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# EFFECT OF CASEIN EDIBLE COATING ON THE POSTHARVEST QUALITY OF FRESH GUAVA FRUITS DURING AMBIENT STORAGE CONDITIONS

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**ABSTRACT**

Maintaining quality of fruits is an important task in fresh food retailing throughout the supply chain. Fruits fall under perishable foods because they quickly respond to chemical, physical, and biological changes which lead to quality aspects. Edible coatings are used to prevent the physicochemical changes in fruits during the storage and transportation. In the present study, Casein was chosen as a bio-based edible coating material, enriched with ascorbic acid and was applied on fresh guava fruits to study the delay of ripening and other quality properties. Different concentrations of casein were fortified with 1% of ascorbic acid and applied on whole guava fruits as coating. Fruits were treated with 5% and 10% casein with and without ascorbic acid, fortification process was established to maintain and enrich the vitamin C content in the fruits to reach maximum levels to the consumers. Experimental samples were coded as S1, S2, S3, S4 and sample (So) without coating is considered as control. The fruit samples were stored at (26±1°C) for a period of 16 days. Various physicochemical, biological parameters and microstructural studies were tested to evaluate freshness, nutritional status, and keeping quality during the storage. Fruit ripening, firmness and various visual quality aspects like appearance, defects, and shrinkage rates were studied to understand the physical quality of the fruits upon storage period. During storage, results shows that all casein treated samples were noted with decreased firmness, titratable acidity and delayed chlorophyll content, microbial load while the pH, TSS, carotenoids were increased along the storage when compared with control sample and all the coated samples were found glossy appearance with acceptable flavor. This study prompt that casein is an ideal, promising coating to preserve the quality and extends the post-harvest life of guava fruits.

## 1. Introduction

Guava (*Psidium guajava* L.) is a well-known subtropical fruit grown widely in tropical and subtropical regions of the world. Fruits are rich in vitamin C (260 mg /100g) and a fair source of calcium, phosphorus, iron, and vitamin A (Rashida et al., 1997). Due to its characteristic nature, ripened fruits are very perishable with a very short shelf life ranging from 2-3 days at ambient temperature. Simple, low-cost technologies with improved post-harvest practices will minimize the qualitative and quantitative loss of harvested fruits from field to consumption. Edible coating (EC) is such type of post-harvest practice used to coat whole fruits to reduce the physical and biological reactions responsible for the quality deterioration. Edible coatings are formed a thin layer on the surface of the fruit, can be eaten as whole along with the fruit or can be removed with gentle washing with

water. A biopolymer layer that formed on the surface of the fruits as active layer can also be served as carrier for several bioactive compound-like preservatives, vitamins, mineral, biochemical markers. etc., for their specific need. Recent years, edible coatings claimed as unique advanced preservation technique due to its antimicrobial, antioxidant nature, barrier against transmission of gases ($O_2$, $CO_2$, and ethylene), vapors, and solutes. Edible coatings perform a key role in maintaining quality, Safety, storage and distribution of fresh and processed foods (Daniel et al., 2007). Past years, several researchers were worked or working on edible coatings for fruits and fruit products (Chiabrando and Giacalone, 2016). According to the published literature from scientific database, most of the research was focused on to develop edible coatings from naturally occurring sources i.e., from bio-origin polymers, and they have been used to increase shelf life, carry/transfer the nutrients, and to enhance the physical appearance of the fruits (Lin and Zhao, 2007). Applications were not only limited for fruits and vegetable, and also used for almost all fresh and processed foods like fish, poultry and meat products, dairy products, and other designer foods and flavors. etc., (Bhagath and Manjula, 2019; Krochta et al., 1994). Thorough observations on biopolymers as edible coatings, present study was planned to investigate casein as edible coating material for preservation and fortification of guava fruits.

Casein, a milk protein performs strong mechanical and barrier properties than polysaccharides with its unique coat forming capability. Addition of ascorbic acid (AA) to the casein as a fortificant plays a vital role to improve antioxidant nature of the coatings as well total to maintain antioxidant capacity of the whole fruits as an essential vitamin for humans throughout the supply chain. In general, ascorbic acid content is gradually decreases during senescence in fruits due to the activity of ascorbic acid oxidase so, fortification of coatings with AA is recommended to meet optimum range within the fruits (Hosseini et al., 2018). According to the published data, effects of different edible coatings have been tested on guava fruits and few of them are milk-proteins (Cerqueira and Alleoni, 2011), starch and chitosan solutions (Soares et al., 2011), coating based on potato starch and pectin (Quezada Gallo et al., 2005); cassava starch (2%) formulation with chitosan (2%) and Lippa gracilis Schauer genotypes (Aquino et al., 2015); cassava starch (2%) with cinnamon essential oil (0.01%) (Botelho et al., 2016); 2% chitosan coating extended 12 days shelf-life of guava at 11º C (Hong et al., 2012). Cashew gum and carboxymethyl cellulose based coatings extend the shelf-life of guava for two weeks at ambient temperature (Forato et al., 2015). McGuire and Hallman. (1995) extended shelf life up to 7 days at 12° C by using hydroxypropyl cellulose or carnauba wax coating. Guava coated with xanthan gum and carnauba wax improved storage stability to about 30 days at 10°C (Zambrano-Zaragoza et al., 2013). Additionally, edible coatings such as carnauba wax (Jacomino et al., 2003); candelilla wax (Salinas-Hernández et al., 2010), wax coating (Pal et al., 2004); and miscellaneous formulations like gelatin, triacetin and lauric acid (Fakhouri et al., 2003) have also been tested on guava fruits. However, to the best of our knowledge, there is no report on the use of casein-based coatings to maintain the quality and extend the storage life of guava fruits. Based on the thorough investigation, casein was selected as coat forming proteinaceous substance for the preservation of fresh guava fruits because of its availability, safety and versatility. Various quality parameters were assessed periodically to understand the qualitative and quantitative changes in guava fruits during the storage. Therefore, the chief objective of our research was to assess the potential effect of casein coatings on the storage life of fresh guava fruits during ambient temperature.

## 2. Materials and methods

### 2.1. Materials

Fresh and matured green colored guava fruits (*Psidium guajava* L.) were procured directly from the farm (single farm) with maximum uniform identity. Immediately fruits were brought to the laboratory in carton boxes with straw, where they were sorted out to remove any further immature, misshaped, bruised, diseased, and insect infested fruits. Casein powder was purchased from HiMedia Laboratories, Mumbai and citric acid, ascorbic acid and glycerol were purchased from Merck spl.Ltd, Mumbai, India. All chemicals were maintained as analytical grade and restricted for a single brand.

### 2.2. Preparation of edible coating solution

Two different casein coating dispersions were prepared, based on concentration and fortification of casein. Among the treatments four different samples were developed as 5% and 10% casein coatings without and with fortification of ascorbic acid. The sample without coating is kept as a control. The casein-based edible coatings were produced by dissolving 5g (5%) and 10g (10%) of casein, 10 g of citric acid and 5ml of glycerol (as plasticizer) in 100 ml of distilled water. The prepared solution was kept in autoclave 15 lbs for 15 min. For fortified samples 10g (10%) of ascorbic acid was added to the previously prepared cooled casein coating solution under stirring and further it is used to coat on the fruit.

### 2.3. Preparation of sample

Fruits were cleaned, washed with water and treated with 1ppm chlorinated water solution for 10 minutes and air-dried at room temperature. The Randomly selected guava fruits were divided into five lots (S0, S1, S2, S3, and S4). Each treatment contained 64 fruits. S1, S2, S3, and S4 were dipped in casein coating solution at 5% and 10% respectively without and with fortification for 1 minute. One lot (C) was left without casein treatment as control. The treated fruits were air dried (Hussain et al., 2012) and kept in ambient storage conditions for 2 hrs to set a coat of edible casein on their surface. The coated and control samples were wrapped in blotting paper (filter paper) and each was coded as sample S1, S2, S3, S4 and control(C) with respect to the days on blotting paper and stored at ambient temperature (26± 1ºC) for a period of 16 days for shelf-life studies in the laboratory.

### 2.4. Determination of weight loss

Guava fruits of coated and control samples were weighed with the electronic weighing balance (Shimadzu- ELB300 NO: D515711067, Japan) at an interval of 4 days for the total 16 days storage period and the results were expressed as percent weight loss by using the following formula.

$$\text{Weight loss}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (1)$$

### 2.5. Measurement of fruit firmness

Firmness of whole fruit was measured in the middle on two opposite sides of each fruit using a penetrometer (Digital fruit firmness tester, T.R.Turoni srl, Italy) with a 8-mm diameter cylindrical plunger was used to determine tissue firmness of the whole guava fruits. The mean values for maximum force were reported in Newtons laws (N). Three guavas were used on each replication. The results of three replicates were averaged to produce a single value.

### 2.6. Visual quality

The visual quality of the guava fruit was evaluated by subjective method using a rating scale of 5 points (Thumula, 2006). The parameters for evaluation of the quality of guavas are shown below:

Visual Appearance (1- very poor, 2- Poor, 3- Fair, 4- Good, 5- Very good)

Defects* (5- None, 4- 10%, 3- 10-25%, 2- 25-50%, 1 >50%)

Shrinkage (5- None, 4- 10%, 3-25%, 2-50%, 1 >50%)

*Defects include microbial spoilage, discoloration, pitting and softening

### 2.7. Determination of pH, total soluble solids, titratable acidity, and ascorbic acid

The pH, total soluble solids (TSS) and titratable acidity (TA) have been measured by Islas-osuna et al. (2010) method with slight modifications. 5g of guava pulp was homogenized in 25 ml of distilled water. Then the mixture was filtered using muslin cloth. An aliquot sample was used to measure pH with a pH meter (Eutech instruments, prod-ECPH70042SEU, Singapore). The TSS was measured using a hand refractometer (Erma Inc. Tokyo, Japan) and expressed as brix$^0$. The titratable acidity was determined with 0.1 N NaOH using phenolphthalein as indicator. Guava pulp (3g) from fruit was homogenized using a mortar and pestle (grinder) and then centrifuged at 3500 rpm (Remi centrifuge, CE model, India) for 10 minutes; The supernatant phase was collected and analyzed to determine ascorbic acid content by 2,6-dichlorophenolindophenol titration (Sucharitha et al., 2018).

### 2.8. Total chlorophyll and total carotenoid content

Guava fruits are extracted for chlorophyll in a mortar pestle to a fine pulp with the addition of 20 ml of 80 % acetone. Absorbance was taken at 645 and 663 nm in UV-Spectrophotometer (Sudhakar et al., 2016**;** Arnon, 1949). Total carotenoid contents were evaluated according to Harborne JB, 1973, based on the absorbance values at 480 nm in UV-Spectrophotometer.

### 2.9. Sensorial properties

The acceptability of the samples was evaluated through the standard sensory evaluation techniques. The sensory attributes such as visual appearance, color, taste, texture, flavor and overall acceptability was carried out by selected panel of judges (10 Members) rated on a five-point hedonic scale (5-Excellent, 4-Very good, 3-Good, 2-Fair, 1-Poor).

### 2.10. Microbial growth rate

Total plate count and yeast & molds were determined by using pour plate method throughout the storage period. 10 g of sample was homogenized in 90 ml ringer's solution; other decimal dilutions were prepared from a $10^{-1}$ dilution. The total bacterial count was assessed by using plate count agar as culture media (HiMedia, M001). Petri plates were incubated at 35°C for 48 hrs. Similarly, the yeast and mold count were assessed by using dextrose agar as the culture media (HiMedia, M403). Petri plates were incubated at 30°C ± 2°C for 3 to 5 days (72 hrs). Samples were analyzed in duplicate from 0 to 16th day at an interval of 4 days and microbial counts were counted and expressed as log CFU/g (Chien et al, 2007).

### 2.11. Micro structure

The sample with 3×3 mm was fixed, dried and mounted on aluminium stubs and coated with gold at 5 mA and 1.5 kV using a sputter coater. The microstructures of coated and uncoated samples were analyzed using a scanning electron microscope (Carl Zeiss EVO MA15).

### 2.12. Statistical analysis

All the experiments were carried out in triplicates for the control and experimental samples. The data was subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for the average value of parameter among the five treatments and Tukey's test is used to compare the mean values between pair of samples. Differences were calculated to compare significant effects at $p \leq 0.05$ level using SPSS statistics 22 (IBM).

## 3. Results and discussion

### 3.1. Weight loss

Figure 1 shows the data for weight loss of whole fruits coated with different concentration of casein stored at ambient conditions (26± 1ºC) for a period of 16 days. Significant increase was noted in weight loss over the storage period in all the experimental samples. But the control sample shows higher weight loss (20.4%) at 12$^{th}$ day of storage when compared with the coated fruits. The results revealed that a lesser weight loss in S1 (16.56%) followed by S2 (16.58%),

S4 (16.58%) and S3 (16.97%) samples. A small difference ($p<0.05$) was observed in weight loss within the coated samples at the end of storage period. The reason might be coated casein formed a layer like structure up on the fruit surface and acted like a barrier for water drips and helps to maintain/control the moisture content in fruits for long time. Similar results were observed in a study conducted by Perez-Gago et.al, 2019 on fresh cut apples coated with composite whey protein isolate and bees wax. Researchers found that reduced weight loss was in coated fruits compared with the control samples. According to the Valverde et al. (2005) grapes coated with *aloe vera* gel noted potential reduction in weight loss in comparison with control, indicating that any biopolymeric material can be formed a layer and acted like a shield to control the fruits moisture loss.

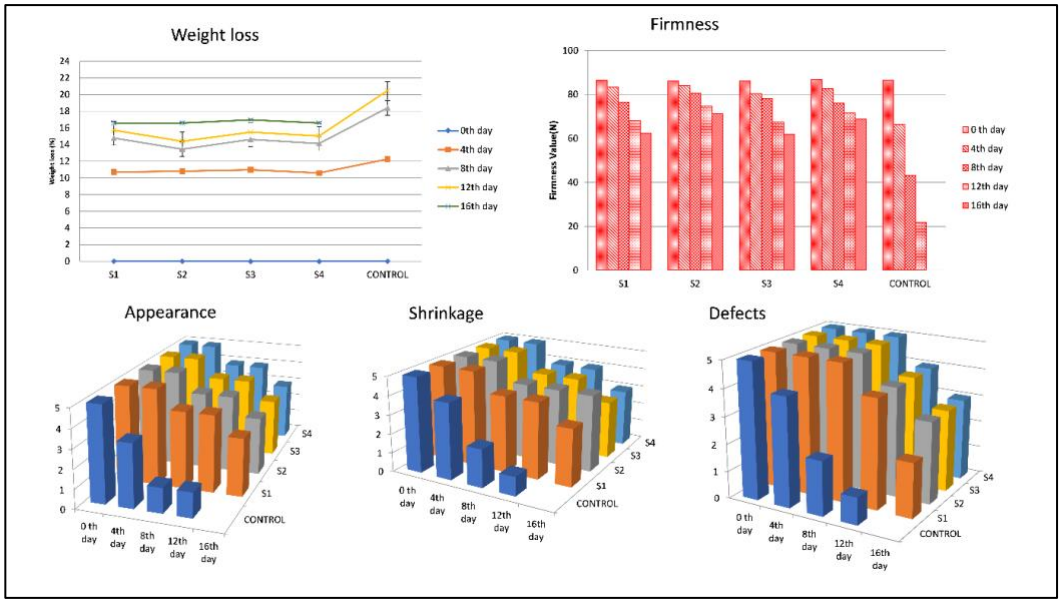


**Figure 1**. Weight loss, firmness, appearance, defects and shrinkage of casein coated whole guava fruits on storage at 26±1$^0$ C.

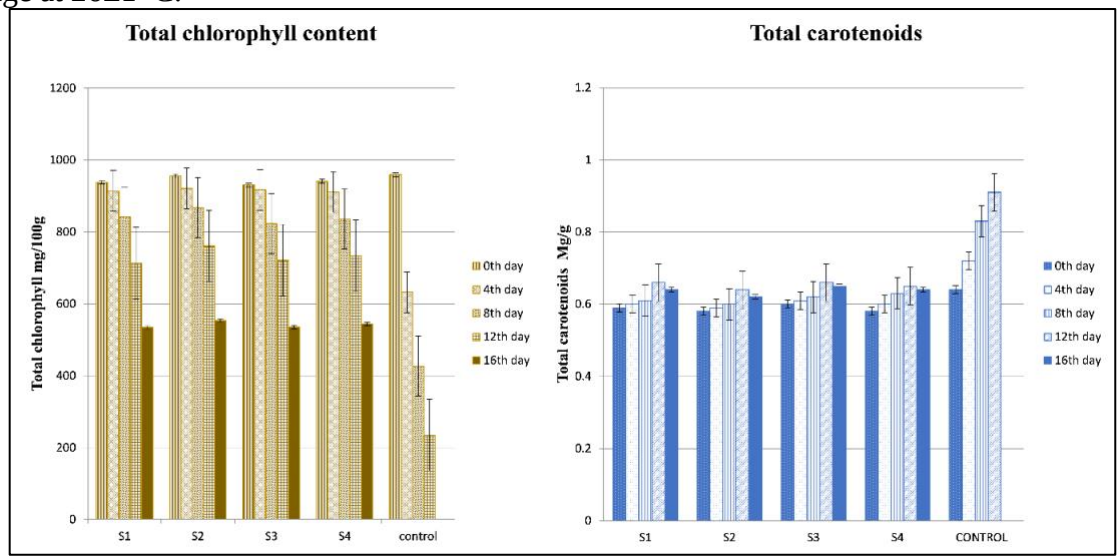


**Figure 2.** Total chlorophyll and carotenoid content of whole guava during the storage at 26±1$^0$ C.

### 3.2. Firmness

The fruit firmness was measured based on the characteristic softening and dissolving of middle lamella in the cell wall. When fruit starts to ripening, the hemicellulose becomes soluble and leads to increased cell wall disruption causes decline in fruit firmness (Wills et al., 1980**;** Wang et al., 2013). In general, firmness of whole guava fruits decreased with the ripening process during the storage (Figure 1). Whereas, casein coated samples maintained stable firmness throughout the storage. During the initial days, control and casein coated samples resembles similar firmness patterns (86.11-86.90 N) in all experimental samples. However, up on storage/end of the storage a significant difference ($P<0.05$) between control and coated samples was observed, difference was also significant in between the different casein concentrations. A slow decreased firmness was noted for all casein coated samples ranges between 61.85 to 71.26 N at $16^{th}$ day of storage. But, when coming to the control sample, firmness was drastically decreased to 21.82 N at $12^{th}$ day of storage which is a un accountable firmness to measure fruit quality. 10% casein (S2) was identified as most effective coating to retain higher value of firmness among the all concentrations. The reason might be the physical shielding of fruits from the external environment give more pleasure to maintain internal biochemical reactions and delayed the production of ethylene and $CO_2$ which accelerates the ethylene production and prevents the shrinkage of fruits mesocarp and stops the sharp firmness decrease in fruits. Coating layer can prevent the water loss leads to the ionic balance in cell walls, and controlled gaseous exchange would help the maintaining the structure and integrity of cell wall components. The results were in accordance with Forato et al., 2015, worked on shelf life of guava fruits coated with cashew gum and methoxy cellulose. Their research study reveals edible coatings can successfully maintained fruits firmness by delaying the ripening process up on storage. In another study, Sánchez et al. (2015) observed that 'Rocha' pear slices coated with high concentrated chitosan resulted higher firmness value indicating the role of edible coatings in preserving textural profile of the fruits.

### 3.3. Visual quality of coated fruits

Visual quality of the casein coated guava fruits were presented in Figure 1 and it shows the optical determination of fruits quality coated with casein at different concentrations and stored at ambient temperature.

