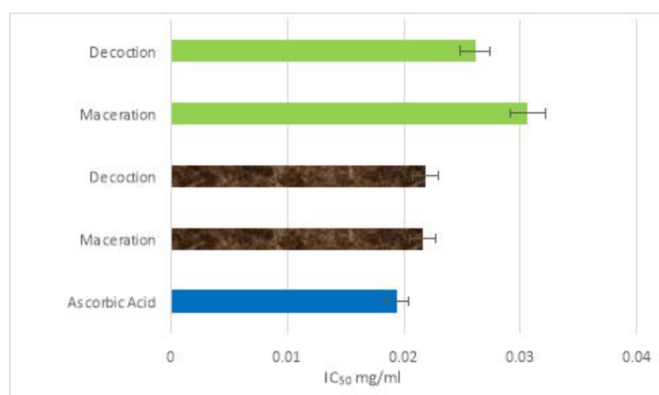

Figure 5. Values of IC_{50} of *J. regia* extracts.

According to the study by Agarwal *et al.,* (2012), the aqueous and alcoholic extract of *J. regia* showed an IC_{50} value of 0.182 mg / ml and 0.238 mg / ml, respectively. While it was found that the aqueous extracts have a lower IC_{50} value. This strong antioxidant activity of walnut could be attributed to the presence of phytochemicals such as phenolic compounds. Recently, Zhang *et al.,* (2009) identified seven phenolic compounds in *J. regia* by spectroscopic methods, which were pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, proto-catechic acid, gallic acid and 3, 4, 8, 9, 10 penta-hydroxy-di-benzo (b, d) pyran-6-one, containing important antioxidant activities. Several studies have shown that walnut consumption can improve human antioxidant capacity (Ma *et al.,* 2010, Mckay *et al.,* 2010).

4.Conclusions

The qualitative and quantitative phytochemical study demonstrated a richness of *J. Regia* species in bioactive compounds by the presence of total and Gallic tannins, saponosides and coumarins. The results of DPPH Radical Scavenging Assay showed that all the extracts have interesting antiradical properties including the macerated extract and even decoction of the bark. It was manifested by low IC_{50} values that were very close to those of standard compounds (ascorbic acid).

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EVALUATION OF BOVINE MILK PROCESSING ON THE DIGESTIBILITY AND ALLERGENICITY OF MILK PROTEINS

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ABSTRACT

The objective of this study was to evaluate whether thermal processes applied in milk, such as pasteurization and UHT affect the protein digestibility leading to changes in the allergenic responses. Samples were subjected to a simulation of the human digestion and subsequently evaluated regarding protein cleavages and enzyme immunoassay for caseins and β-lactoglobulin immunogenicity. Among the different samples, protein digestibility was mainly affected in the gastric phase. α-lactalbumin and caseins showed high susceptibility to gastrointestinal enzymes, while a partial β-lactoglobulin resistance to pepsin was observed. Concerning *in vitro* allergenicity, a tendency of reduction was demonstrated in UHT and powdered milk samples after digestion in the stomach. Following the intestinal digestion, all milk samples presented low allergenicity, over 96% reduction of antibody binding. These data corroborates to the understanding of the effects of the world´s most used heat treatments in cow's milk protein digestibility and allergenicity.

1.Introduction

Bovine milk is an important source of proteins, lactose, calcium, vitamins and bioactive peptides (Villa *et al*., 2018). In Western diets cow's milk consumption is also a habit among humans and its regular intake is associated with the prevention of several chronic diseases, including cardiovascular, diabetes, obesity and osteoporosis (Willett and Ludwig 2020). Despite its nutritional relevance and its whole on health preservation, bovine milk is on the list of the eight most allergenic foods (FAO and WHO, 2018). Currently, several allergenic epitopes have already been identified within the structure of the main milk proteins – caseins, alpha lactalbumin (α-La), beta-lactoglobulin (β-Lg). Cow's milk allergy affects 4% of children and 0.5% of adults worldwide, causing symptoms such as atopic dermatitis, acute urticaria, rhinitis, asthma exacerbation, vomiting, diarrhea and abdominal pain (Villa *et al*., 2018, Willett and Ludwig, 2020). It is known that some food processes, including pasteurization and UHT process can modify the structure of proteins, either by glycosylation, Maillard reaction, aggregation or unfolding. These processes may alter the allergenic epitopes of proteins, influencing their binding to immunoglobulins, consequently modulating the immunological response (Bogahawaththa *et al*., 2017, Bu *et al*., 2013; Villa *et al*., 2018). Different thermal processes such as pasteurization, ultra-high temperature (UHT) and UHT followed by atomization (milk powder) are used by milk industries to reduce microbiological contamination. However, the relation between these processes, especially UHT and atomization, with milk protein