#### *3.3.1. Appearance*

For good marketability of any fresh produces like fruits and vegetables, appearance is an important visual determinant quality factor decides consumer perception. In present study the guava fruits were evaluated for quality by their physical appearance. Figure 1 show that the mean score for appearance began to fall from $8^{th}$ day of storage. During the $4^{th}$ day, the uncoated fruit samples scored significantly lower ($p< 0.05$) than the four casein coated samples and continued up to $12^{th}$ day of storage. It was found that the S1, S2, S3, S4 coatings continued better scores at the end of the storage period. This shows that the casein coatings were more compatible to preserve the freshness of the guava fruits.

#### *3.3.2. Defects*

Defects include microbial spoilage, discoloration; pitting and softening of the fruits were evaluated and depicted in Figure 1. According to the picture, higher mean score indicates the lesser the defects and the same was used to determine the degree of defects. No decay was recorded in coated fruits until $11^{th}$ day of storage but, coated fruit samples began to change color from $12^{th}$ day and it is early in the case of control samples. The decaying process was attained maximum in control sample at $12^{th}$ day where coated samples were not observed decaying. There is a significant difference was observed in all coated samples in comparison with control up to $12^{th}$ day. S2, S3, S4 scored better results than the S1, though the means are not statistically different at $16^{th}$ day and it is clear that there are no signs of microbial spoilage till the end of the storage period in casein coated fruits.

### *3.3.3. Shrinkage*

Shrinkage is a physical and optical determinant that decides the physical appearance and freshness of the fruits. Statistical means for shrinkage of whole fruits treated with different concentration of casein with and without fortification of ascorbic acid were presented in the Figure 1. Increased shrinkage was observed during the storage in control samples while coated samples showed without any shrinkage during the initial days. However, shrinking starts from 8$^{th}$ day onwards in all coated fruits, moreover little difference was noted in between coated fruits. Because, concentration and the composition of the casein coatings seems to be influenced the control of shrinkage process in the fruits. Up to the 12$^{th}$ day, it was noted that all coated samples were significantly different when compared to control. However, coated samples also started to shrink during the later stage of storage and it is evident that casein coatings successfully controlled the shrinkage of fruits by creating low relative humidity and preventing moisture and gaseous escape from the fruit tissues during the storage. Due to the physical properties and the plasticizer of thick coatings/films shows wrinkling during storage (Miranda et al., 2004). However, all coated samples did not differ significantly within the storage period. But the S2 casein coated sample scored low when compare with other coated samples.

### *3.3.4. pH of the fruits*

The results from the Table 1 concluded that increased storage period increases the pH from 0 to 16 days with respect to all coated and control fruit samples. However, when compared to control sample, there is a significant difference ($P$<0.05) was noted between the four coated samples from 4$^{th}$ to 16$^{th}$ day of storage. Control sample which was not coated with casein have been completely spoiled on 13th day of storage. Above observations may be concluded that casein coatings showed a significant effect on pH control in whole guava fruits. Coatings can lower the increase in pH during storage period because, it creates an internal modified atmosphere that helps in delay of fruit ripening by arresting various metabolic reactions leads to the control effect on pH of coated fruits. Similar results were reported by Togrul and Arslan. (2004), Maftoonazad et al. (2008), Wani et al. (2014), results shows that edible coatings can controlled the pH levels in both cut and whole fruits.

**Table 1.** Effect of casein coatings and control samples in storage period on p$^{H}$, Total soluble solids (TSS), titratable acidity (TA), Vitamin C (Ascorbic acid) of whole guava

| Parameters | Storage periods (days) | Samples | | | | |
|---|---|---|---|---|---|---|
| | | Control | S1 | S2 | S3 | S4 |
| pH | 0 | 3.71±0.04$^{a}$ | 3.60±0.05$^{a}$ | 3.67±0.00$^{a}$ | 3.59±0.02$^{a}$ | 3.68±0.01$^{a}$ |
| | 4 | 4.23±0.03$^{a}$ | 3.93±0.01$^{bA}$ | 3.85±0.02$^{CA}$ | 3.94±0.01$^{dA}$ | 3.86±0.01$^{eA}$ |
| | 8 | 5.12±0.01$^{a}$ | 4.90±0.00$^{bA}$ | 4.79±0.00$^{CBA}$ | 4.92±0.01$^{dAB}$ | 4.79±0.01$^{eCA}$ |
| | 12 | 5.92±0.02$^{a}$ | 5.72±0.02$^{bA}$ | 5.60±0.00$^{cBC}$ | 5.71±0.00$^{dADE}$ | 5.59±0.00$^{eCCE}$ |
| | 16 | * | 5.92±0.00$^{a}$ | 5.81±0.01$^{bA}$ | 5.91±0.01$^{cBA}$ | 5.82±0.01$^{dAB}$ |
| TSS (%) | 0 | 10.30±0.34$^{a}$ | 10.30±0.28$^{a}$ | 10.10±0.34$^{a}$ | 10.30±0.34$^{a}$ | 10.0±0.15$^{a}$ |
| | 4 | 12.80±0.34$^{a}$ | 10.50±0.34$^{bA}$ | 10.30±0.28$^{cA}$ | 10.50±0.28$^{dA}$ | 10.40±0.28$^{eA}$ |
| | 8 | 15.31±0.28$^{a}$ | 12.47±0.28$^{bA}$ | 12.31±0.28$^{cA}$ | 12.54±0.28$^{dA}$ | 12.46±0.34$^{eA}$ |
| | 12 | 17.44±0.28$^{a}$ | 14.30±0.23$^{bA}$ | 14.0±0.34$^{cA}$ | 14.40±0.28$^{dA}$ | 14.20±0.28$^{eA}$ |
| | 16 | * | 15.31±0.28$^{a}$ | 15.29±0.34$^{a}$ | 15.34±0.28$^{a}$ | 15.32±0.34$^{a}$ |
| | 0 | 1.15±0.34$^{a}$ | 1.17±0.34$^{a}$ | 1.19±0.28$^{a}$ | 1.18±0.34$^{a}$ | 1.15±0.28$^{a}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Titrable acidity(mg /100g) | 4 | 0.98±0.23$^{a}$ | 1.11±0.34$^{a}$ | 1.12±0.28$^{a}$ | 1.13±0.34$^{a}$ | 1.09±0.28$^{a}$ |
| | 8 | 0.81±0.46$^{a}$ | 0.96±0.28$^{a}$ | 0.98±0.23$^{a}$ | 0.97±0.23$^{a}$ | 0.99±0.23$^{a}$ |
| | 12 | 0.69±0.23$^{a}$ | 0.74±0.28$^{a}$ | 0.76±0.34$^{a}$ | 0.75±0.34$^{a}$ | 0.74±0.28$^{a}$ |
| | 16 | * | 0.52±0.28$^{a}$ | 0.59±0.28$^{a}$ | 0.56±0.34$^{a}$ | 0.54±0.17$^{a}$ |
| Vitamin C (mg/100g) | 0 | 238±0.34$^{a}$ | 234±0.40$^{bA}$ | 237±0.46$^{aB}$ | 464±0.40$^{cC}$ | 472±0.40$^{dD}$ |
| | 4 | 208±0.28$^{a}$ | 200±0.28$^{b}$ | 204±0.23$^{c}$ | 412±0.28$^{d}$ | 418±0.34$^{e}$ |
| | 8 | 168±0.28$^{a}$ | 189±0.34$^{b}$ | 196±0.34$^{c}$ | 382±0.46$^{d}$ | 390±0.23$^{e}$ |
| | 12 | 118±0.40$^{a}$ | 152±0.46$^{b}$ | 158±0.23$^{c}$ | 314±0.34$^{d}$ | 316±0.46$^{e}$ |
| | 16 | * | 113±0.46$^{a}$ | 126±0.28$^{b}$ | 290±0.28$^{c}$ | 301±0.23$^{d}$ |

Mean values± standard error. Means with the same letters within a period of storage (rows) are not significant ($p<0.05$), *sample deteriorated

Note: - S1 (casein 5%) and S2 (casein 10%), S3 (casein-5% fortified with ASA) and S4 (casein-10% fortified with ASA) samples.

### 3.4. Total soluble solids (TSS)

Mean values for total soluble solids of whole guava fruits coated with different concentration of casein with and without fortification of ascorbic acid were shown in Table 1, a gradual increase in all samples throughout the storage period was noted. The highest value for TSS was noted in control sample (17. $44^{0}$ brix) in which general ripening process was continued without any hurdles. TSS values for casein coated samples at the end of the storage were likely 15.31 and 15.29º brix for S1 (casein 5%) and S2 (casein 10%) samples, and it is 15.34, 15.32º brix for S3 (casein-5% fortified with ASA) and S4 (casein-10% fortified with ASA) samples. According to the results, a significant difference was observed in all coated samples when compared to control samples from $4^{th}$ to $12^{th}$ day of storage. A research study carried out by Keqian Hong et al. (2012) stated that the edible coated guava fruits resulted lower TSS value than uncoated samples at the end of the storage. The reason may be controlled ripening process was achieved in coated fruits by inhibiting/slow down the production of ethylene, abscisic acid and other growth hormones with in the fruit tissues (Bashir and Abu-Goukh, 2003; Krishna and Rao, 2014). According to the literature, it is well reported that edible coatings can slow down the gaseous exchange and create a modified atmosphere inside the coating further leads to the unavailability of optimum level of $O_2$ and $CO_2$ required for ripening process (Dang et al., 2008; Taşdelen and Bayindirli, 1998). However, a quite suitable edible coatings can slow down the ripening process when compared to uncoated fruits but not completely, and defined phenomena was quite sufficient for fresh whole guava fruits for retailing.

### 3.5. Titratable acidity

Table 1 shows the mean values for titratable acidity (%) of casein coated and uncoated guava fruits stored at ambient storage condition (26±1ºC) upto16 days. The results revealed that there is a significant decline in acidity of fruits up on storage. The maximum value for acidity was observed in S2 (casein 10%) as 0.59% followed by S3 (casein-5 % fortified with ASA) 0.56%. However, recorded values for the S4 (casein-10% fortified with ASA) and S1 (casein 5%) were 0.54 and 0.52% which were little lower than S2 and S3 indicated a little decline in acidity for casein coated samples. The maximum drop was found in control sample (0.69%) at $12^{th}$ day of storage was an evidence for the acidity regulation of casein coating by delaying senescence of the fruits. In general, acidity was drastically increased up on ripening of fruits which was slow down by delaying various growth factors related to the senescence. Han et al. (2004) reported the faster reduction in acidity gives rise to a faster senescence which leads to the rapid quality deterioration of the fruits. However, the different concentrations of casein can effective to maintaining and controlling the

acidity in guava fruits. However, the acidity changes were not significantly differing in coated samples but when compared to the control, drastic acidity changes were noted. Above observations stated that casein edible coatings can be delayed the maturation and ripening as much as possible required in maintaining fruits quality during the storage.

### 3.6. Vitamin C

Ascorbic acid is a well-known organic antioxidant compound named as vitamin C which is an essential vitamin required for the human body. Fruits and vegetables are general and natural source for the vitamin C required for daily intake. Ascorbic acid content in fruits gradually decreases during senescence (Lentheric et al., 1999) and made them unavailable for some times as its heat sensitivity and photolytic nature. So, to maintain stable ascorbic acid levels in whole fruits, casein edible coatings were fortified with different concentrations of Vitamin C. According to the experimental data, vitamin C content was slowly decreased in all experimental samples during the storage. Decreasing trend was little accelerated in control when compared to the coated samples and the difference was significant at $p<0.05$. Changes in ascorbic acid content for the casein coated and control fruits during the storage period were shown in Table 1. The results show gradual decline during storage. The initial ascorbic acid content for the experimental samples was ranged from 234-472 mg/100g. Compared with the control, the casein coating significantly increased the amount of ascorbic acid in the guavas. After 16 days of storage, the ascorbic acid retention for the guavas treated with casein (S1, S2) was 113 and 126 mg/100g. The vitamin C content of fortified ascorbic and casein treatment (S3, S4) was 290 and 301mg/100g, respectively, whereas the control samples maintained 118 mg/100g of the ascorbic content at 12$^{th}$ day of storage period. The addition of ascorbic (10% w/v) to the casein based edible coatings helped to preserve the natural ascorbic acid content in fresh guavas, thus helps to maintain its nutritional quality throughout storage. Same results were identified in a study conducted by Tapia et al. (2008). This concludes that the casein coating creates a modified atmosphere and slowed down the loss of vitamin C during ambient storage temperature. Similar results were reported by Mathooko. (2003) and Ayranci and Tunc. (2004), according to their study edible coatings formed a shield like structure on fruit surface and controlled the vitamin C loss during the storages.

### 3.7. Total chlorophyll and carotenoids

Figure 2 shows the values for Chlorophyll and carotenoid content of whole guavas coated with different concentration of casein edible coatings, In the present study the Chlorophyll content decreased while carotenoid content increased during ripening in guava fruits.

Total chlorophyll value in control sample was reduced rapidly from 959.5 mg/100g to 235mg/100g. Casein coated fruits shows slow decrease in total chlorophyll content from 955 ± 0.34 mg/100 g, fresh weight on initial day to 544.3 ±0.34 mg/g, on 16$^{th}$ day of storage period. The coated fruit samples (S1, S2, S3, S4) significantly ($p<0.05$) delayed the reduction in chlorophyll pigment at end of the storage period in guava fruits. The reduction of chlorophyll content in the guava fruit treated with casein is similar with the result of Keqian Hong et al. (2012) and Soares et al. (2007). The decline in chlorophyll is due to increase in chlorophyll degrading enzymes (chlorophyllase, chlorophyll oxidase, and peroxidase) during ripening. There was a significant difference of carotenoid content in all coated and control sample. In the present study carotenoid content of 0.65 mg/g was observed for control fruit on initial day whereas similar value of 0.65 was observed in coated fruit on 16$^{th}$ day of storage period. There was no significant difference in carotenoid contents among the coated samples at the end of the storage period.

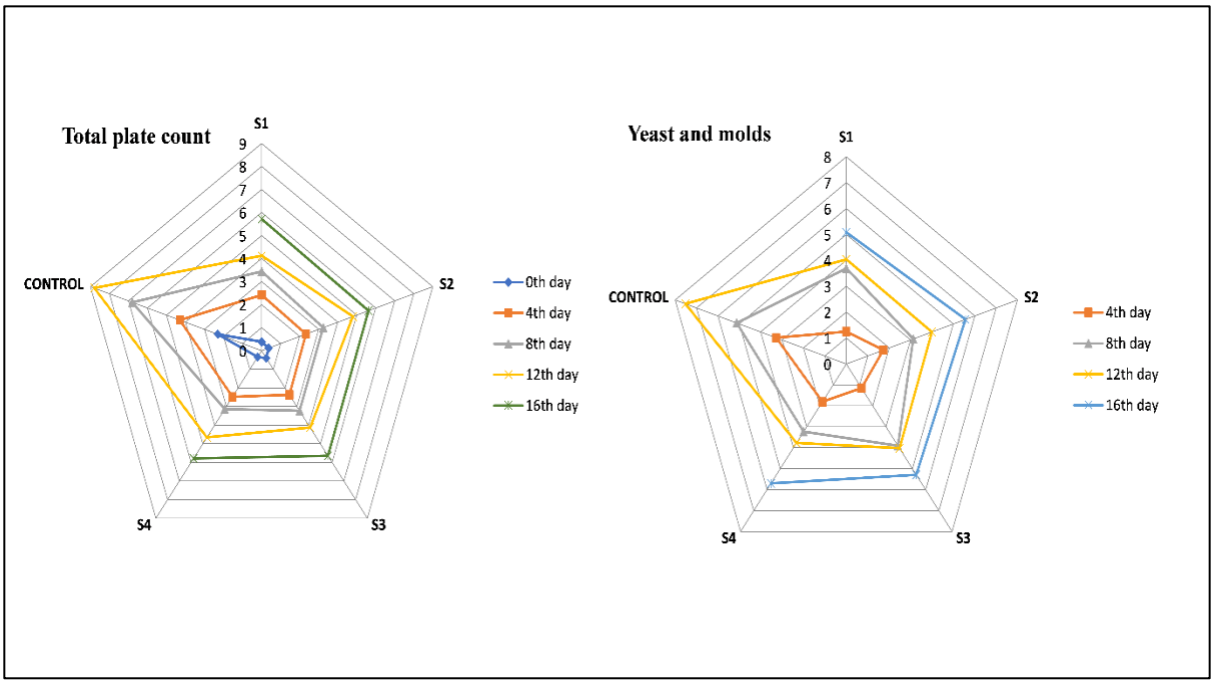


**Figure 3.** TPC and yeast & Molds of casein coated guava fruits during the storage

### 3.6. Sensory quality of casein coated guava fruits on storage

#### *3.6.1. Appearance*

**Table 2.** Effect of different casein coatings and control sample on sensory scores of whole guava fruits during 16 days of storage period.

| Sensory evaluation attributes | Storage periods (days) | Samples | | | | |
|---|---|---|---|---|---|---|
| | | Control | S1 | S2 | S3 | S4 |
| Appearance | 0 | 5±0.46$^{a}$ | 5±0.28$^{a}$ | 5±0.34$^{a}$ | 5±0.40$^{a}$ | 5±0.23$^{a}$ |
| | 4 | 3±0.46$^{a}$ | 4±0.28$^{a}$ | 4±0.34$^{a}$ | 4±0.40$^{a}$ | 4±0.23$^{a}$ |
| | 8 | 2±0.46$^{a}$ | 4±0.34$^{bA}$ | 4±0.23$^{cA}$ | 4±0.40$^{dA}$ | 4±0.28$^{eA}$ |
| | 12 | 1±0.11$^{a}$ | 3±0.28$^{bA}$ | 3±0.34$^{cA}$ | 3±0.40$^{dA}$ | 3±0.23$^{eA}$ |
| Colour | 0 | 5±0.28$^{a}$ | 5±0.28$^{a}$ | 5±0.34$^{a}$ | 5±0.40$^{a}$ | 5±0.23$^{a}$ |
| | 4 | 3±0.46$^{a}$ | 4±0.34$^{a}$ | 4±0.40$^{a}$ | 4±0.23$^{a}$ | 4±0.28$^{a}$ |
| | 8 | 2±0.28$^{a}$ | 4±0.46$^{bA}$ | 4±0.34$^{cA}$ | 4±0.28$^{dA}$ | 4±0.40$^{eA}$ |
| | 12 | 1±0.11$^{a}$ | 3±0.40$^{bA}$ | 3±0.34$^{cA}$ | 3±0.28$^{dA}$ | 3±0.46$^{eA}$ |
| Taste | 0 | 5±0.46$^{a}$ | 5±0.34$^{a}$ | 5±0.28$^{a}$ | 5±0.40$^{a}$ | 5±0.23$^{a}$ |
| | 4 | 3±0.28$^{a}$ | 4±0.34$^{a}$ | 4±0.28$^{a}$ | 4±0.23$^{a}$ | 4±0.40$^{a}$ |
| | 8 | 2±0.28$^{a}$ | 4±0.23$^{bA}$ | 4±0.46$^{cA}$ | 4±0.34$^{dA}$ | 4±0.40$^{eA}$ |
| | 12 | 1±0.11$^{a}$ | 3±0.46$^{bA}$ | 3±0.28$^{cA}$ | 3±0.34$^{dA}$ | 3±0.40$^{eA}$ |
| Texture | 0 | 5±0.46$^{a}$ | 5±0.17$^{a}$ | 5±0.28$^{a}$ | 5±0.40$^{a}$ | 5±0.23$^{a}$ |
| | 4 | 3±0.28$^{a}$ | 4±0.28$^{a}$ | 4±0.23$^{a}$ | 4±0.40$^{a}$ | 4±0.34$^{a}$ |
| | 8 | 2±0.35$^{aA}$ | 4±0.$^{23aA}$ | 4±0.46$^{aA}$ | 4±0.40$^{aA}$ | 4±0.34$^{aA}$ |
| | 12 | 1±0.11$^{a}$ | 3±0.40$^{b}$ | 3±0.46$^{c}$ | 3±0.28$^{d}$ | 3±0.34$^{e}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Flavour | 0 | $5\pm0.28^{a}$ | $5\pm0.17^{a}$ | $5\pm0.40^{a}$ | $5\pm0.46^{a}$ | $5\pm0.23^{a}$ |
| | 4 | $3\pm0.34^{a}$ | $4\pm0.23^{a}$ | $4\pm0.40^{a}$ | $4\pm0.34^{a}$ | $4\pm0.46^{a}$ |
| | 8 | $2.6\pm0.35^{a}$ | $4\pm0.40^{aA}$ | $4\pm0.46^{aA}$ | $4\pm0.23^{aA}$ | $4\pm0.34^{aA}$ |
| | 12 | $1\pm0.11^{a}$ | $3\pm0.34^{bA}$ | $3\pm0.28^{cA}$ | $3\pm0.17^{aA}$ | $3\pm0.28^{eA}$ |
| Overall acceptability | 0 | $5\pm0.28^{a}$ | $5\pm0.23^{a}$ | $5\pm0.34^{a}$ | $5\pm0.40^{a}$ | $5\pm0.46^{a}$ |
| | 4 | $3\pm0.34^{a}$ | $4\pm0.46^{a}$ | $4\pm0.40^{a}$ | $4\pm0.23^{a}$ | $4\pm0.34^{a}$ |
| | 8 | $2\pm0.28^{a}$ | $4\pm0.34^{bA}$ | $4\pm0.40^{cA}$ | $4\pm0.23^{dA}$ | $4\pm0.28^{eA}$ |
| | 12 | $1\pm0.11^{a}$ | $3\pm0.34^{bA}$ | $3\pm0.28^{cA}$ | $3\pm0.34^{dA}$ | $3\pm0.34^{eA}$ |

Mean values± standard error. Means with the same letters within a period of storage(row) are not significant ($p<0.05$), *sample deteriorated
Note: - S1 (casein 5%) and S2 (casein 10%), S3 (casein-5% fortified with ASA) and S4 (casein-10% fortified with ASA) samples.

Means regarding appearance of coated and control samples of guavas kept at ambient storage conditions are given in Table 2. The mean values for S1 and S2 were 5±0.28 and 5±0.34 at 0 day that decreases to 3±0.28 and 3±0.34 at 12th day, respectively. However, the mean values for S3 and S4 were 5±0.40 and 5±0.23 at 0 day that decreases to 3±0.40 and 3±0.23 at the same storage intervals. Maximum decline in appearance was found in control sample as values are ranging from 5±0.46 to 1±0.11 from initial to 12th day of storage period respectively, control sample maintained its shelf life up to 12 days.

***3.6.2. Colour***

Mean values of casein coated guavas for attribute colour was given in Table 2. Maximum decline in colour values was found in S1 as described by values ranging from beginning (Score 5) to the end of the day (Score 4) respectively. All the treatment delayed the decline in sensory quality and extended the shelf life. However, after 12 days of storage the control sample became unacceptable for consumption. The casein coated samples retained the sensory quality. Edible coatings were proven in maintaining the moisture content and keep a fresh appearance (Alikhani, 2014). Coatings can act as the carriers of anti-browning agents and improves the water vapour properties by the addition of glycerol (Rojas-grau et al., 2008)

***3.6.3. Taste***

Mean values regarding taste of coated and control samples of guavas kept at ambient storage conditions are given in Table 2. The mean values for S1 and S2 were 5±0.34 and 5±0.28 at 0 day that decreases to 3±0.46 and 3±0.28 at 12th day, respectively. However, the mean values for S3 and S4 were 5±0.40 and 5±0.23 at 0 day that decreases to 3±0.23 and 3±0.40 at the same storage intervals. Maximum decline in taste was found in control sample as values are ranging from 5±0.46 to 1±0.11 from initial to 12th day of storage period respectively. The control sample maintained its shelf life up to 12 days of storage period.

***3.6.4. Texture***

Mean values of casein coated guavas for attribute texture was given in Table 2. Maximum decline in texture values was found in S1 as described by values ranging from beginning (Score 5) to the end of the day (Score 4) respectively. All the treatments delayed the decline in sensory quality and extended the shelf life. However, after 12 days of storage the control sample became unacceptable for consumption. The casein coated samples retained the sensory quality.

***3.6.5. Flavour***

Mean values of flavour of coated and control samples of guavas kept at ambient storage conditions are given in Table 2. The mean values for S1 and S2 were 5±0.17 and 5±0.40 at 0 day that decreases to 3±0.34 and 3±0.28 at 12th day, respectively. However, the mean values for S3 and S4 were 5±0.46 and 5±0.23 at 0 day that decreases to 3±0.17 and 3±0.28 at the same storage intervals. Maximum decline in flavour was found in control sample as values are ranging from 5±0.28 to 1±0.11 from initial to 12th day of storage period respectively, control sample maintained its shelf life up to 12 days.

***3.6.6. Overall acceptability***

Results of sensory evaluation of guavas coated with casein edible coatings were plotted in Table 2. All the coated samples delayed the

decline in sensory quality and extended the shelf life. Maximum fruit overall acceptability score (3.00) was recorded in all coated samples. With irrespective of the control and coatings there was decrease in overall acceptability as the storage period advanced. There was a significant difference between the control and coated samples at the end of the storage period. The overall acceptability of casein coated guava fruits was best as compared to control. Guava fruits coated with casein edible coatings have greater retention of green colour than the uncoated fruits, shows delay in ripening in coated fruits. The control sample was spoiled on 12$^{th}$ day of storage period. During storage period the panel members found glossy appearance with acceptable flavor.

### 3.7. Microbial quality of casein coated fruits

Fruits are rich in nutrients and contains highest amount of moisture which creates a better environment for the growth of microorganism. The Figure 3 shows the growth curve of total bacterial count in fresh guavas with and without casein coatings, stored for 16 days. Results of the present study shows there was a significant increase ($p<0.05$) in the total plate counts (TPC) and yeast and mold counts (Y&M) during storage period for all samples. There was a significant difference between the TPC and Y&M for casein-based coating samples and uncoated control sample at all storage time. This shows that the incorporation of casein-based formulation into guavas significantly reduced the TPC and Y&M count of coated samples during storage period. However, the growth of microorganisms in the control sample (S0) was higher than the growth with coatings in the 8$^{th}$ day of storage reaching 6.80 log cfu/g for (TPC) and 7.52 log cfu/g for (Y&M) at 12$^{th}$ day of storage. According to the IFST (Institute of food science and technology), $10^6$ cfu/g is considered as limit of acceptance of fruit-based products during the shelf-life period. (Bierhals et al, 2011). The casein coated samples reached $10^5$ cfu/g at 16$^{th}$ day of storage. In the present study the incorporation of 5% and 10% casein without and with fortification decreased the microbial load and extended the shelf life of guava samples (S1, S2, S3, S4) to 16 days at ambient storage conditions.

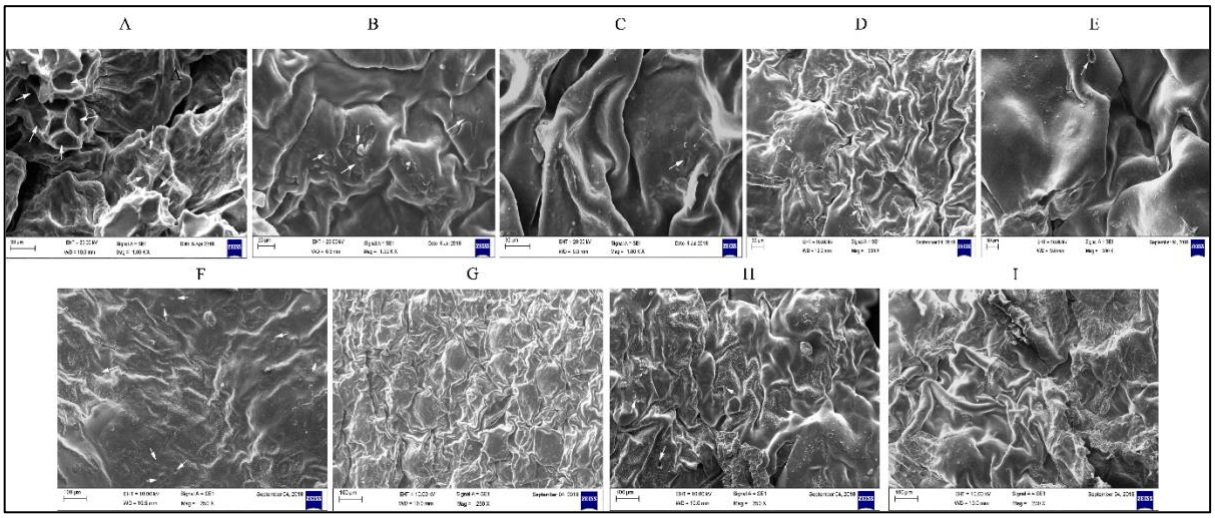


**Figure 4.** SEM micrographs of guava fruits coated with casein and fortified casein at different concentrations (A- control; B-5% casein initial; C-5% casein stored; D-10% casein initial; E-10% casein stored; F-5% casein fortified initial; G-5% casein fortified and stored; H- 10% casein fortified initial; I-10% casein fortified and stored).

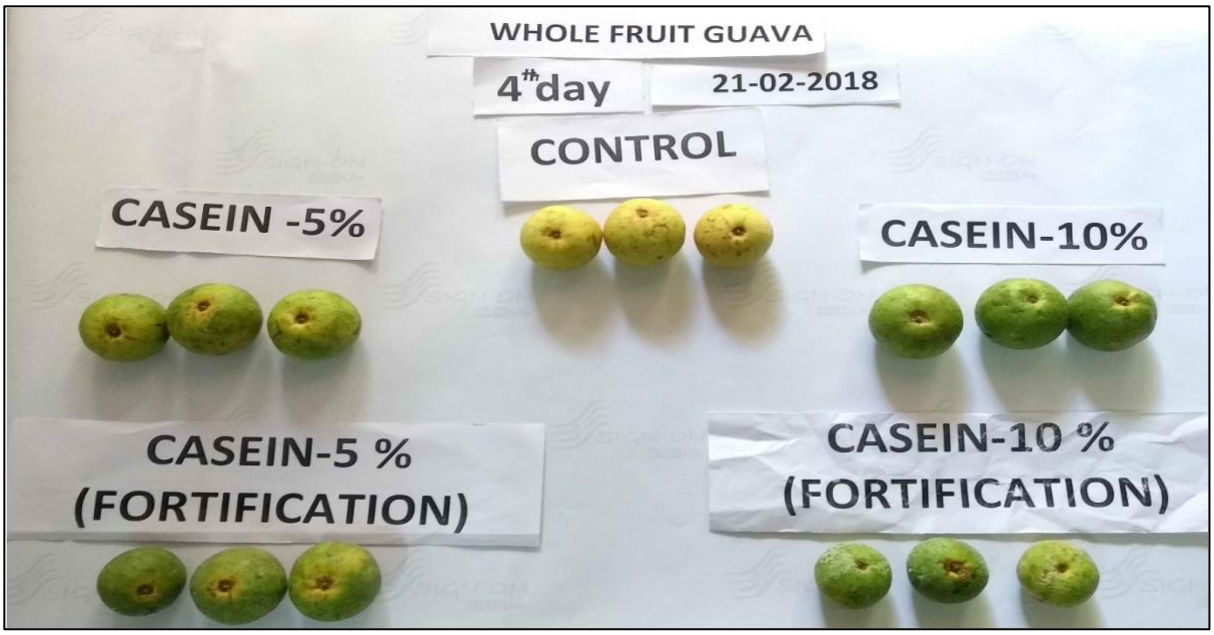


**Figure 5.** Appearance and physical state of casein coated guava fruits at various concentrations during the storage at ambient temperature.

### 3.8. Micro structure and morphology of casein coated fruits

The arrows in Figure 4 indicate micropores and stomatal aperture on guava skin in control, coated and fortified 5% and 10% initial and stored casein coated fruit. The results of scanning electron microscopy clearly show several micropores, rough and disordered surface with cavities on the guava fruit skin surface (S0) which helps in transpiration (Figure 4 (A)). While the guavas coated with 5% (S1) and 10% (S2) casein on the initial day (Figure 4 (B and D)) showed tightly compacted, less pores but few stomatal apertures are still open. After storing for sixteen days the surface of the guava (Figure 4 (C and E)) showed the stomata was entirely sealed and covered with casein coating on outer surface of the guava. The casein coating on the fruit surface produces a more uniform covering which appeared to be smoother, rigid and more ordered structure without cavities. Pereira-Kechinski et al. (2012) proposed that if the natural openings were covered by coating, this could physically prevent the invasion of the pathogen, and therefore reduce disease incidence.

The arrows in Figure 4 (F and H) indicate stomatal aperture on guava fruit surface in casein 5% and 10% fortified (S3) and (S4) initial samples. After storing for sixteen days Figure 4(G and I) the casein coatings on the fruit surface produces a more uniform covering which appeared to be smoother without opening of stomatal aperture. It resembles formation of casein coatings was homogeneously distributed on the surface of guava fruit.

## 4. Conclusions

Casein based coatings with the addition of plasticizer like glycerol and fortifying agent as ascorbic acid was applied as coating for fresh whole guava fruits. All casein coatings act as barrier in retention of fruit quality and helps in loss of vitamin C through fortification. The milk based edible coating is most emerging technique in preserving and extending the shelf life of guavas upto 16 days at ambient storage conditions (26± 1ºC) and also helpful for commercial storage without affecting the nutritional quality during preservation and storage process.

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# PHYTOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF DEVELOPED FREEZE DRIED WATERMELON AND TOMATO POWDER

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**ABSTRACT**

Fruits and vegetables are the prominent source of various nutrients as well as phytochemicals. Due to their higher water activity, they are prone to deterioration. The present study was conducted to prepare freeze dried powder mix of Watermelon(*Citrullus vulgaris*) and Tomato(*Lycopersicon esculentum Mill*).Freeze drying or lyophilization is a method of food dehydration to make high quality food products without altering their colour, texture, flavour and nutritive contents. In this study, the phytochemical(qualitative and quantitative),microbiological and antioxidant capacity and lycopene content have been evaluated. For this analysis the variations of 10%,20%,30%,40% and 50% were prepared. Result shows a gradual increase in TPC, antioxidant capacity and lycopene content. The microbiological (bacterial and fungal) load for each variation was found to be constant for 45 days during 15 days interval.

## 1.Introduction

Fruits and vegetables are the essential sources of vitamins,minerals,fibers and other phytochemicals.A regular consumption of these shortens the risk of chronic diseases.Watermelon belongs to the Cucurbitaceae family, which include melons,cucumbers and pumpkins.Watermelon (*Citrullus vulgaris*) is widely cultivated throughout India . The sweet pulpy flesh of watermelon is highly refreshing and consumed fresh. The watermelon juice forms a cooling and refreshing beverage highly valued during summers. Juice is reported to have diuretic properties and is a good source of potassium and other minerals.Lycopene, a red pigment of the carotenoid class found in only a few fruits and vegetables, is a powerful oxygen radical scavenger and highly effective antioxidant . Watermelon and tomatoes are the most familiar sources of lycopene in the Western diet, containing on average 48.6 and 30.1 µg lycopene/g fresh weight, respectively.The amino acids citrulline and arginine have been studied widely by medical researchers for their usefulness in sickle cell anemia, immune function, wound healing, and cardiovascular health (Watermelons and Health January ;2007).

Tomato (*Lycopersicon esculentum Mill*) belongs to family "Solanaceae" and is a worldwide important agricultural commodity..In terms of area, tomato is the second horticultural product cultivated and the first in industrilized volume.(Filhoz C,et al.;1996).Tomato is a climacteric fruit,with a short shelf-stability under ambient storage conditions.(Shahnawaz M.,et al.; 2012).Drying is the most suitable method to comply with the above requirements. Dried tomato products are used as important ingredients for pizza, various vegetables, spicy dishes.Tomato is an important vegetable crop which grows worldwide. During their peak

season farmer doesn't get good price moreover a big share of crop produce is spoiled and become a waste due to lack of proper processing and storage facilities. However, it can be converted in to some value added products to achieve the greater value in the market .By drying to a certain moisture level the dried fruit and vegetable powders can be an ideal addition to soups, sauces, marinades, baby foods, dips, extruded cereal products, fruit purees, and fillings for frozen toaster snacks. (Srivastava,S,et al.;2013).The antioxidant lycopene is the most abundant carotenoid in tomatoes,which is the point of increasing interest. Presence of lycopene promoted research activities on fresh tomatoes and tomato products.(Bashir, N, et al.;2014).In recent years, the interest is inclining towards the use of these elements as micro-nutrient supplements or functional foods in medical treatment to prevent various diseases such as cancer,cardiovascular diseases, AIDS, Alzheimer's disease, osteoporosis, osteoarthritis, asthma, cataract, fatigue and ageing (Hunt, C.D;1996) .Freeze drying also known as Lyophilization is one of the techniques of dehydration which is suitable for the preparation of high value dried products.In this technique, the product quality did not undergo any change (color, shape, aroma and nutritional value), which is higher than other products obtained by different drying techniques. The method works at low temperature and occurs in the lack of oxygen which help in reducing the degradation reaction (Litvin et al.1998). The freeze dried material are hygroscopic and can be rehydrated more quickly and completely then other dried samples (Giri ,S.K.,et al;2007). There is no such loss of bioactive compounds in this method like flavonoids, flavonols, flavones,catechins and phenolics (Asami ,D.K.,et al, 2003).In freeze drying the mechanism of ice sublimation under low pressure Freeze drying is a costly and labor intensive process but retains the flavor, taste and color of the product when re-introduced into the water.Vegetable and fruit juice powders have many advantages and economic potentials over their liquid counterparts such as reduced volume or weight, reduced packaging, easier handling and transportation, much longer shelf life and nutritional supplements (Pradyuman Kumar (2018). Freeze drying has been widely used in a number of applications for many years. It is commonly used in the food and pharmaceutical industries. There are, however, many other uses for the process including the stabilization of living materials such as microbial cultures, preservation of whole animal specimens for museum display, restoration of books, stabilization of perishable food products by dehydration and other items damaged by water, and the concentration and recovery of reaction products.

## 2. Materials and method.

**2.1.Preparation of sample:**For the preparation of dried fruit powder the watermelon and tomatoes were procured from the local market of Gwalior(Madhya pradesh).The fresh red and ripened tomatoes without any bruises were selected for the sample preparation.The sample was properly washed with clean potable water (with 1% NaCl solution for surface cleaning). The watermelon was cut to remove the rind and converted in to small pieces.The samples were frozen at -35°C for 24 hrs and dried under vacuum condition at -20°C for 24 hrs(only for tomato) and 48 hrs (for watermelon).The freeze dried samples were packed into HDPE pouches and stored at normal conditions.

**2.2.Working sample preparation:** For further analysis the prepared samples were taken in an appropriate quantity and mixed with butanol,methanol and water. The samples were kept on orbital shaker for 24 hrs and filtered by whatsmann filter paper no.4 to be used for further analysis.