allergenicity and digestibility is not fully understood, requiring further investigation (Bogahawaththa *et al.*, 2017, Bu *et al.*, 2013, Villa *et al.*, 2018). Milk caseins are stable proteins when milk is treated by thermal processing, which generates a small attenuation of allergenicity and a slight increase in its digestibility. In contrast, the main whey proteins (α-La and β-Lg) are more susceptible to heat, especially when temperatures over 90 C° are applied as conformational changes leads to epitopes exposition and further destruction by the gastrointestinal enzymes (Rahaman *et al.*, 2016, Villa *et al.*, 2018). During protein digestion several chemical and enzymatic reactions occur, generating changes in the structure of proteins, which can either lower or increase their allergenicity. Not all proteins are fully cleaved in amino acids during digestion. Some of them are cleaved into larger peptides preserving allergenic epitopes, which may intensify certain immune system stimulations, such as IgE binding (Benede *et al.*, 2014, Villa *et al.*, 2018). Considering all mentioned above, the present study aimed to investigate different types of bovine milk processing – pasteurized, UHT and powdered (UHT followed by spray-drying atomization) – regarding the digestibility and allergenicity of milk proteins.

2. Materials and methods

2.1. Materials

Different commercially bovine milk, processed by pasteurization, UHT associated with homogenization and powder were selected. Samples of raw milk from local milk producers were also used for comparative purposes. The milk samples were selected from the dairy basin in the Southeast of Brazil, more specifically from Rio de Janeiro and Minas Gerais.

2.2. *In vitro* gastrointestinal digestion of milk samples

Digestion simulation was carried out in accordance to the international consensus on static in vitro digestion, Infogest (Brodkorb *et al.*, 2019, Minekus *et al.*, 2014). As recommended by the method for liquid foods, the salivary phase of the digestion was not performed. Therefore, simulated gastric fluid containing porcine pepsin (423 U.mg-1, Sigma-Aldrich, St. Louis, MO, USA) was added to the different milk samples. Gastric digestion occurred for 2 hours in a water bath (Banho Dubnoff NT 232, Novatecnica, Piracicaba, SP, Brazil) under constant agitation. To stop the reaction, the pH was adjusted to 7 with hydrochloric acid. Following to the intestinal phase, the solution was mixed with a simulated intestinal fluid containing porcine pancreatin (7.05 U.mg-1, Sigma-Aldrich, St. Louis, MO, USA) and bile (1.00 mmol.g-1, Sigma-Aldrich, St. Louis, MO, USA). The reaction occurred for 2 hours and it was stopped with ice bath. Samples were kept at -20°C until further analysis.

2.3. Degree of hydrolysis

The soluble protein content was quantified according to Bradford (1976), in a digital spectrophotometer SP-220 (Biospectro, Curitiba, PR, Brazil). The spectrophotometric measurement of aromatic amino acids was carried out according Goodwin and Morton (1946). A tyrosine standard curve was used and samples were read at 280nm. Results were analyzed by one-way ANOVA and submitted to the Tukey t-test in Microsoft Excel 2019 software with a significance level of $p = 0.05$.

2.4. Protein electrophoresis

One-dimensional protein electrophoresis was performed (Laemmli, 1970) using polyacrylamide gel. The stacking and running gels were prepared with 8% and 12% acrylamide solutions, respectively. Undigested and digested milk samples, as well as a wide molecular weight standard (Bio-Rad Laboratories, Inc, United States) were applied to the gels. The electrophoretic run was carried out in the Mini PROTEAN® Tetra Cell (Bio-Rad Laboratories, Inc, United States) at 100V for 2.5 hours. After running, the electrophoretic gels were fixed and stained in a solution containing acetic acid (10%), methanol (40%) and Coomassie Brillant Blue R 250 (1%) overnight.