### 2.3.Evaluation of secondary metabolites:

#### *2.3.1.Qualitative analysis for tannin:*

Crude extract was mixed with 2 ml of 2% solution of Ferric Chloride. A blue-green black

colouration indicated the presence of phenols & tannins. (1ml sample + 500µl FeCl3)

***2.3.2.Qualitative analysis for saponins:***

Dried powder (1mg.) was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 seconds. 1cm layer of froth stable for 1 min indicates the presence of saponin. (1ml sample + 5ml distilled water).

***2.3.3.Qualitative analysis for flavonoids:***

Extract were treated with few drops of sodium hydroxide solution. Formation of yellow colour which become colourless on addition of dilute acid, indicate the presence of flavonoid. 10% NaOH solution (2ml) + 100 µl sample gives yellow colour.

***2.3.4.Qualitative analysis for Alkaloids:***

To 1 ml of extract, 2-3 drops of Wagner's reagent were added. The appearance of pale or white precipitate indicated the presence of alkaloids.

***2.3.5.Qualitative analysis for phenolics :***

To 1m.g of freeze dried sample, 2 ml of distilled water and few drops of 10% ferric chloride solution were added. Formation of blue or green colour indicates the presence of phenols.(Johri S.,et al.;2017).

***2.3.6.Quantitative estimation of TPC content and Antioxidant activity:***

**Total Phenolic Content:**

The total phenolics in the samples were determined using Folin-Ciocalteu method (Habila J D ., et al.;2010). To each sample solution (1.0 ml) and the standard (gallic acid) was added 5 ml of folin-ciocalteu (sigma-aldrich) and 4 ml sodium carbonate (7% w/v) and shaken. The solution could stand for 30 min in the dark at room temperature, after which absorbance was measured at 765 nm using spectrophotometer (UV-VISIBLE parkin elmer Lambda 25). The phenolic content was calculated from the standard curve of gallic acid.

$$C = (c \times V)/ m \quad (1)$$

Where,
C = total content of phenolic compounds mg/gm plant extract
c = the concentration of gallic acid established from the calibration curve (mg/ml)
V = the volume of extract in ml.
m = weight of sample in g.

**Reducing power estimation :**

The reducing power of methanolic extracts increases with the increased in concentration. The reducing power was best observed at 1mg/ml concentration. The presence of reducers (i.e antioxidants) causes the reduction of Fe3+ or ferricyanide complex to the ferrous form. Therefore, measuring the formation of Prussian blue at 700 nm gives an indication of Fe2+ concentration and the reducing capability (Chauhan N.,et al.; 2019).

The electron donating capacity of the bioactive compounds is defined as the reducing power or the antioxidant activity. The antioxidant property of a compound or extract could be described as a redox reaction in which a reactant species (antioxidant) is reduced by the exposure of the oxidant. Different fraction of aqueous plant extract at various concentration (200 - 1000µg/ml) were prepared.2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferrocyanide was mixed in 1 ml of different extract prepared. The test tubes were incubated in water bath for 10 minutes at 50°C followed by addition of 2.5 ml of 10% TCA and was centrifuged at 3000rpm for 10 minutes. 2.5 ml of upper layer obtained was collected and mixed with 2.5 ml of distilled water followed by addition of freshly prepared 0.5 ml of 0.1% FeCl3. Absorbance was noted at 700nm against a suitable blank.(Ijaz F.,et al.;2017)

**Nitric oxide scavenging activity :**

Extract of different dilutions (0.1 to 1 mg/ml) dissolved in PBS (25mM, pH7.4) were prepared. 200µl sodium nitroprusside (5mM) was added to 800µl of the prepared dilutions. The mixture was then incubated at 37 °C for 2.5 hours under normal light followed by incubation of 20 minutes in dark. 600µl Griess reagent was added followed by incubation for 40 minutes at room temperature and absorbance was noted at 540 nm against a suitable blank(Chauhan N.,et al.; 2019)and

percent of inhibition was calculated by using this equation:

**% Inhibition = (OD of control- OD of extract/OD of control)×100.** (2)

**Total Antioxidant Activity:**

Different dilutions of 1 mg/ml of freeze dried powder were prepared and to it 4 ml of 28 mM sodium phosphate, 4mM Ammonium molybdate and 0.6 M Sulphuric acid was added and were put in capped test tube and were left for incubation in a water bath for 90 min at 95℃. The incubated sample was thereafter cooled to room temperature and the absorbance was measured at 695 nm against blank. Antioxidant activity was expressed relative to that of BHT which was used as standard (Capek I.;2004).

**Lycopene content estimation of freeze dried powder:**

Lycopene has absorption maxima at 473 nm and 503 nm.The standard procedure has been followed to estimate the lycopene content in freeze dried powder. For the analysis 5 gm.of sample was taken.samples were extracted repeatedly with acetone in pestle and mortar until the residue became colourless.The mixture was transferred to a separating funnel containing 10-15 ml. Of petroleum ether.To the petroleum ether extract small quantity of anhydrous $Na_2SO_4$ was added.The collected extracts were measured by Spectrophotometer (Perkin elmer uv vis, lambda 25) at and 473 nm and 503 nm (Slightly modified) using petroleum ether as blank (Rangana S.;2011).

$$mg\ of\ lycopene\ per\ 100\ g = \frac{3.1206 \times OD\ of\ sample \times Volume\ made\ up \times 100}{1 \times Wt\ of\ sample \times 1000} \quad (3)$$

**Statistical Analysis:** All data were reported as mean and standard deviation of three measurements.

**Microbial load:**

Microbiological analysis included isolation,enumeration and identification of bacteria and fungus using standard protocol. Media used for this analysis included nutrient agar, peptone water,EMB agar, mannitol salt agar and sabouraud dextrose agar.For isolation and enumeration of pathogens in samples, lyophilized powder of different concentration is used with 9ml.of sterile peptone water.The 10-fold serial dilution was performed and 0.1ml.of last two dilutions($10^{-4}$ and $10^{-5}$) were inoculated on desired media using plate count technique. Spread plate technique was done by inoculating 0.1ml of the appropriate dilutions on plate count agar plate for enumeration of bacteria and on potato dextrose agar for fungal count. The agar plates were incubated at 30ºC for 24-48h for bacterial count and at 26ºC for 3-5 days for fungal count. Each sample was inoculated in duplicate agar plates and the mean values of bacterial and fungal counts were recorded as colony forming unit per ml (cfu/ml) (Braide W.,et al.;2014).

## 3.Results and discussions

### 3.1.Qualitative estimation of secondary metabolites

Secondary metabolites are numerous organic molecules produced by plant cells through metabolic pathways.These metabolites possess various biological effects.For the present study the qualitative estimation has been carried out for tannins, saponins, flavonoids, phenolics, alkaloids and terpenoids .The methanoic and hot aqueous extractions have been prepared for analysis.Result shows difference in both the of extracts.

**Table 1.** Qualitative phytochemical screening of various dilutions of freeze dried powder**.**

| **Qualitative tests** | **Methanol extract** | **Hot aqueous extract** |
|---|---|---|
| Tannins | ++ | +++ |
| Saponins | + | ++ |
| Phenolics | +++ | +++ |
| Flavonoids | ++ | +++ |
| Alkaloids | ++ | ++ |
| Terpenoids | +++ | ++ |

(+)Fairly present , (++) moderately present,(+++) highly present, (-) indicates absence of compounds

**Table 2.**Quantitative estimation of TPC content and antioxidant activity of various dilutions of freeze dried powder.

| **%Compositions** | **TPC content (GAE/gm.)** | | | **Reducing power(%)** | | | **Total Antioxidant Activity(%)** | | |
|---|---|---|---|---|---|---|---|---|---|
| Extraction | Butanol | Methanol | Aqueous | Butanol | Methanol | Aqueous | Butanol | Methanol | Aqueous |
| 10%(VFI) | 592.5±0.577 | 593.15±2.624 | 550.27±0.573 | 0.351±0.002 | 0.271±0.010 | 0.161±0.003 | 0.107±0009 | 0.200±1.055 | 0.100±0.134 |
| 20%(VFII) | 573.75±0.500 | 582.4±0.969 | 454.02±2.085 | 0.425±0.023 | 0.310±0.416 | 0.192±000 | 0.147±0.018 | 0.110±0.008 | 0.122±0.164 |
| 30%(VFIII) | 460.25±0.500 | 511.37±1.065 | 420.25±0.465 | 0.44±0.014 | 0.352±0.001 | 0.26±0.004 | 0.307±0.412 | 0.212±0.322 | 0.247±0.006 |
| 40%(VFIV) | 421.25±0.957 | 497.75±0.506 | 353.67±0.855 | 0.52±0.016 | 0.444±0.001 | 0.363±0.004 | 0.327±0.005 | 0.277±0.416 | 0.320±0.429 |
| 50%(VFV) | 372±0.816 | 421.97±0.05 | 321.5±0.298 | 0.617±0.828 | 0.607±0.002 | 1.455±0.488 | 0.355±0.01 | 0.310±0.008 | 0.337±0.005 |

*VF=Vegetable and fruit powder

*Data expressed as a mean±standard deviation for three experiments.

**Table 3.** Nitric oxide scavenging activity.

| %Compositions | Nitric oxide scavenging activity(%) | | |
|---|---|---|---|
| | Butanol | Methanol | Aqueous |
| 10% (VFI) | 0.727±0.01 | 0.632±0.009 | 0.501±0.000 |
| 20% (VFII) | 0.612±0.001 | 0.5815±0.004 | 0.490±0.001 |
| 30% (VFIII) | 0.548±0.004 | 0.435±0.000 | 0.435±0.584 |
| 40% (VFIV) | 0.439±0.004 | 0.375±0.001 | 0.3522±0.002 |
| 50% (VFV) | 0.375±000 | 0.281±0.377 | 0.294±0.395 |

*VF=Vegetable and fruit powder

*Data expressed as a mean±standard deviation for three experiments.

**Table 4.** Quantitative estimation of Lycopene content

| S.no | % Compositions | Lycopene content (mg/100g) absorbance at 503nm | Lycopene content (mg/100g) absorbance at 473 nm |
|---|---|---|---|
| 1. | 10%(VFI) | 10.656±0.007 | 9.506±0.228 |
| 2. | 20%(VFII) | 10.812±0.006 | 12.588±0.013 |
| 3. | 30%(VFIII) | 12.107±0.053 | 13.389±0.355 |
| 4. | 40%(VFIV) | 12.529±0.005 | 14.616±0.014 |
| 5. | 50%(VFV) | 12.794±0.002 | 14.664±0.004 |

*VF=Vegetable and fruit powder

*Data expressed as a mean±standard deviation for three experiments.

**Table 5. Microbiological analysis of Freeze dried fruit powder**

| Storage (days) | VFI | | VFII | | VFIII | | VFIV | | VFV | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial Isolates | Fungal Isolates | Bacterial Isolates | Fungal Isolates | Bacterial Isolates | Fungal Isolates | Bacterial Isolates | Fungal isolates | Bacterial Isolates | Fungal Isolates |
| 0 | - | - | - | - | - | - | - | - | - | - |
| 15 | $0.12X10^5$ | $0.09X10^5$ | $0.12X10^5$ | $0.10X10^5$ | $0.14X10^5$ | $0.10X10^5$ | $0.16X10^5$ | $0.10X10^5$ | $0.16X10^5$ | $0.10X10^5$ |
| 30 | $0.12X10^5$ | $0.09X10^5$ | $0.12X10^5$ | $0.10X10^5$ | $0.14X10^5$ | $0.10X10^5$ | $0.16X10^5$ | $0.10X10^5$ | $0.16X10^5$ | $0.10X10^5$ |
| 45 | $0.12X10^5$ | $0.09X10^5$ | $0.12X10^5$ | $0.10X10^5$ | $0.14X10^5$ | $0.10X10^5$ | $0.16X10^5$ | $0.10X10^5$ | $0.16X10^5$ | $0.10X10^5$ |

***VF=Vegetable and fruit powder**

### 3.2.Quantitative estimation Total Phenolic content

The total phenolic content was calculated as mg. Gallic acid equivalent (GAE) of freeze dried powder.The extract was made in butanol, methanol and aqueous solution.As results shown in Table:II,the total phenol content was obtained in the range of 592.50±0.577(10%composition) to 372.00±0.816(50%composition) in butanoic extract.In metanoic and aqueous extracts the phenolic content was analyzed from 593.115±2.624 to 421.97±0.05 and 550.27±0.573 to 321.50±0.298 (in 10-50% composition) respectively.The result in Table:II shows five respective formulations, the total phenolic content decreases with the percent of watermelon percent in the composition.The highest percent of total phenol has been observed in methanoic extract and lowest in aqueous extract.

### 3.3.Reducing power estimation

The electron donating capacity of a bioactive compound is defined as reducing power.The reducing power of freeze dried powder was measured by preparing butanoic,methanoic and aqueous extracts.It was found that the butanoic extract had the highest reducing power while the aqueous extract had the lowest.In the results Table:II, the reducing power was found from 0.351±00.02 to 0.617±0.828 in butanoic extract,0.271±0.010 to 0.607±0.002 in methanoic extract and 0.161±0.003 to 1.455±0.488 in aqueous extract respectively.

### 3.4.Nitric Oxide scavenging activity :

Nitric Oxide reacts with oxygen to produce stable products like nitrate and nitrite,under aerobic conditions.By using Griess reagent we can determine the quantities of them.Free radical scavengers are substance like antioxidants,that help to prevent cells from being damaged by free radicals.As above the extracts were prepared in different solvents.From observations shown in Table:III, the increase in concentration leads to decrease in absorbance for all the five variations.Therefore it can be presumed that tomato and watermelon have Nitric Oxide scavenging activity.

### 3.5.Total antioxidant capacity and Lycopene content:

The total antioxidant capacity is the capacity of an antioxidant to suppress the harmful effect of free radicals in blood and cells.The results of total antioxidant capacity can be observed in Table:IV(observations in percent inhibition),For extraction butanol,methanol and distilled water were taken as solvents.The antioxidant capacity of different compositions was found in increasing order.The highest value was found in butanoic extract and lowest in methanoic extract.The range was observed from 0.107±0.009 to 0.355±0.01(butanoic extract),0.200±1.055 to 0.310±0.008 (methanoic extract) and 0100±0.134 to 0.337±0.005 (aqueous extract) respectively.

From the above observations it can be concluded that the antioxidant capacity is due to the presence of lycopene content and other secondary metabolites present in tomato as well as in watermelon.

Lycopene is a red pigment generally found in fruits and vegetables. It is a carotenoid pigment which has antioxidant properties.Fresh tomato or tomato products and watermelon both are prominent sources of lycopene pigment.The results shown in Table:IV,a gradual increase was found in different compositions.

The highest content of lycopene was observed in 50% composition (12.794±0.002).The lycopene content of freeze dried powder varied in the range of 10.656±0.007-12.794±0.002 respectively at 530nm.At 473nm. The observations were noted in the range of 9.506±0.228 -14.664±0.004. It can be stated that with increase in tomato powder the lycopene content also increases.A transmittance spectrum of lycopene in petroleum ether acquired by the Spectrophotometer (Perkin elmer uv vis, lambda 25) 473 nm and 503 nm, and the maximum absorbance was at 473 nm. (Davies, 1976).

### 3.6.Microbiological analysis

For microbiological study standard procedures have been followed to identify bacterial and fungal load.The microbiological

analysis is a tool to establish the relationship between development and handling of the finished product to identify minimal load of microorganisms.The standard load count has been observed at a duration of 15 days(the observations has been taken for 45 days).Number of bacteria and fungus were calculated per ml.or grams of sample by dividing the number of colonies by dilution factor.Colony forming unit(cfu) is a measure of viable bacterial and fungal cell.As shown in Table:V the bacterial count at 15 days interval remained constant ($0.12X10^5$) for variation I, ($0.12X10^5$) variation II,($0.14X10^5$ )Variation III, 0.($16X10^5$) Variation IV and ( $0.16X10^5$) Variation V.The fungal count at 15 days interval also remained constant. It was found ($0.09X10^5$)Variation I, ($0.10X10^5$ ) Variation II, ($0.10X10^5$) Variation III,( $0.10X10^5$) Variation IV and ( $0.10X10^5$) V till 45 days.

## 4.Conclusions

Food preservation is an essential process to increase the shelf life of food.Fruits and vegetables are susceptible to deterioration due to the presence of high water activity.Freeze drying or lyophilization is a technique to suppress the water activity by sublimation of water.As compared to other drying techniques this method applies low temperature and pressure which automatically reduces the chance of reduction in nutritional as well as esthetic qualities of food product.In the present study the observation shows that the freeze dried powder is containing saponins, flavonoids, tannins, phenols, alkaloids and terpenoids respectively.

The quantitative analysis has also been done which evaluates the moderate quantity of secondary metabolites and antioxidant activity.Tomato and watermelon both are rich in lycopene content naturally,which has been reported in this study.This can be concluded that such powders can be consumed by different age groups which may help to bring natural health benefits to the society.

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# DRYING OF ONION PASTE TO DEVELOP POWDERS BY FOAM-MAT DRYING PROCESS USING SOY PROTEIN AS FOAMING AGENT

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**ABSTRACT**
Onion (*Allium cepa*) is locally called piaz. It is a biennial, herbaceous, winter seasoned and cross-pollinated bulb crop belonging to Family *Alliaceae.* High moisture content of onion render it to be affected by microbial and enzymatic spoilage. Drying is a very effective way to preserve onion for a long time. Onion powder was prepared by foam mat drying technique in which onion paste was treated with different concentration of soy protein (0%, 4%, 8% and 12%) as foaming agent and Carboxyl methylcellulose (0.5%) as foam stabilizer and these were dried in hot air tray drier at different temperatures (55°C, 65°C and 75°C) with 3mm sheet thickness of onion foams. Effect of different concentration of foaming agent and drying temperature was studied on moisture loss drying rate of onion paste. Increase in concentration of foaming agent significantly increased the drying rate from 0.422± 0.169 (Control) to 0.744± 0.169 (soy protein). Foamed onion paste were dried faster than un-foamed which decreased the drying time of 5 hours for foamed onion paste at 65°C and 75°C. Foamed onion pastes were dried in 300, 240 and 300 min at 55°C, 65°C and 75°C temperature respectively, with 12 % concentration of soy protein as foaming agent while un-foamed pastes were dried in 600, 420 and 480 mints at 55°C, 65°C and 75°C temperature respectively. Soy protein 12% and 65°C drying temperature was found best for drying of onion paste to develop powder.

## 1. Introduction

Preservation of food has been a keen interest of human beings to increase the shelf life and to make the availability of food material for a long time. In the season fruits and vegetables are available in surplus amount and if these food materials are not preserved by any mean then it may result into wastage off these food materials. It is reported that in developing countries almost 40% of our agricultural products are wasted due to lack of proper processing, preservation and storage facilities for these produce (Lombard, *et al*., 2008). These food spoilages may come from many sources during harvesting, handling, processing and storage of foods but most important are microbial and chemical spoilages (Gram, *et al*., 2002). Vegetables are considered most susceptible to spoilage due to the microbial attack and various kind of chemical changes (Tournas. 2005). And if these spoiled foods which harbor a high load of microbes are consumed result in to several kind of human health complication because of foodborne pathogens in spoiled food materials (Abadias, *et al*., 2008). Different kind of the microbes highly dependent upon the availability of water and the vegetables containing higher water activity have much more chances to be spoiled because of these microorganisms.

Dehydration or drying is the most efficient way to reduce the water activity of these kind of vegetables to prevent their spoilage (Mayor, *et al*., 2004; Koc, *et al*., 2008). There are number of food preservation techniques such as canning, curing, fermenting or acidifying and dehydration. Among all of these dehydration is most important and widely used because of being cost effective in term of packaging, storage and transportation of food material (Chavan and Amarowicz. 2012). Dehydration is one of the oldest method of food preservation in which water is removed or made unavailable in food materials. As water is the main component of the food which is required for microbial and enzymatic activity, more over chemical reactions also take place in availability of water. So, that is a water activity of different vegetables which define their stability (Farkas. 2007).

Market value of dehydrated vegetables is increasing in many countries due their stability and longer shelf life as compare to fresh vegetables (Zhang *et al*., 2006). Vegetables are dried by application of heat which evaporate their water contents. There are different methods for drying of vegetables like as sun drying, freeze drying, microwave drying, vacuum drying and infrared drying. Vegetables have many compounds such as phenolic and vitamins and these compounds are very sensitive to high temperature that's why selection of the drying technique depends upon final quality of end product, cost and many others factors, which should be kept in mind during selection of appropriate drying technique (Sagar and Kumar. 2010). A new technique name as foam-mat drying which is highly suitable for those foods which are sticky, very viscous and sensitive to high temperature, variety of food material can be dried by this technique with minimum quality changes (Kadam *et al.,* 2010).

Many reserachers conducted experiments to study the foam-mat drying process for (Kadam *et al.,* 2012) pineapple, (Dehghannya *al.,* 2019) lime juice, (Sankat *et al*., 2004) banana, (Zheng *et al.,* 2009) black currant pulp and (Alakali*et al.,* 2009) mango pulp to develop powders. All they have found that drying with foaming treatment increased the drying rate and minimized the quality changes by decreasing the water activity of powders. Our present study is also about foam-mat drying of onion paste and to investigate the effect of different concentration of soy protein as a foaming agent on moisture loss and drying rate of onion paste.

## 2. Material and method

### 2.1. Procurement and preparation of raw material

Onions were purchased from a local vegetable market in Faisalabad, sorted for good quality without bruises, cuts and microbial attacks. Which were peeled off, washed and grinded with grinder and converted into paste in fruits and vegetable lab at National Institute of Food Science and Technology, University of Agriculture Faisalabad. Soy protein was purchased from a scientific store (Abdullha Traders) Faisalabad and used as a foaming agent in different concentration.

**Figure. 1.** Cutting of onion into pieces

**Figure 2.** Grinding of onion

**Figure 3.** Onion paste

### 2.2. Development of onion foams by soy protein

Onion paste weighing 200g was taken for each experiment and treated with different concentration of soy protein (0%, 4%, 8% and 12%) as foaming agent. Carboxyl Methyl cellulose (0.5%) was used as a foam stabilizer. Onion paste, foaming agent and foam stabilizer in determined concentration were mixed in a 1000ml beaker and beating was done for 3 minutes to increase surface area of onion paste by developing stable foams with incorporation of maximum amount of air in onion paste by using a small scale hand beater used in kitchen for beating of eggs.

### 2.3. Foam spreading in trays and drying

Foams of onion paste subjected to different concentration of soy protein (0%, 4%, 8% and 12%) as foaming agent were spread to 3mm sheet thickness on aluminum foils and placed in stainless steel trays. Commercially available hot air tray dryer (Model # R-5A, Serial # 10-213, Commercial dehydrator systems, Inc.) was used for drying experiment in fruits and vegetable lab at National institute of food science and technology, University of Agriculture Faisalabad, Punjab, Pakistan. Drying was carried out in 3 batches, first batch was dried at 55°C comprising on four samples, one (Controlled) not treated with any foaming agent while three others which were treated with 4%, 8% and 12% concentration of soy protein respectively. All the four samples were prepared again with same above mentioned concentrations of foaming agent and dried at 65°C and 75°C. During drying experiment after each 60 minutes' weight of all samples were recorded and when constant weight was appeared, all the samples from the dryer were removed and placed in desiccator.

**Figure 4.** Onion foams

**Figure 5.** Foam spreading in trays


**Figure 6.** Drying of onion foams in tray dryer

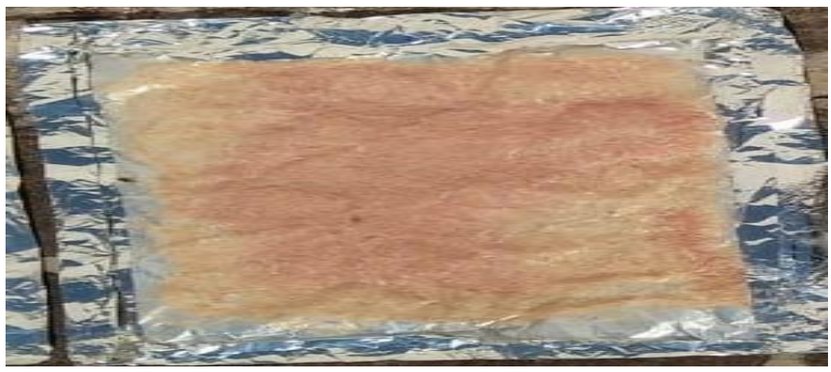

**Figure 7.** Dried onion foam sheet

### 2.4. Milling and storage of powder

All the samples were removed from the desiccator and grounded in a grinder to develop free flowing powders. All the samples were stored at room temperature in polythene bags.

**Figure 8.** Dried onion powder samples

### 2.5. Moisture loss and drying rate

During the drying experiments of onion foams developed by different concentration of soy protein as foaming agent at different drying temperatures. Weight of each sample was recoded after an hour by a weighing balance, which was used to determine the decrease in moisture content of onion foams during drying process. Initial moisture content in onion foams were calculated by AOAC. (2017) standard method of moisture calculation. Final moisture content of onion powders was also measured. By using the data of decrease in moisture of different samples drying rate was calculated by using $\Delta X/\Delta t$.

### 2.6. Drying curves

Drying curves for moisture loss and drying rate was plotted by using the data of moisture loss X from onion foams and drying rate $\Delta X/\Delta t$ verses time. Drying curves give information to know about exact drying time where these onion foams are dried. By these drying curves we can have idea about the best concentration of soy protein and temperature where sample dried in minimum time.

### 2.7. Physicochemical analysis of onion powder

**Moisture content**

Moisture content of all onion powder samples were determined by a standard method for moisture determination described by AOAC. (2017). 5g of powder sample was taken into known weight of china dish. China dish along with sample was placed in an oven for overnight at 105°C or till constant weight of sample. Then moisture content was calculated by following formula.

$$\text{Moisture content \%} = \frac{\text{Weight of fresh sample} - \text{weight of dried sample}}{\text{Weight of fresh sample}} \times 100 \quad (1)$$

### 2.8. Total Soluble Solids (TSS)

Refractometer was used for the measurement of Total soluble solid (°B, Degree brix) in foam-mat dried rehydrated onion powder samples.

**Figure 9.** Rehydrated onion powder samples

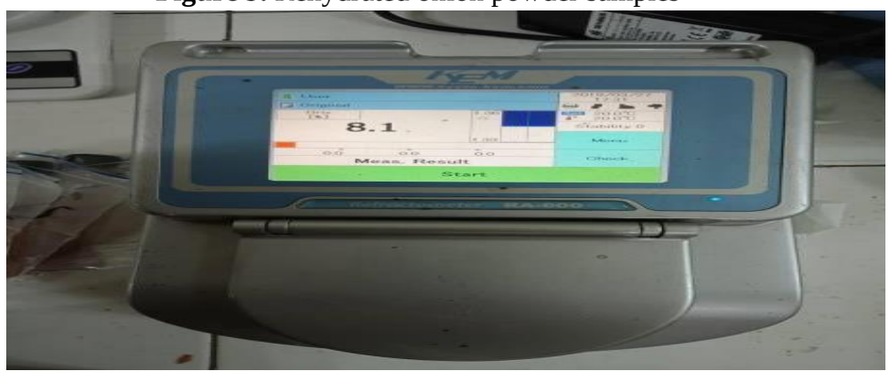


**Fig. 10.** Refractometer for total soluble solids determination

### 2.9. Water solubility index (WSI)

Solubility of the foam-mat dried onion powders was calculated by a method used by Flade and okocha. (2010). Solubility of onion powders which were developed by treatment of different concentration soy protein as foaming agent was determined at Food and Nutraceutical lab, Food Science department of University of Agriculture Faisalabad, Pakistan. Onion powder weighing of 2g was taken and dissolved in 20ml of distilled water by stirring for 3 mints. Then poured rehydrated onion powder solution into 20ml centrifuge tube and performed centrifugation for 3 mints at 3000 rpm. Then after centrifugation wet samples were dried in oven at 105°C.Dried samples were weighted and recorded. Weight of dried samples were used to calculated water solubility.

### 2.10. Water absorption index (WAI)

Water absorption capacity was determined by a method described by Falade, *et al.* (2010). Water absorption of onion powders was determined at Food and Nutraceutical lab, Food Science department of University of Agriculture Faisalabad, Pakistan. Onion powder weighing of 1.5g was taken and dissolved in 20ml of distilled water by stirring for 3 mints. Then poured rehydrated onion powder solution into 20ml centrifuge tube and performed centrifugation for 3 mints at 3000 rpm. Then after centrifugation wet samples were weighted and dried in oven at 105°C.Dried samples were weighted and recorded. Weight of dried samples were used to calculate water solubility.

### 2.11. Statistical analysis

The data of each parameter was analyzed by two-way ANOVA statistical analysis to determine the level of significance according to the method defined by Montgomery, (2008).

## 3. Results and discussions

Onion powder was produced by using soy protein in different concentration levels. Data for moisture loss for onion powders developed by different concentration levels of soy protein as foaming agent for which values are given in table 1, 2 and 3 which were used to draw drying curves for moisture loss which can be seen in fig. 11,12 and 13 for drying at 55°C, 65°C, and 75°C. From the available data it was observed that moisture content decreased with time. Analysis of variance (ANOVA) showed highly significant effect of foaming agent and temperature ($P<0.01$). Foaming treatment resulted in faster drying as compare to non-foamed onion paste drying. Drying was fast for all above experiments in which foaming agents was used. Initially 200g onion paste sample was taken which contained 184g of moisture content and dried to achieve constant weight in try drier at 55°C, 65°C and 75°C. At 55°C onion paste without foaming agent (Control) take 10 hours to dry while onion pastes subjected to different concentration of soy protein dried faster and saved 5 hours which can be seen in table 1. As foaming treatment increases the surface area for drying which is resulted in better and faster removal of moisture content from onion paste. While removal of moisture from un-foamed onion paste was slow because of dense structure which resulted in slow moisture reduction. Increase in concentration of foaming agent resulted in faster drying. Fastest drying of onion paste at 55°C was observed for 12% concentration of soy protein as foaming agent. As indicated in fig. 11, 12 and 13 increase in temperature resulted in significantly drop in moisture content of onion powders.

Dehghannya. (2019); Kadam *et al*., (2011) reported similar results. In this research descending trend in moisture content of onion powders were observed with increase in temperature for which values are given in table 1, 2 and 3. Drying rate was calculated and used to draw drying rate curves for which data is given in table 4, 5 and 6. Drying rate for the onion powder at 55°C, 65°C and 75°C were evaluated by drying curves shown in fig. 14, 15 and 16 for drying at 55°C, 65°C and 75°C. Drying curves were drawing with the data on the rate of drying versus time. Falling rate period was observed with passage of time because in start of the drying onion paste contains very high water content while with the passage of time during drying water content decrease and it became hard to remove moisture from inside of the sample. Hence, drying rate decreases with time. Mean values for drying rate of onion paste dried at 55°C, 65°C and 75°C by using different concentration of soy protein (4%,8% and 12%) as foaming agents are given in table 7. From the data it is shown that highest drying rate for soy protein 0.744± 0.169 at 65°C with 12% concentration.

**Table 1.** Moisture loss from onion paste drying at 55°C temperature using different concentration of soy protein as foaming agent

| **Drying of onion paste at 55°C temperature** | | | | | |
|---|---|---|---|---|---|
| **Sr.No.** | **Time,t(min)** | **Moisture content , X ( g moisture/g dry solid)** | | | |
| **Treatments** | | **$T_0$ (Control)** | **$T_1$(SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 0 | 183 | 183 | 183 | 183 |
| 2 | 60 | 154 | 143 | 135 | 123 |
| 3 | 120 | 127 | 112 | 101 | 73 |
| 4 | 180 | 105.3 | 82 | 67 | 36 |
| 5 | 240 | 84 | 62 | 44 | 8 |
| 6 | 300 | 64.7 | 42 | 21 | 0 |
| 7 | 360 | 48.4 | 24 | 0 | |
| 8 | 420 | 32 | 4 | | |
| 9 | 480 | 18.5 | 0 | | |
| 10 | 540 | 9 | | | |
| 11 | 600 | 0 | | | |

**Table 2.** Moisture loss from onion paste drying at 65°C temperature using different concentration of soy protein as foaming agent

| **Drying of onion paste at 65°C temperature** | | | | | |
|---|---|---|---|---|---|
| **Sr.No.** | **Time,t(min)** | **Moisture content , X ( g moisture/g dry solid)** | | | |
| **Treatments** | | **$T_0$ (Control)** | **$T_1$ (SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 0 | 183 | 183 | 183 | 183 |
| 2 | 60 | 143.2 | 135 | 123 | 121 |
| 3 | 120 | 109 | 95.12 | 81 | 78 |
| 4 | 180 | 78 | 55.7 | 42 | 35 |
| 5 | 240 | 53 | 19.7 | 6 | 0 |
| 6 | 300 | 31 | 0 | 0 | |
| 7 | 360 | 11 | | | |
| 8 | 420 | 0 | | | |

**Table 3.** Moisture loss from onion paste drying at 75°C temperature using different concentration of soy protein as foaming agent

| **Drying of onion paste at 75°C temperature** | | | | | |
|---|---|---|---|---|---|
| **Sr.No.** | **Time, t (min)** | **Moisture Loss, X ( g moisture/g dry solid)** | | | |
| **Treatments** | | **$T_0$ (Control)** | **$T_1$ (SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 0 | 183 | 183 | 183 | 183 |
| 2 | 60 | 140.3 | 125 | 121 | 113 |
| 3 | 120 | 113 | 93 | 85 | 71 |
| 4 | 180 | 88 | 63 | 53 | 35 |

| | | | | | |
|---|---|---|---|---|---|
| 5 | 240 | 67 | 39 | 25 | 6 |
| 6 | 300 | 46 | 15 | 0 | 0 |
| 7 | 360 | 26 | 0 | | |
| 8 | 420 | 8 | | | |
| 9 | 480 | 0 | | | |

**Table 4.** Drying rate of onion paste at 55°C using different concentration of soy protein as foaming agent

| **Sr. No.** | **Time** | **Drying rate at 55°C, N (g/cm$^2$min)** | | | |
|---|---|---|---|---|---|
| **Treatments** | | **$T_0$ (Control)** | **$T_1$ (SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 60 | 0.453± 0.025 | 0.656± 0.012 | 0.790± 0.010 | 0.986± 0.015 |
| 3 | 120 | 0.423± 0.025 | 0.520± 0.020 | 0.560± 0.010 | 0.783± 0.042 |
| 4 | 180 | 0.350± 0.017 | 0.503± 0.015 | 0.563± 0.012 | 0.660± 0.035 |
| 5 | 240 | 0.346± 0.025 | 0.306± 0.021 | 0.380± 0.020 | 0.440± 0.017 |
| 6 | 300 | 0.330± 0.017 | 0.303± 0.023 | 0.393± 0.015 | 0.120± 0.017 |
| 7 | 360 | 0.286± 0.016 | 0.283± 0.015 | 0.350± 0.020 | 0 |
| 8 | 420 | 0.243± 0.025 | 0.330± 0.010 | 0 | |
| 9 | 480 | 0.193± 0.023 | 0.070± 0.010 | | |
| 10 | 540 | 0.130± 0.026 | 0 | | |
| 11 | 600 | 0.156± 0.011 | | | |
| 12 | 660 | 0 | | | |

**Table 5.** Drying rate of onion paste at 65°C using different concentration of soy protein as foaming agent

| **Sr. No.** | **Time** | **Drying rate at 65°C , N (g/cm$^2$min)** | | | |
|---|---|---|---|---|---|
| **Treatments** | | **$T_0$ (Control)** | **$T_1$ (SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 60 | 0.650± 0.010 | 0.806± 0.020 | 1.013± 0.015 | 1.006± 0.020 |
| 3 | 120 | 0.570± 0.010 | 0.630±0.026 | 0.650± 0.026 | 0.710± 0.017 |
| 4 | 180 | 0.503± 0.015 | 0.663± 0.005 | 0.626± 0.025 | 0.693± 0.023 |
| 5 | 240 | 0.423± 0.050 | 0.593± 0.005 | 0.610± 0.034 | 0.566± 0.011 |
| 6 | 300 | 0.346± 0.025 | 0.323± 0.030 | 0.596± 0.015 | 0 |
| 7 | 360 | 0.306± 0.020 | 0 | 0 | |
| 8 | 420 | 0.153± 0.025 | | | |
| 9 | 480 | 0 | | | |

**Table 6.** Drying rate of onion paste at 75°C using different concentration of soy protein as foaming agent

| **Sr.No.** | **Time** | **Drying rate at 75°C, N (g/cm$^2$min)** | | | |
|---|---|---|---|---|---|
| **Treatments** | | **$T_0$ (Control)** | **$T_1$ (SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 60 | 0.700± 0.010 | 0.970± 0.020 | 1.016± 0.011 | 1.143± 0.030 |
| 3 | 120 | 0.453± 0.011 | 0.516±0.023 | 0.600± 0.020 | 0.703± 0.015 |
| 4 | 180 | 0.436± 0.015 | 0.493± 0.011 | 0.523± 0.011 | 0.586± 0.011 |
| 5 | 240 | 0.340± 0.017 | 0.416± 0.015 | 0.473± 0.015 | 0.486± 0.005 |
| 6 | 300 | 0.333± 0.015 | 0.413± 0.032 | 0.416± 0.005 | 0.103± 0.015 |
| 7 | 360 | 0.296± 0.012 | 0.220± 0.030 | 0 | 0 |
| 8 | 420 | 0.136± 0.011 | 0 | | |
| 9 | 480 | 0 | | | |