2.5. Allergenicity of Milk Proteins

The allergenicity of milk samples and their digested products was determined by enzymatic immunoassay using sandwich ELISA kits for caseins and β-Lg (RIDACREEN FAST Milk, R-Biopharm AG, Darmstadt, Germany). The reaction was read in Multiskan FC (ThermoScentific, Waltham, MA, USA) at a wavelength of 450 nm. Casein and β-Lg concentrations were calculated by the RIDA®SOFT Win.net software (R-Biopharm AG, Darmstadt, Germany). The results correspond to the average of 4 experiments that were submitted to statistical analysis using Tukey's t-test in Microsoft Excel 2019 software with a significance level of p = 0.05 to compare the results.

3.Results and discussions

3.1. Milk Proteins Hydrolysis

Protein digestion began in the stomach by the activity of pepsin under acidic pH conditions. Pancreatic and intestinal proteases followed the digestion process, hydrolyzing the remaining protein fragments (Sah *et al*., 2016). In the present study, the soluble protein content dramatically reduced after the simulated gastric digestion achieving reductions of 24.3-, 19.5-, 25.7- and 27.3-times fold for raw, UHT, pasteurized and powdered samples, respectively (Figure 1A). After enteric digestion the soluble protein content of all bovine milk samples remained stable as the method is able to quantify proteins and peptides with molecular weight higher than 3 kDa. In contrast, no significant increase in the concentration of aromatic amino acids was observed from the undigested to gastric digested samples, showing that the peptic digestion was able to convert part of the protein into higher molecular weight peptides. Meanwhile, intestinal enzymes were able to release small peptides and amino acids and increments of 2.6-, 6.1-, 5.3- and 4.8-times fold for raw, UHT, pasteurized, and powdered milk samples, respectively, were observed after enteric digestion (Figure 1B).

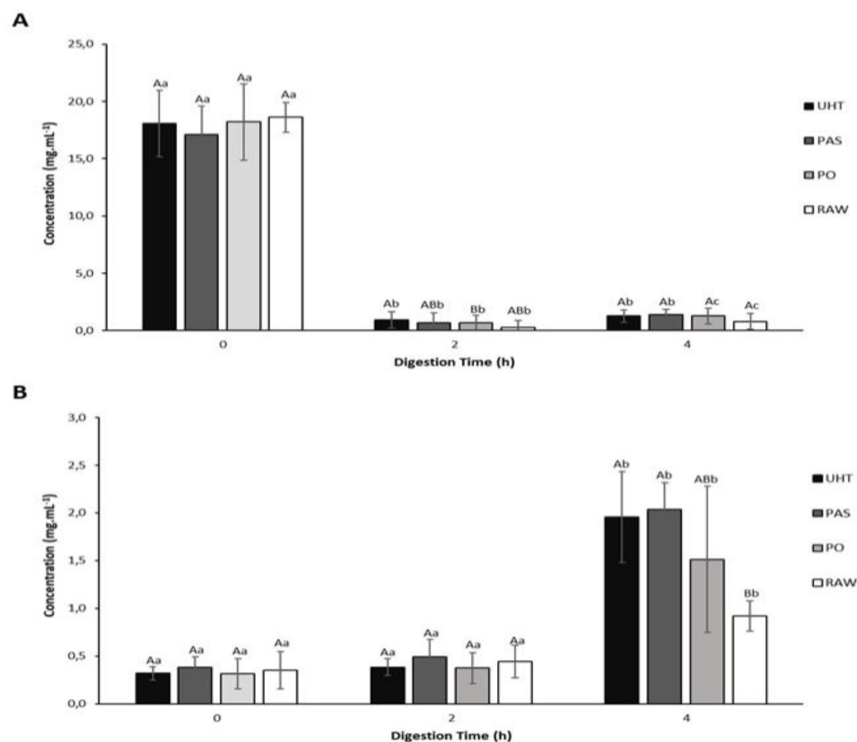

Figure 1. Soluble protein content (A) and aromatic amino acids (B) quantification of UHT, pasteurized (PAS), powdered (PO) and raw undigested and *in vitro* digested milk samples. Capital letters: significant difference between milk samples at the same time of digestion, $p < 0.05$; Lower-case letters: significant difference between digestion times in the same type of milk, $p < 0.05$.