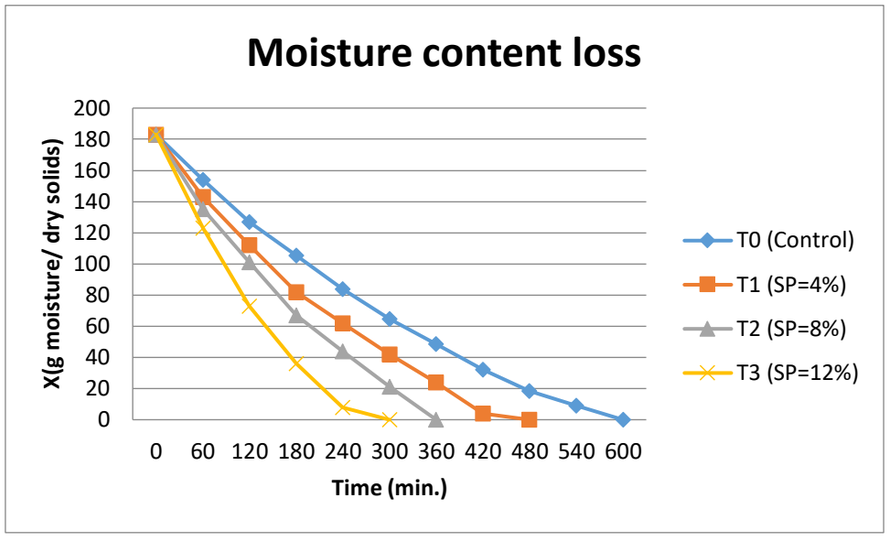


**Figure 11.** Effect of soy protein concentration level on moisture during foam mat drying of onion paste at 55°C.

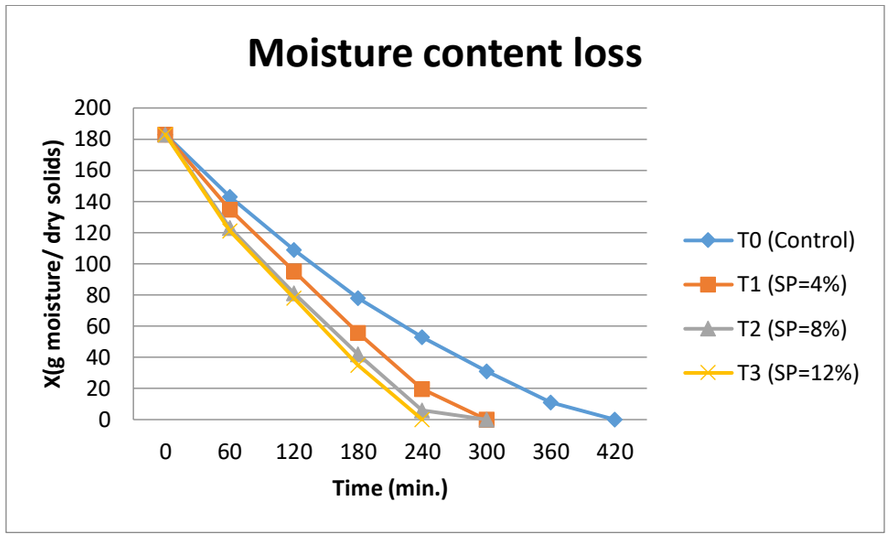


**Figure 12.** Effect of soy protein concentration level on moisture during foam mat drying of onion paste at 65°C.

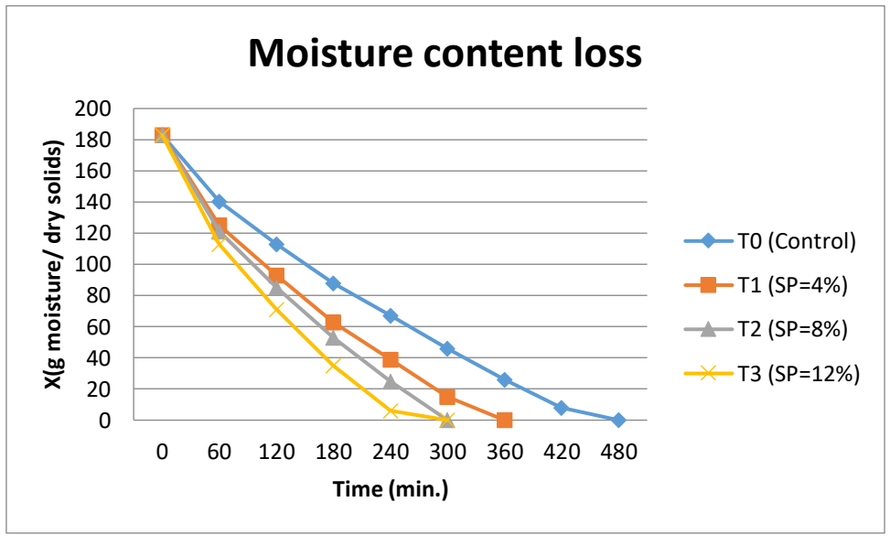


**Figure 13.** Effect of soy protein concentration level on moisture during foam mat drying of onion paste at 75°C.

### 3.1.Moisture of onion powder

In our daily life we always need to preserve food materials to make their availability throughout the year. Main purpose of food preservation is to prevent the food spoilage and maintain the nutritional characteristics of the food material. Spoilage can be caused by physical, chemical and microbial changes. But mostly chemical and microbial spoilage are most important. Most of chemical reactions take place in the water. Although all the microbes needed water to cause spoilage. Drying is a most

important phenomena of food preservation to reduce the water content in our food material to keep them safe from chemical and microbial spoilage. Analysis of variance (ANOVA) showed significant effect ($p<0.05$) of foaming agent on moisture content of onion powder while temperature showed highly significant ($P<0.01$) effects on moisture content. Effect of soy protein concentration levels on final moisture content in onion powders developed through foam mat drying process at 55°C, 65°C and 75°C was significant. Kadam *et al*., (2011) reported similar results as in our present study. In that research a descending trend of moisture % was observed as temperature increased from 50°C to 70°C. Increase in concentration of soy protein resulted in decreasing moisture content of onion powder. For samples in which soy protein was used as foaming agent moisture content was 7.967± 0.266 and 4.593± 0.102 with 4 % and 12 % concentration at 65°C. From moisture data of foam mat dried onion powder 28.636± 0.474 was the highest value of moisture content at 55°C in control sample (un-foamed) and 4.593± 0.102 was lowest value at 75°C in T3 sample treated with 12% soy protein as a foaming agent. Hence, temperature influenced the moisture content very significantly for soy protein treatments.

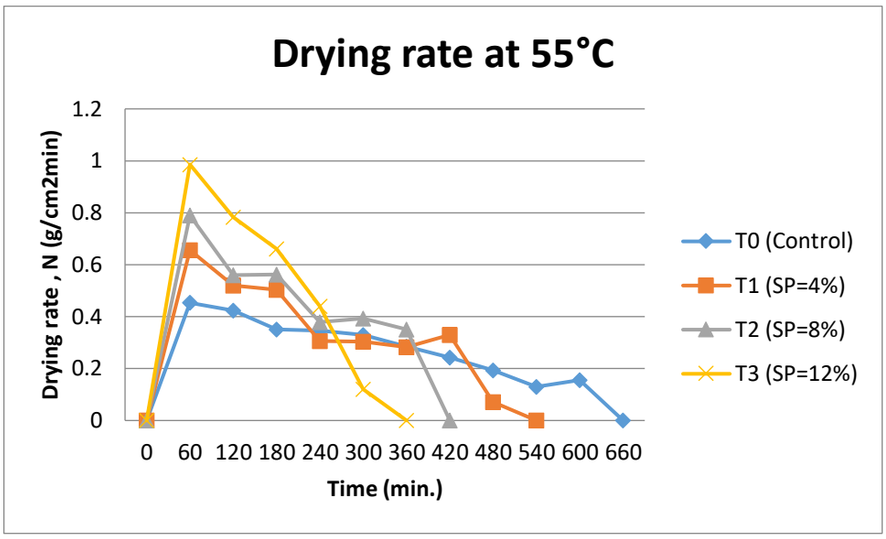


**Figure 14.** Effect of soy protein concentration level on drying rate of foam mat dried onion paste at 55°C.

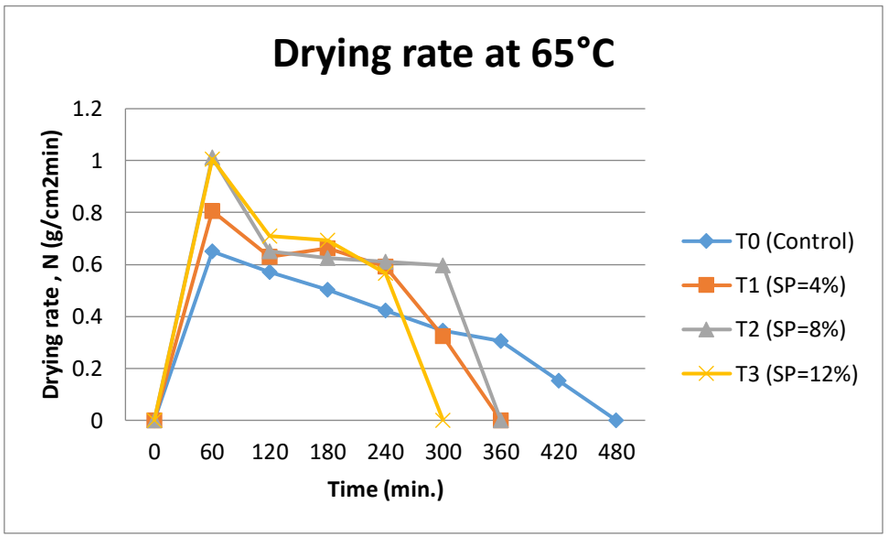


**Figure 15.** Effect of soy protein concentration level on drying rate of foam mat dried onion paste at 65°C

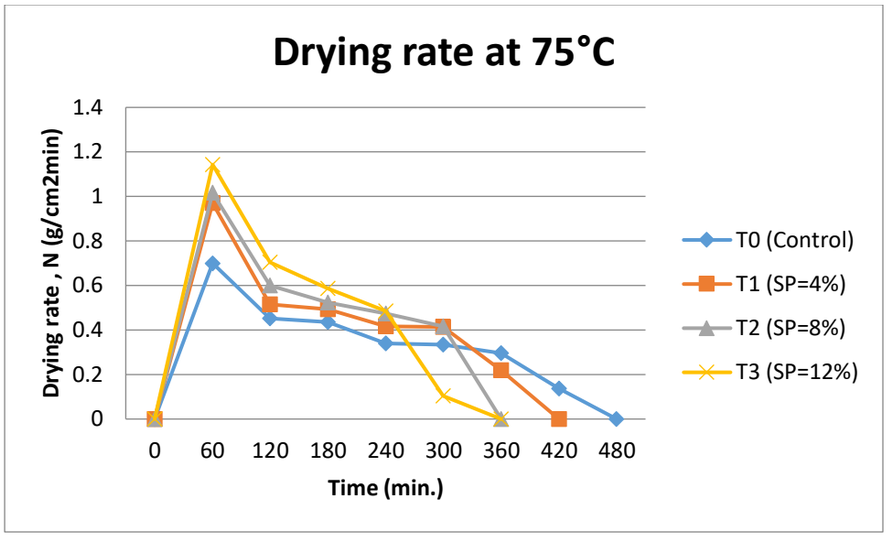


**Figure 16.** Effect of soy protein concentration level on drying rate of foam mat dried onion paste at 75°C.

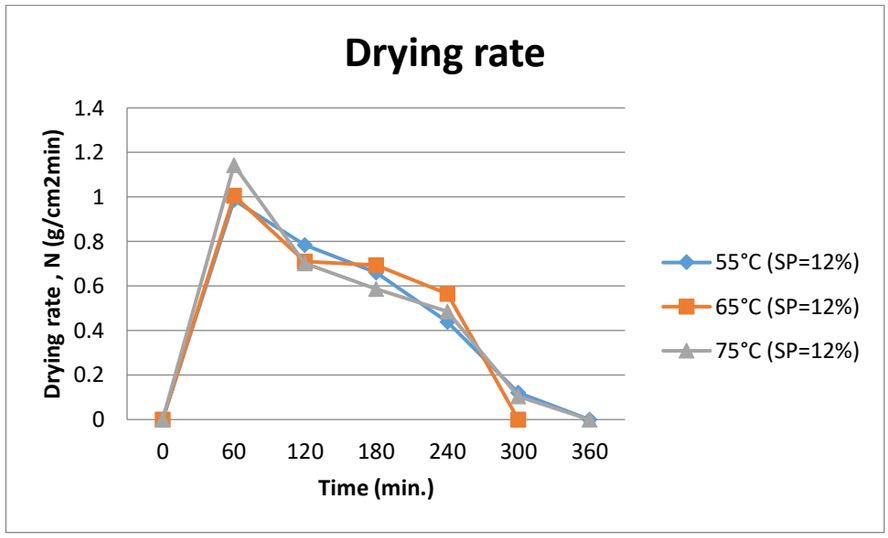


**Figure 17.** Effect of different temperatures (55°C, 65°C and 75°C) on drying rate of onion powders developed by 12% soy protein as foaming agent.

### 3.2. TSS (Brix %) of onion powders

TSS of fresh onion paste was 12 % while TSS of reconstituted foam mat dried onion powder was ranged from 11.666± 1.155 to 4.800± 0.200 which can be seen from table, 8. TSS of onion powder has significantly decreasing trend with increase in concentration of foaming agent. Analysis of variance (ANOVA) showed that only foaming agent have significant ($P<0.05$) effect onion total soluble solids of onion powder, while temperature effect was non-significant ($P>0.05$. As the soy protein concentration increased from ($T_1$) 4% to ($T_3$)12 % TSS of onion powder was decreased from 7.753± 1.946 to 5.333± 0.757at 65°C. Our results were in compliance with Rajkumar, *et al*. (2007). Many researchers have reported that this decline in TSS could be due to some heat sensitive compounds in food materials but use of these foaming agent has reduced the drying time and minimized the quality losses due to temperature effects. Only foaming agent concentration showed significant effects on TSS of onion powder while effect of temperature was non-significant.

**Table 7.** Mean drying rate of onion powder dried at different temperatures (55°C, 65°C and 75°C) using different concentration of soy protein as foaming agent

| Mean drying rate | | | | | |
|---|---|---|---|---|---|
| **Sr. No** | **Temperature** | **$T_0$ (Control)** | **$T_1$ (SP=4%)** | **$T_2$ (SP=8%)** | **$T_3$ (SP=12%)** |
| 1 | 55°C | 0.291± 0.109 | 0.371± 0.273 | 0.506± 0.273 | 0.598± 0.273 |
| 2 | 65°C | 0.422± 0.169 | 0.603± 0.169 | 0.699± 0.169 | 0.744± 0.169 |
| 3 | 75°C | 0.385±0.174 | 0.512± 0.174 | 0.606± 0.174 | 0.604± 0.174 |

**Table 8.** Effect of different concentration of egg albumin as foaming agent and drying temperatures on moisture content and total soluble solids (Brix %) of onion powder

| Temperature | Moisture content | | | | Total soluble solids (Brix %) TSS | | | |
|---|---|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | $T_0$ | $T_1$ | $T_2$ | $T_3$ |
| **55°C** | 28.636± 0.474 | 19.423± 0.425 | 14.347± 0.538 | 6.396± 0.176 | 11.666± 1.155 | 8.220± 1.680 | 8.100± 0.361 | 4.800± 0.200 |
| **65°C** | 8.410± 0.370 | 7.967± 0.266 | 7.216± 0.300 | 4.593± 0.102 | 9.966± 1.761 | 7.753± 1.946 | 8.133± 0.321 | 5.333± 0.757 |
| **75°C** | 8.183± 0.318 | 6.693± 0.331 | 5.646± 0.593 | 4.593± 0.102 | 10.667± 1.528 | 8.253± 1.675 | 7.833± 0.764 | 5.300± 0.650 |

**Table 9.** Effect of different concentration of egg albumin as foaming agent and drying temperatures on water solubility index (WSI) and water absorption index (WAI) of onion powder

| Temperature | Water solubility index (WSI) | | | | Water absorption index (WAI) | | | |
|---|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T0 | $T_1$ | $T2_2$ | $T_3$ |
| **55°C** | 20.330± 1.527 | 10.830± 2.254 | 27.330± 2.517 | 14.500± 0.500 | 2.683± 0.025 | 1.790± 0.010 | 1.690± 0.017 | 1.307± 0.023 |
| **65°C** | 9.500± 1.500 | 19.667± 1.547 | 29.333± 2.082 | 30.833± 1.607 | 2.543± 0.012 | 1.786± 0.006 | 1.570± 0.017 | 1.183± 0.023 |
| **75°C** | 29.333± 1.528 | 60.167± 1.258 | 54.667± 0.577 | 73.833± 2.466 | 2.490± 0.051 | 1.576± 0.015 | 1.493± 0.015 | 1.093± 0.080 |

### 3.3. Water solubility and absorption index

Water solubility index is the property of powders to show their ability of being homogenously mixed with water. Excellent powder is that which easily and instantly wet and submerged rather than float or diffuse without swelling. Analysis of variance (ANOVA) showed that temperature have highly significant ($p<0.01$) effect on water solubility index of onion powder while effect of foaming agents was non-significant ($p>0.05$) for water solubility index of onion powder. The effect of different concentration levels of soy protein on onion powder is given in table 9. From results it was found that with increase in concentration of soy protein resulted in increase in solubility of onion powder. It is because with increase in concentration of foaming agent structural stability of foams raised, which increased the porosity of onion powders. Hence, with increase in stability of foams in drying process, more number of bubbles will remain during whole drying process and these bubbles will rise porosity and ultimately solubility of powders will increase. Moreover, lower moisture content in foamed onion powders resulted in less sticky nature of powder with high surface area which increase water binding capacity of these powders. From the data which is given in table, 9, it was found that 9.500± 1.500 was WSI of un-foamed $T_0$ (Control) treatment, while 30.833± 1.607 was the value of WSI of the onion powder which was treated with soy protein ($T_3$)12% concentration dried at 65°C. Increase in temperature from 55°C to 75°C solubility of onion powder was significantly increased. It is because at 55°C the drying rate is very low as compare to drying at 65°C and 75°C. At 55°C bubbles have to stand for more time during entire drying process, while with increase in temperature drying taken place a short time so bubbles have less time to get collapsed. Therefore, at higher temperature reduction in drying time decreased the bubble collapse. As bubbles increases the porosity by which solubility of onion powders increased at higher temperature (Abbasi, *et al.*, 2016; Goula and Adamopoulos (2005). Maximum values for WSI was 73.833± 2.466 with ($T_3$)12% concentration of soy protein as foaming agent at 75°C.

Water absorption index is related to capacity of powders to absorb water; it is also known as hydration capacity. Several other properties of powders like moisture content, TSS, porosity and texture of powders will be altered due to increase in water absorption index. Moreover, all microbes are water loving so increase in WAI results into microbial attack and loss of quality attributes. Analysis of variance (ANOVA) showed highly significant ($p<0.01$) effect of foaming agent and temperature on water absorption index of onion powder. As temperature increased from 55°C to 75°C water absorption index was significantly decreased from 2.683± 0.025 to 2.490± 0.051 for ($T_0$) control, 1.307± 0.023 to 1.093± 0.080 soy protein ($T_3$)12% used as foaming agent. Maximum and minimum values of water absorption index was 2.683± 0.025 at 55°C $T_0$(Control) and 1.093± 0.080 at 75°C Soy protein ($T_3$)12% as foaming agent was used which can be seen in table, 9. This decrease in water absorption index due to increase in temperature is because of denaturation of protein molecules. Similarly, Wilson, *et al.* (2012) reported that with increase in temperature from 65°C to 85°C results into significant decrease in WAI. Azizpour, *et al.* (2016) reported, with

increase in temperature from 45°C to 90°C WAI decreased significantly. In another study, Franco, *et al*. (2015) also reported similarly that with increase in temperature from 50°C to 70°C WAI decreased.

## 4. Conclusions

Onion powder was prepared by foam-mat drying technique using soy protein in different concentration as foaming agent. Effect of foaming agent and different drying temperature was studied on moisture loss and drying rate of onion paste. From the results it was found that onion paste which was treated with different concentration of soy protein as foaming agent was dried in short time as compare to un-foamed onion paste. Moisture loss from foamed onion paste was higher because with foaming treatment surface area for drying was increased which resulted into faster and easy removal of moisture of foamed onion pastes. Moreover, drying rate of onion pastes which were treated with different concentration of soy protein was higher as compare to un-foamed onion pastes.

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# A COMPARATIVE STUDY ON IMPACT OF BLANCHING AND AUTOCLAVING ON NUTRACEUTICAL PROFILE OF *HELIANTHUS TUBEROSUS* L. (JERUSALEM ARTICHOKE)

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**ABSTRACT**

*Helianthus tuberosus*, a kind of herbaceous perennial tuber has high amount of soluble fibres and biologically active components that possesses strong antioxidant activity, anti-inflammatory, antifungal, antimicrobial, anti-diabetic, anti-obesity and anticancer activities. The present study was undertaken to explore the influence of blanching and autoclaving processing methods on nutraceutical profile of *Helianthus tuberosus* (*Ht*). Soluble fibres content (inulin and fructo-oligosaccharides) and antioxidant profile (total phenols content, total flavonoids content, ascorbic acid and DPPH radical scavenging activity as well as FRAP activity) were performed with slight modification in standard protocol. The study results revealed that blanched-*Ht* extract had significantly decrease inulin (21.53±0.16g/100ml) and fructo-oligosaccharides content (4.28±0.17g/100g) followed by autoclaving (17.43±0.25g/100ml and 3.76±0.19g/100g) when compared with unprocessed-*Ht* extract (23.29±0.16g/100ml and 5.31±0.45g/100g) at $p<0.05$ level. Unlike this, blanched-*Ht* extract had significantly higher total phenols content (9.36±0.12mgGAE/100g), total flavonoids content (3.30±0.36mgQE/100g) and ascorbic acid (17.71±0.81) followed by autoclaving (8.93±0.16mgGAE/100g, 4.38±0.22mgQE/100g and 14.36±0.31mg/100g) as compared to unprocessed-*Ht* extract (7.91±0.09mgGAE/100g, 3.30±0.28mgQE/100g and 21.83±0.64g/100g). Likewise, blanched-*Ht* extract exhibits highest antioxidant capacity with $IC_{50}$value (21.07µg/ml) followed by autoclaved-*Ht* extract (23.1µg/ml) when compared with unprocessed-Ht extract (26.2µg/ml). The FRAP activity of Unprocessed-*Ht* was 16.40±0.33 which was significantly increased by 57.5% (Blanched-*Ht*) and 15.5% (Autoclaved-*Ht*). Hence, the present study suggests that blanched *Ht* aqueous extract would be appropriate to possess pharmaceutical properties due to high nutraceutical content.

## 1.Introduction

It is commonly known that oxidative stress caused by free radicals and their derivatives is responsible for disturbing redox homeostasis (Hybertson et *al.* 2011). It is also one of the primary factors involved in the development of chronic metabolic disorders and degenerative diseases. Reactive oxygen species are a group of unstable molecules that are generated in all cells during normal physiological and biochemical processes. These radicals may cause DNA damage, leading to mutagenic changes and cell death (Redza-Dutordoir and Averill-Bates, 2016). An extremely important role in the fight against damage caused by free radicals play nutraceutical derived from diet.

However, some epidemiological studies stated the protective association between nutraceutical and chronic ailments. Nutraceuticals, a combination of nutrition and pharmaceutical are the naturally occurring compounds derived from foods and associated with improving health, delaying the aging process, increasing life expectancy and supporting the structure and function of body (Nasri et *al.* 2014).

*Helianthus tuberosus* L. (Jerusalem artichoke) belongs to family *Asteraceae*, is a herbaceous perennial tuber that is cultivated worldwide in the temperate regions (Slimestad et *al.* 2010). It contains good amount of nutrients and excellent amount of soluble dietary fibres (inulin and fructo-oligosaccharides) instead of starch. It has high amount of biologically active components including sesquiterpenes, flavonoids, isoflavonoids, phenols, phenolic acids, glycoalkaloids, phytic acids, coumarins, organic acids, polyacetylenes, and their derivatives. The tubers are also rich source of naturally occurring isomers of caffeoylquinic acid namely neochlorogenic acid, chlorogenic acid, crypto chlorogenic acid and 4 isomeric di-caffeoylquinic acids (Kapusta et *al.* 2013). Bioactive compounds in tubers are secondary metabolites associated with various pharmacological activities, such as cholagogue, aphrodisiac, aperient, stomachic, diuretics, and tonic effects. Moreover, it also possesses strong antioxidant activity, anti-inflammatory, antifungal, antimicrobial, anti-diabetic, anti-obesity and anticancer activities, which may be associated with its highest level of phenolic content (Zhang and Hye-Young, 2015). Hence, in the light of the above research facts, the present investigation was undertaken to assess the impact of processing on nutraceutical profile of *Helianthus tuberosus*.

## 2. Materials and methods

### 2.1.Collection of plant material and extraction procedure of *Helianthus tuberosus*

Freshly cultivated *Helianthus tuberosus* were collected from Indian Institute of Vegetable Research (IIVR), Varanasi. The tubers were selected considering the absence of any visual damage and infection as well as the uniform size, colour and maturity. Sorted and cleaned tubers were stump trimmed off and weighed. The tubers were peeled, washed with tap water and cut into thin slices and then blanched with hot water (95ºC±2) for 2 minutes whereas in autoclaving, tubers were kept at 121ºC for 15 min and cooled for 10 min at room temperature. Blanched and autoclaved tubers were exposed directly to sunlight until samples reached constant weight.

20g of powdered tuber was kept in 200ml conical flask and 100ml of distilled water was added. The mouth of the conical flask was covered with the aluminium foil and kept in a reciprocating shaker for 25 min for continuous agitation at 150 rpm for thorough mixing. The extracts were filtered by using muslin cloth followed by Whatman filter paper No. 42 (125mm) and kept in amber colored screw capped bottle at 18ºC for further analysis.

### 2.2.Determination of inulin

#### *2.2.1.Free fructose content ($F_f$)*

150µl tuber extract was pipetted into 10ml volumetric flask containing 20 mmol $L^{-1}$ citrate buffer 6 (5ml) and diluted with water up to 10ml. After 5 min, 150µl of 100 mmol $L^{-1}$ potassium iodide was added, and mixture was left for an additional 5 min. The absorption of solution was measured at 350nm using a UV-Vis spectrophotometer.

#### 2.2.2. Total fructose content ($F_{tot}$)

0.50ml of tuber extract was acidified with HCL (0.2 mol $L^{-1}$) in a final volume of 25ml and kept for acid hydrolysis at 97±2ºC for 45 min. The solution was then adjusted to 7 pH with NaOH before dilution with water to 25ml. The absorption of neutral hydrolysate was measured at 350nm using a UV-Vis spectrophotometer.

The inulin content was calculated using the following equation:

$$I = k(F_{tot}-F_f) \quad (1)$$

Where I is inulin content, $F_{tot}$ is total fructose content, $F_f$ is free fructose content, k is 0.995, it is a correction factor for the glucose part of water and inulin loss during hydrolysis (Saengkanuk et *al.* 2011; Srinameb et *al.* 2015).

## 2.3.Determination of fructo-oligosaccharides (FOS)

Total sugars were estimated by phenol sulphuric method (Agrawal et *al.* 2015). Whereas, reducing sugars was estimated by Di-Nitro Salicylic Method (DNS) (Dangeti *et al.,* 2013). The FOS content was calculated as non-reducing sugars which derived from total sugars subtracted by reducing sugars (Ngampanya et *al.* 2016).

## 2.4.Preliminary phytochemical screening of *Helianthus tuberous*

The filtrate of unprocessed and processed tuber were tested for the presence of various bioactive compounds namely saponins (Froth Test**),** tannins (Ferric chloride test), alkaloids (Mayer's reagents), flavonoids (Shinoda test), Terpenoids (Salkowski test), glycosides (Legal's test), steroids (Libermann Burchard test), phenols (Ferric Chloride) and anthroquinones (Borntrager's reaction) (Gul et *al.* 2017).

## 2.5.Determination of antioxidant potential

### *2.5.1.Determination of total phenols content*

Total phenols content were determined by Folin-Ciocalteu Reagent using gallic acid as a standard phenolic compound. A dilute extract of tuber (0.5 ml of 1:10g/ml) or Gallic acid was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and the mixture was stirred vigorously. 4ml of aqueous sodium carbonate (1M) was added after 3 minutes and then allowed to stand for 2 h with intermittent shaking. After that, the absorbance was measured at 765 nm in spectrophotometer against blank consisting of all the reaction agents except the extract. Total phenols content values are expressed in terms of Gallic acid equivalent (mgGAE/g of dry mass) (Kim et *al.* 2019).

### *2.5.2.Determination of total flavonoids content*

Total flavonoids content were determined by using aluminium chloride colorimetric assay. A volume of 125µl of tuber extract is added to 75µl of a 5% $NaNO_2$ solution. The mixture was allowed to stand for 6 min. 150µl of aluminium trichloride (10%) was added in it and incubated for 5 min, followed by the addition of 750µl of NaOH (1M). The final volume of the solution was adjusted to 2500µl with distilled water. After 15 min of incubation of mixture turned to pink and the absorbance was measured at 510nm using spectrophotometer. The total flavonoids content was expressed as mg of quercetin equivalent (mgQE/g dry mass) (Aryal et *al.* 2019).

### *2.5.3.Ascorbic acid content*

10g tuber powder was ground in 40ml of metaphosphoric acid to stabilize ascorbic acid content of the sample. The content was made upto 100ml by using 6% metaphosphoric acid. 20ml of standard ascorbic acid solution was taken in a conical flask and titrated against 2, 6 -dichlorophenol indophenols solution. Faint pink color resisting for at least 15seconds marked the completion of titration (Dinesh et *al.* 2015).

### *2.5.4.DPPH radical scavenging activity*

The ability of the aqueous extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined Spectrometric method. Aliquots of the sample extract at different concentrations 20-200 µg/ml were added to 1 mm aqueous solutions of DPPH. Each mixture was vortexes vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage. $IC_{50}$ value was also determined by graph (Sandiya and Munniappan, 2015).

### 2.5.5. *FRAP (ferric reducing antioxidant powder) radical scavenging activity*

FRAP assay is based on the ability of antioxidants to reduce $Fe^{3+}$ to $Fe^{2+}$ in the presence of TPTZ, forming an intense blue $Fe^{2+}$-TPTZ complex with an absorption maximum at 750nm.This reaction is pH-dependent (optimum pH 3.6). 0.1ml extract is added to 3.0ml FRAP reagent (10 parts 300mM sodium acetate buffer at pH 3.6, 1 part 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) in 40mM HCl and 1 part 20mM FeCl3) and the reaction mixture is kept in a water bath at 50°C for 20min. The absorbance was measured at 595nm. FeSO4 (100 to 1000µM ml-1) was used as a positive control (Jung et *al.* 2011).

### 2.6.Statistical analysis

The results obtained were expressed as Mean ±SD and student t-test of three determinations and also statistically analysed to ascertain its significance at $p \leq 0.05$ levels.

## 3.Results and discussions

**Table 1.** Effect of processing on phytochemical screening of *Helianthus tuberosus* aqueous extract

| Phytochemicals | Unprocessed-JAT | Processed-HtAqE | |
|---|---|---|---|
| | | Blanching | Autoclaving |
| **Saponins** | + | ++++ | +++ |
| **Tannins** | + | ++ | + |
| **Alkaloids** | + | +++ | ++ |
| **Flavonoids** | ++ | +++ | ++ |
| **Terpenoids** | + | +++ | ++ |
| **Glycosides** | + | ++ | + |
| **Steroids** | _ | _ | _ |
| **Phenols** | ++++ | +++++ | ++++ |
| **Anthroquinones** | _ | + | + |

HtAqE: *Helianthus tuberosus* Aqueous Extract. - Absence, + Present, ++ Fairly Good, +++ Good, ++++ Very Good, +++++ Excellent.

The preliminary phytochemical analysis revealed that unprocessed-HtAqE contained saponins, tannins, alkaloids, flavonoids, terpenoids, glycosides and phenols except steroids and anthroquinones as depicted in Table 1. However, anthroquinones were found to be positive in processed-HtAqE. Though, blanched-*Ht* aqueous extract contained very good number of secondary metabolites followed by autoclaved-*Ht* aqueous extract. The present study is comparable with Krishnapriya and Suganthi, (2017) stated that aqueous and methanolic extracts of *Colocasia esculenta* tubers showed the presence of alkaloids, glycosides, terpenoids, flavonoids, phenols and the absence of tannins, quinones and steroids. According to Gul et *al.* (2017), saponins, glycosides, alkaloids, phenols and flavonoids were found to be positive and tannins was found to be negative in methanol and ethanol extract of *Ephedra intermedia*.

**Table 2.** Effect of processing methods on nutraceutical profile of *Helianthus tuberosus* aqueous extract

| Parameters | Unprocessed-*Ht* Aqueous Extarct | Processed-*Ht* Aqueous Extract | |
|---|---|---|---|
| | | Blanching | Autoclaving |
| **Inulin (g/100ml)** | 23.29±0.16 | 21.53±0.07* (7.29%↓) | 17.43±0.25*a (25.16%↓) 19.04%↓ |
| **Fructo-oligosaccharides (g/100g)** | 5.31±0.45 | 4.28±0.17* (19.41%↓) | 3.76±0.19*a (29.14%↓) 12.14%↓ |
| **Total Phenols Content (mgGAE/g)** | 8.10±0.09 | 9.36±0.12* (15.5%↑) | 8.93±0.16*NS (10.24%↑) 4.59%↓ |
| **Total Flavonoids Content (mgQE/g)** | 3.30±0.28 | 4.94±0.36* (49.6%↑) | 4.11±0.22*NS (24.5%↑) 16.8%↓ |
| **Ascorbic Acid (mg/100g)** | 21.83±0.64 | 17.71±0.81* (18.8%↑) | 14.36±0.31*a (34.2%↑) 18.91%↓ |

Values are Mean±SD of triplicate determinations. *Ht*: *Helianthus tuberosus*
*denotes significant difference when compared with unprocessed-JATF at p<0.05 level
a denotes significant difference and NS shows non-significant difference when autoclaved-*Ht* aqueous extract compared with blanched-*Ht* aqueous extract

Table 2 shows the nutraceutical profile of (inulin, fructo-oligosaccharides (FOS), Total phenols content (TPC), total flavonoids content (TFC) and ascorbic acid content) of unprocessed and processed-*Ht* aqueous extract. The data showed that inulin content (g/100ml) of unprocessed-HtAqE was 23.19±0.16 which agrees with El-Kholy and Mahrous, (2015) who reported that *Helianthus tuberosus* had 21.46g/100g of inulin content. Inulin content of blanched-*Ht* aqueous extract (21.53±0.07) and autoclaved-*Ht* aqueous extract (17.43±0.25) was significantly decreased by 7.29% and 25.16% at p<0.05 level when compared to unprocessed-*Ht* extract. On the other hand, autoclaving resulted significantly decrease in inulin content by 19.4% when compared with blanched-*Ht* extract. Likewise, Takeuchi and Nagashima, (2011) revealed that Jerusalem artichoke chips treated for 120 seconds lost 20-30% inulin in hot water. The fructo-oligosaccharides content (g/100g) results indicated that unprocessed-*Ht* aqueous extract had highest value (5.31±0.45) while blanched-*Ht* extract and autoclaved-*Ht* extract had lowest value i.e. 4.27±0.17 and 3.76±0.19 which was significantly decreased by 19.41% and 29.14%. On the other hand, autoclaving resulted significantly decrease in fructo-oligosaccharides content by 12.4% when compared with blanched-*Ht* extract. The processed samples registered significant difference at p<0.05 level when compared to unprocessed-*Ht* aqueous extract. The present data is comparable with Khuenpet et *al.* (2015) who stated that *Helianthus tuberosus* had

6.71±0.06g/100g fructo-oligosaccharides content and also revealed that blanching reported significantly decrease in inulin (26.14±0.87g/100g) and fructo-oligosaccharides content (4.97±0.005g/100g) when compared with unblanched *Helianthus tuberosus* (33.81±1.44g/100g and 7.35±0.07g/100g). The loss of inulin and fructo-oligosaccharides content during thermal treatment is associated with its solubility in the hot water (Vendrell-Pascuas et *al.* 2000).

The data showed that total phenols (mgGAE/100g) and total flavonoids content (mgQE/100g) of unprocessed-*Ht* aqueous extract was 7.91±0.09 and 3.30±0.28 which agrees with Niziol-Lukaszewska et *al.* (2018) who reported that *Helianthus tuberosus* had 76.84±4.96mgGA/g (TPC) and 6.05±0.32mgQE/g (TFC) content. TPC content of processed-*Ht* extract i.e. blanched (9.36±0.12) and autoclaved (8.93±0.16) was significantly increased ($p<0.05$ level) by 18.4% and 13.03% when compared with unprocessed-*Ht* aqueous extract (8.10±0.09).

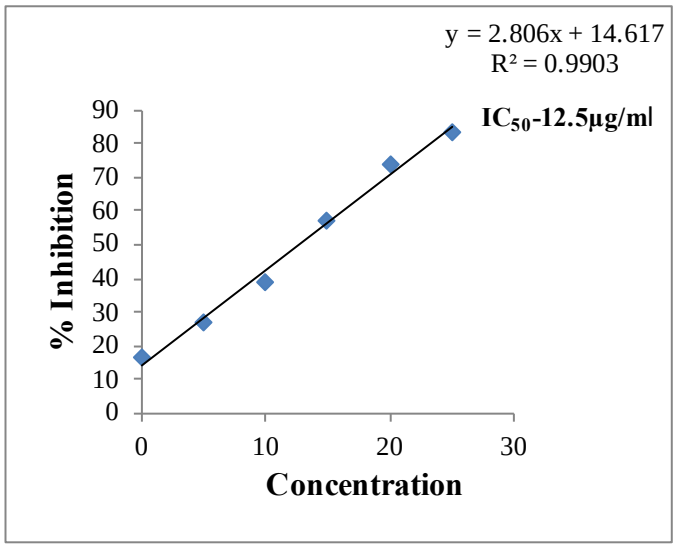


**Figure 1.a.** DPPH radical scavenging activity of ascorbic acid

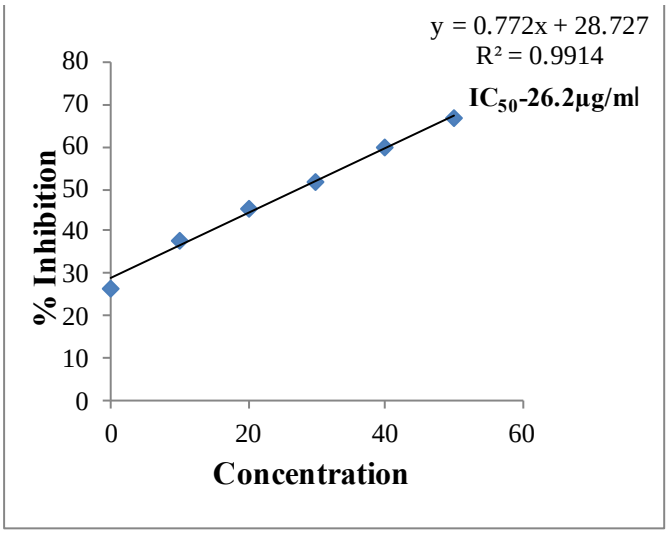


**Figure 1.b.** DPPH radical scavenging activity of unprocessed-*Ht* methanol extract

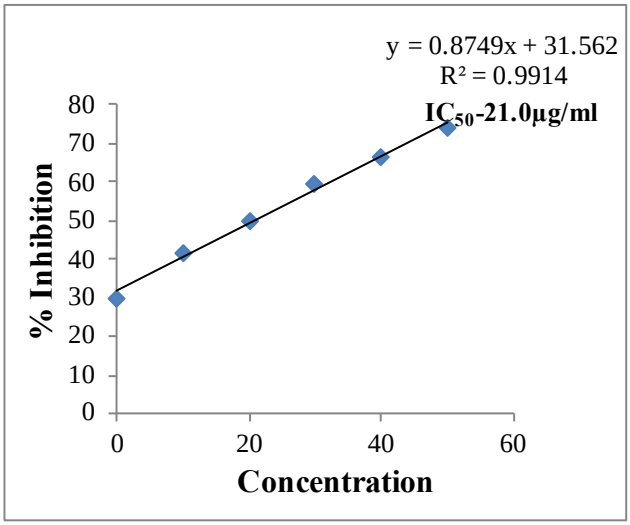


**Figure 1.c.** DPPH radical scavenging activity of blanched-*Ht* methanol extract

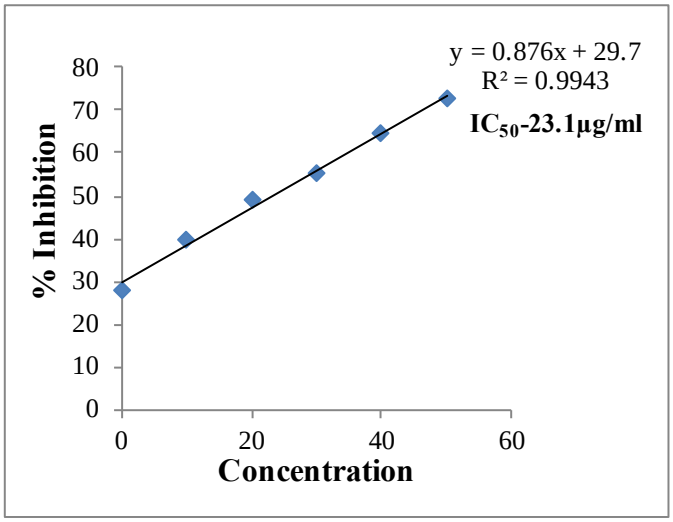


**Figure 1.d.** DPPH radical scavenging activity of autoclaved-*Ht* methanol extract

On the other hand, autoclaving resulted insignificantly decrease in TPC content by 4.59% when compared with blanched-*Ht* extract. Similarly, TFC content of blanched-*Ht* aqueous extract (4.94±0.36) and autoclaved-*Ht* extract (4.38±0.22) was significantly increased by 49.6% and 32.7% at $p<0.05$ level as compared to unprocessed-*Ht* aqueous extract (3.30±0.28). On the other hand, autoclaving resulted insignificantly decrease in TFC content by 11.3% when compared with blanched-*Ht* extract. The present data is comparable with Bembem and Sadana, (2013) who stated that the TPC content (mg/100g) of boiled (26.38) and pressure cooked (32.72) potato tuber was significantly increased ($p<0.05$) by 11% and 38% when compared with unprocessed tubers (23.75). Similarly, Kamalaja et *al.* (2018) reported that pressure cooked beans (577.13±2.02) had higher TPC content as compared to unprocessed beans (501.4±0.01). Data for TFC reported by Saetan et *al.* (2016) indicated that blanched *C. porrectum* herbal tea had significantly increased TFC value (mgCE/g) i.e. 57.05±8.62 when compared with unblanched (45.32±1.58). Thermal processing

at low temperature releases more bound phenols due to breakdown of the cellular components of the tuber, thus increasing TPC during blanching.