3.2. Electrophoretic Profile

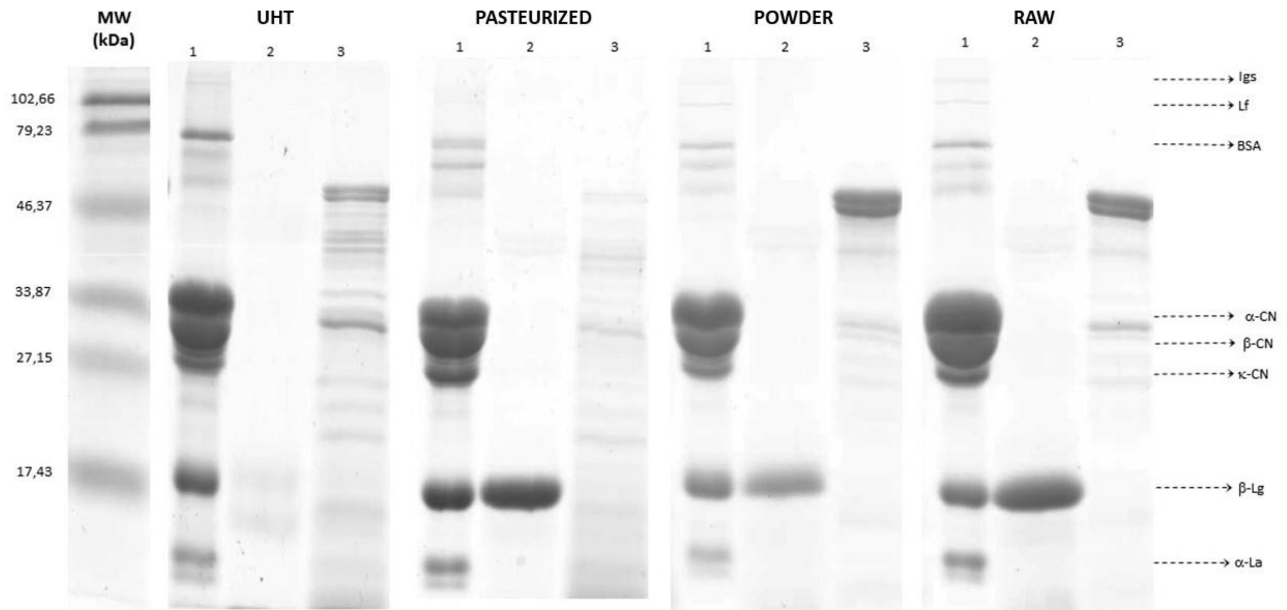

Figure 2. SDS-PAGE electrophoretic gel of UHT, pasteurized (PAS), powdered (PO) and raw milk during simulated digestion. MW: molecular weight; 1: undigested; 2: digested in the stomach; 3: digested in the small intestine; Igs: immunoglobulins; Lf: lactoferrin; BSA: bovine serum albumin.

The Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Figure 2) revealed that all cow's milk samples presented similar protein profiles, indicating that the different thermal processes applied did not affect their protein composition. According to Bouzerzour *et al.* (2012), approximately 77% of milk caseins are degraded before 30 minutes of digestion by pepsin and after the enteric phase of digestion they could no longer be found. Corroborating with this statement, in the present study the electrophoretic profile showed an intense reduction in casein bands for all milk samples after the gastric phase of digestion. Regarding whey proteins, the second most abundant protein in whey (α-La) was rapidly hydrolyzed by pepsin after the simulated gastric digestion for all milk treatments. Kopf-Bolanz *et al.* (2014) found similar results for raw, pasteurized and UHT whole milk and Mellinger-Silva *et al.* (2015) in pepsin hydrolysates of whey protein isolate. The susceptibility of α-La to pepsin can be attributed to conformational alterations occurred in α-La structure at low pH, exposing its hydrophobic interior and increasing its proteolysis by pepsin (Kamau *et al.*, 2010, Nik *et al.*, 2010). The major whey protein (β-Lg) presents a strong globular conformation at low pH, hiding pepsin's target amino acids (Ozorio *et al.*, 2020). Bateman *et al.* (2010) and Ozorio *et al.* (2020) also reported a β-Lg resistance to simulated gastric digestion. In Figure 2 is possible to observe that in milk types treated with severe heat – UHT and powder – β-Lg was susceptible to pepsin, which is probably related to the thermal sensitivity of the β-Lg structure to high temperatures (Villa *et al.*, 2018), exposing pepsin target sites. However, in milk samples that received mild or no heat treatment – pasteurized and raw milk, respectively – β-Lg was further hydrolyzed by intestinal enzymes. The susceptibility of β-Lg to enteric digestion can be associated to conformational alterations at pH above 7, uncovering amino acid residues

that are sensitive to intestinal enzymes (Ozorio *et al.*, 2020).

3.3. Allergenicity of caseins and β-Lg

The sensitivity of an individual to a food allergen is very particular, explaining the lack of agreement regarding safe concentration for ingestion or minimum intake recommended to avoid allergic reactions (Villa *et al.*, 2018). Figure 3 shows that prior in vitro digestion, all milk samples presented similar immunoreactivities to casein and β-Lg through ELISA tests. If IgE-mediated responses to milk proteins may cause symptoms usually right after ingestion or within the following 2h (Villa *et al.*, 2018), all types of milk may generate immediate reactions in allergic people, since undigested samples presented high immunoreactivity. After gastric digestion, milk powder achieved the lowest allergenicity, followed by UHT, while pasteurized and raw milk showed similar profiles. Once β-Lg was completely and partially hydrolyzed in UHT and powdered milk (Figure 2), respectively, the most prominent reduction in the allergenicity of these both milk types may be related to a combination of severe heat treatment with pepsin digestion, as reported by Rahaman *et al.* (2016) and Villa *et al.* (2018). When compared to the intestinal digested, milk gastric digested samples presented higher allergenicity, which may be attributed to the preservation of allergenic epitopes in proteins and polypeptides not digested in this phase. Although in the present study the combination of alkali pH and intestinal enzymes had been able to drastically reduce the allergenicity in all cow's milk samples, some non-IgE-mediated allergic late reactions may still occur (Villa *et al.*, 2018).

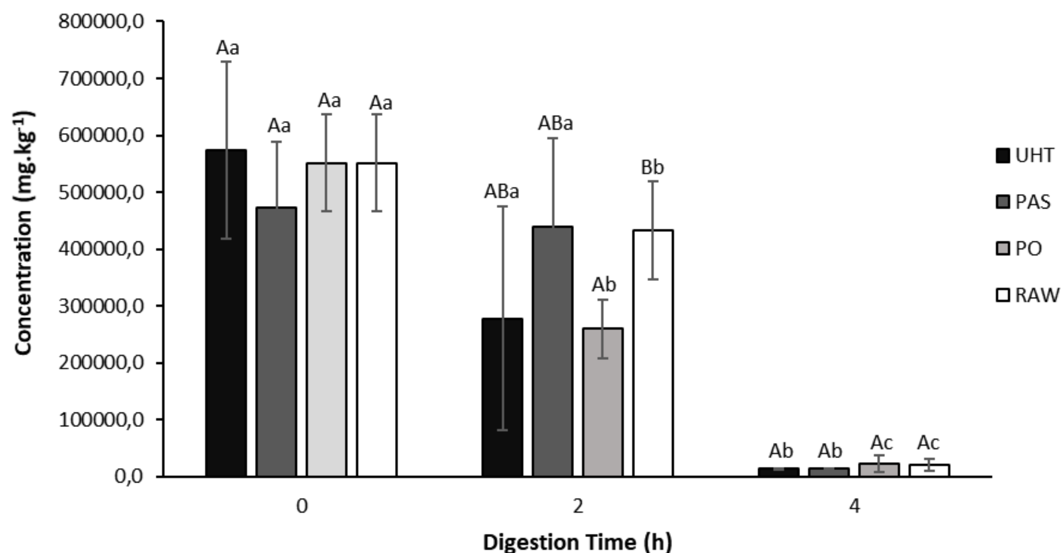

Figure 3. Concentration of casein and β-Lg in undigested and *in vitro* digested milk samples as an indicative of milk allergenicity. PAS – pasteurized milk; PO – powdered milk. Different capital letters: significant difference between milk samples at the same time of digestion; $p < 0.05$ Different lower-case letters: significant difference between digestion times in the same type of milk; $p < 0.05$.

4. Conclusions

Considering the results presented, undigested and in vitro digested milk samples evaluated showed similar degrees of hydrolysis. Through the electrophoretic gel it was possible to confirm the high susceptibility of cow's milk caseins to gastrointestinal digestion. The β-Lg of bovine milk types treated with intense heat (UHT and powder) showed more sensitivity to pepsin digestion than in raw and pasteurized ones. This can also be related to the tendential lower allergenicity of both UHT and powder milk gastric digests in comparison to pasteurized and raw gastric samples. Enteric enzymes could

hydrolyze the remaining proteins in all intestinal digested milk samples, which can be related to the small allergenicity observed for them. In this sense, the different thermal processes applied by industries to allow milk distribution worldwide besides providing a safe product, regarding microbiological contamination, also demonstrated small influence on the digestibility and allergenicity of the main milk proteins.

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