Secondary, the less obtained value in autoclaving is probably due to a degradation of some phenolic compounds at high temperature (Dewanto et *al.* 2002). The ascorbic acid content (mg/100g) of unprocessed-*Ht* was 21.83±0.64 which was comparable with Mahrous et *al.* (2016) who reported that *Helianthus tuberosus* had 17.07mg/100g of ascorbic acid. Ascorbic content of processed-*Ht* i.e. blanched (17.71±0.81) and autoclaved (14.36±0.31) was significantly decreased by 18.8% and 34.2% at $p<0.05$ level when compared with unprocessed-*Ht* (21.83±0.64). On the other hand, autoclaving resulted significantly decrease in ascorbic acid content by 18.91% when compared with blanched-*Ht* extract. Likewise, Sinha et *al.* (2015) reported that steamed sweet potato had significantly lower ascorbic acid content i.e. 15.85±0.35mg/100g when compared with unprocessed (21.23±1.22mg/100g). The loss in ascorbic acid content during processing might be due to its sensitivity towards water, heat and air (El-Ishaq and Obirinakem, 2015).

The DPPH radical scavenging activity for ascorbic acid, unprocessed and processed-*Ht* methanolc extract is shown in Figure 2(a, b, c and d). DPPH is a stable free radical that is deep purple in color. This assay measures the ability of biological samples to reduce 1,1-diphenyl-2-picryl hydrazyle radical to 1,1-diphenyl-2-picryl hydrazine, therefore a reduction in purple color indicates a reduction in free radicals (Willis et *al.* 2019). The activity was estimated by comparing the % inhibition of DPPH radical formation by the extracts and ascorbic acid acted as positive control. It was found that the radical scavenging activity of control and samples extract increased with increasing concentration and a lower value of $IC_{50}$ value indicates higher antioxidant activity. The data indicated that blanched-*Ht* extract exhibits significantly highest antioxidant capacity with $IC_{50}$value (21.07µg/ml) followed by autoclaved-*Ht* extract (23.1µg/ml) while unprocessed-*Ht* extract showed lowest scavenging activity 26.2µg/ml when compared to control (12.5 µg/ml). The present study is comparable with Oboh, (2005) who reported that blanched *Telfairia occidentalis* had highest free radical scavenging (16.4%) when compared with unprocessed (20.0%). The decrease free radical scavenging during autoclaving occur due to the loss of functional groups as a result of polymerization reactions arising at high temperature while the increase scavenging during blanching is associated with high level of phenolic compounds (Carciochi et *al.* 2016).

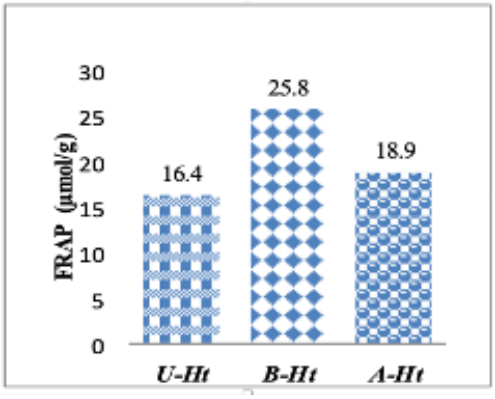


**Figure 2.** FRAP activity of *Ht* aquous extract

Figure 2 depicts antioxidant estimation for FRAP (µmol/g) of unprocessed (U) and blanched (B) and autoclaved (A) *Helianthus tuberosus* aqueous extract. The FRAP activity of U-*Ht* was 16.40±0.33 which was significantly increased by 57.5% (B-*Ht*) and 15.5% (A-*Ht*). Similar study was reported by Halvorsen, et *al.* (2006) that blanching of vegetables resulted in increased FRAP value. Sreeramulu and Raghunath, (2010) reported that unprocessed *Tryphonium trilobatum*, *Solanum tuberosum* and *Ipomoes batatas* had 2891.47±310.24, 704.73±102.28 and

422.56±315.34mg/100g FRAP values which were higher than the present data.

## 4.Conclusions

The present study uncovered the fact that blanching and autoclaving had significantly affected the nutraceutical profile of *Ht* aqueous extract. Blanching resulted significantly decrease in inulin, fructo-oligosaccharides and ascorbic acid content but less than autoclaving. Unlike this, it resulted significantly increase in total phenols and flavonoids content. Likewise, blanching exhibits high antioxidant capacity than autoclaving. Hence, in an overall consideration of these treatments the present study suggests that blanched tuber would be appropriate to possess pharmaceutical properties due to high nutraceutical content.

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# IMPACT OF REFRIGERATED CURD ON KASHKAVAL QUALITY I. CHEMICAL CHARACTERISTICS

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**ABSTRACT**

The present study examined the impact of refrigerated curd (1 °C, 2 months), so called "Cagliata" on Kashkaval cheese chemical characteristics. Changes of cheese components during ripening as well as proteolysis, biogenic amines formation, fatty acid profile, lipolysis and oxidation of milk fat were investigated. Kashkaval cheese samples were characterized by similar dry matters and protein contents but the results obtained for the components such as milk fat, minerals, salt and active acidity were significantly different ($P<0.05$). A more pronounced proteolysis was found in the cheese produced from refrigerated curd but no accumulation of biogenic amines in both analyzed samples was established. A greater lipolysis and oxidation of milk fat was observed in the cheese obtained from fresh milk. Cheese produced from refrigerated curd could be successfully used as a cheaper alternative of traditional Kashkaval or when a fresh curd is not available without compromising on its quality.

## 1. Introduction

In recent years, technologies are expanding, in order to meet the increased demands of the consumer for a safe, high-quality and affordable product. This can be done by value-added products as cheese supplemented with different compounds (Bukvicki *et al*., 2018; Ullah *et al*., 2018; El-Sayed *et al*., 2020), cost-effective technologies (Simov and Ivanov, 2005; Marcuzzo *et al*., 2012; Beykont and Kilic-Akyilmaz, 2013; Sharma *et al*., 2018; Alinovi *et al*., 2020a; 2020b) or innovative technologies (Johnson, 2017; Cabral *et al*., 2019; Alinovi and Mucchetti, 2020a; 2020b).

In individual European countries, the production of raw cow milk varies widely (Bórawski *et al*., 2020). There are producers on the market from the regions generating more quantities of cow milk at a cheaper price. They offer quality intermediate semi-finished product or so-called "Cagliata", which can be used as a raw material for the production of various type of cheese. This is an opportunity for manufacturers in countries, where milk is at high cost, to obtain a product with characteristics similar to the traditional product, but at a lower price.

The storage of raw materials in a refrigerated or frozen state is a common approach in food industry in order to extend their shelf life (Prakash, 2018). Different studies suggested the use of frozen milk or deep frozen curd as alternative to produce different kind of cheeses as Teleme cheese (Alichanidis *et al*., 1981), soft caprine cheese (Van Hekken *et al*., 2005), sheep cheese (Zhang *et al*., 2006; Pazzola et al., 2013; Fava *et al*., 2014), Mozzarella from buffalo milk (Manzo *et al*., 2017), white brined goat cheese (Kljajevic *et al*., 2017), Hispánico cheese from ewe milk (Alonso *et al*., 2012), high-pressure treated raw goat milk curd (Picon *et al*., 2012)

etc. They reported that the production of cheese from frozen curd is possible with some minor changes in their composition and quality.

Kashkaval cheese is a typical hard cheese (Bulgarian National Standard (BNS) 14:2010). It is produced in the region of the Balkans and Eastern Europe. It is frequently associated with the "Pasta filata" cheese production technology because it includes cheddaring, cooking, and stretching of the fresh curd as well as molding and ripening of fresh cheese (Bylund, 2015).

The possibility to obtain cost effective production of Kashkaval by storing it in a frozen state before or after ripening is discussed by Simov and Ivanov (2005). They studied the proteolytic processes in frozen Kashkaval cheese and found enhanced proteolysis during ripening of thawed Kashkaval cheese. To the best of our knowledge any scientific studies about the possibility to obtain Kashkaval cheese from refrigerated curd were not available. Given that, we hypothesized that different raw material than fresh cow milk can represent a cost effective technology for Kashkaval production and similar cheeses, saving the chemical properties of the product.

Therefore, the aim of the present research was to determine the effect of using refrigerated curd on the chemical composition of Kashkaval cheese.

## 2. Materials and methods

### 2.1. Materials

Fresh cow milk, meeting the national and European requirements, was obtained by the local farmers supplying milk to the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo" was used in order to obtain a fresh curd with a studied composition (Table 1). A refrigerated (at 1 °C for 2 months) "Cagliata" curd was used as a raw material supplied by a foreign company with a composition presented in Table 1.

**Table 1**. Chemical composition of fresh ad refrigerated curd

| Component | Refrigerated curd | Fresh curd |
|---|---|---|
| Dry matter, % | $62.90 \pm 1.95^a$ | $57.21 \pm 1.35^b$ |
| Milk fat, % | $27.5 \pm 0.5^a$ | $25.5 \pm 0.5^b$ |
| Total protein, % | $24.69 \pm 1.24^a$ | $24.50 \pm 1.23^a$ |
| Ash, % | $3.11 \pm 0.08^a$ | $2.01 \pm 0.01^b$ |
| Milk fat in dry matter, % | $43.75 \pm 1.22^a$ | $44.60 \pm 1.04^a$ |
| pH | $5.57 \pm 0.01^a$ | $5.23 \pm 0.05^b$ |

[a, b] Means with different letters within a row are significantly different ($p<0.05$)

Starter culture containing *Str. thermophilus*, *Lb. delbrueckii* ssp. *bulgaricus* and *Lb. helveticus* was supplied by LB Bulgaricum Ltd. Calcium dichloride solution (50%) was purchased from Biokom Trendafilov Ltd. Rennet enzyme CHY-MAX® M was delivered by Chr. Hansen. The chemicals and reagents used for analysis were analytical grade and were used without further purification.

### 2.2. Cheese preparation

#### *2.2.1. Kashkaval cheese produced from fresh curd*

Kashkaval cheese was prepared from two batches of cow milk. It was produced in the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo". A classic production process included the following technological operations (Ivanova *et al.*, 2020): cow milk qualification; clarification and standardisation of milk fat (at 35-45 °C) in order to achieve casein to fat content ratio 0.70; thermisation (at 63 ± 2 °C for 15-20 s); cooling (at 33 ± 1 °C); addition of starter culture in amount of 1%, calcium dichloride solution (50%) in amount of 30 $cm^3$ per 100 $dm^3$ of milk (previously diluted in water in 1:10 ratio) and rennet (previously diluted in water in 1:10 ratio) in such amount that the coagulation started 10-12 min after enzyme addition and a set coagulum was formed after 45 min; cutting the obtained coagulum at two stages - into 3-4 cm grains and after 5-10 min into 6-8 mm grains; stirring and stabilizing

the grains for 5-15 min; heating the formed grains (at 40-42 °C for 40-60 min); draining (separated whey titratable acidity 16-24 °T); collecting of curd for pressing (6 kg weight for 1 kg curd for 15-20 min, when pH reached 5.8-5.9); cutting the curd in 50-60 cm parallelepiped slices; cheddaring (1-1.5 h, pH 5.2-5.3); milling of the curd (slices with length of 20-30 cm and width 8-10 mm) and salting in a hot water solution (at 72 °C and 14% salt content); forming in 0.5 kg mould; stabilizing the forms by 3-4 turnings of the moulds; drying the fresh cheese (at 8-10 °C for 15 h); unmoulding and drying the cheese (at 6-8 °C for 2 days); packing and ripening (at 8-10 °C and relative humidity *80-85% for 60 days).*

#### *2.2.2. Kashkaval cheese produced from refrigerated curd*

Kashkaval cheese was prepared from two batches of refrigerated "Cagliata" curd. It was produced in the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo". The production process included the following technological operations: refrigerated curd qualification; cutting the curd in slices; milling of the curd and salting in a hot water solution (at 72 °C and 14% salt content); forming in 0.5 kg mould; stabilizing the forms by 3-4 turning of the moulds; drying fresh cheese (at 8-10 °C for 15 h); unmoulding and drying (at 6-8 °C for 2 days); packing and ripening (at 8-10 °C and relative humidity 80-85% for 60 days).

### 2.3. Analysis

#### *2.3.1. Chemical analysis*

Determination of cheese dry matter – Bulgarian National Standard (BNS) EN ISO 5534:2005; Determination of cheese fat content ISO 3433:2008; Determination of fat in dry matter – empirical calculation; Determination of cheese total protein content - BNS EN ISO 8968-1:2014; Determination of water soluble nitrogen (WSN), noncasein nitrogen (NCN), nonprotein nitrogen (NPN) and total nitrogen (TN) content - Vakaleris and Price (1959) with some slight modifications by Ivanova et al. (2021); Determination of biogenic amines – Ivanova et al. (2021); Determination of cheese ash content - BNS 6154:1974; Determination of cheese salt (NaCl) content – BNS 8274:1982; Determination of salt in moisture – empirically calculated; Determination of cheese fat fatty acids – milk fat extraction - ISO 1735/IDF 5:2004, preparation of methyl esters of fatty acids - ISO 5509:2001, chromatographic analysis of methyl esters of the fatty acids - ISO 5508:2000; Determination of fat indices - milk fat extraction – Bligh and Dyer (1959), peroxide value determination - ISO EN 3960:2008, acid value determination - ISO EN 660:2009; Potentiometric determination of active acidity (pH).

#### *2.3.2. Statistical analysis*

Statistical processing of obtained results was performed by Microsoft Excel 2010 according to one-way ANOVA method for analysis of variance. Multiple comparisons were made with the LSD (Least Significant Difference) procedure. Results were presented as the mean ± SD (standard deviation) of four replicates (n=4) and were considered significantly different when $P<0.05$.

## 3. Results and discussions

### 3.1. Chemical composition

As shown in Table 2 Kashkaval produced from refrigerated curd differed from the same produced from a fresh curd by some chemical parameters.

Despite the difference with the dry matter of the raw materials, no statistically significant difference ($P>0.05$) was observed of this component in the analyzed samples during the repining period. This can be explained by the higher hydrophilicity of the proteins in the refrigerated curd and their ability to absorb more water than the proteins in the fresh curd during the salting process in the cooker-stretcher production unit. Kljajevic *et al.* (2017) suggested that heating the curds in whey was an attempt to rehydrate proteins after thawing frozen curd. Our study showed that when cheese was produced from refrigerated curd, milk casein kept its ability to retain water – an effect

clearly demonstrated after heating the curd in the salting solution. This property of the milk protein was lost when the cheese was produced from frozen curd and significant water losses were reported (Alichanidis *et al*., 1981). Greater fat losses were obtained in the sample with refrigerated curd compared to the control ($P<0.05$). It was likely that the protein-fat matrix in the curd obtained from chilled curd was less dense and had a reduced ability to retain milk fat. Similar explanation was given by Kljajevic *et al.* (2017) who suggested that proteins, damaged during frozen (refrigerated) storage, released more fat into whey (salting solution) if the curd was not pressed before freezing (refrigeration), because a significant whey separation occurred after thawing (tempering the curd in the production unit). No statistical difference in fat content during ripening period was observed in the both samples ($P>0.05$). The salt content in the experimental sample produced from chilled curd was higher than the control one. This phenomenon could be explained by the higher initial pH of this curd as well as its lower fat content (Kozhev, 2006). This tendency continued until the end of the ripening period of the cheese. According to Kozhev (2006) the salt dissolved in the water phase was continuously redistributed in the colloidal bound water during ripening which explained the decrease in the salt in moisture content. The active acidity of the control sample was found to be lower than the experimental one which was due to the lower initial pH of the fresh curd. Increased active acidity in the beginning of maturation was explained by the partial separation of significant part of the lactic acid present in the curd after cheddaring. This tendency persisted during the ripening process despite the lactose fermentation process, because protein proteolysis took place and its products increased cheese buffer capacity. Similar results were obtained for cheese produced from frozen curd (Alichanidis *et al*., 1981; Kljajevic *et al*., 2017).

**Table 2.** Chemical composition of Kashkaval from refrigerated and fresh curd

| Component | Kashkaval from refrigerated curd | | Kashkaval from fresh curd | |
|---|---|---|---|---|
| | Beginning of ripening | End of ripening (60 days) | Beginning of ripening | End of ripening (60 days) |
| Dry matter, % | $57.57 \pm 2.67^{a}$ | $57.33 \pm 3.50^{a}$ | $59.89 \pm 2.08^{a}$ | $58.21 \pm 1.99^{a}$ |
| Milk fat, % | $25.0 \pm 0.5^{a}$ | $24.0 \pm 0.5^{a}$ | $27.0 \pm 0.3^{b}$ | $27.5 \pm 0.3^{b}$ |
| Total protein, % | $24.37 \pm 1.22^{a}$ | $24.75 \pm 1.24^{a}$ | $24.56 \pm 1.23^{a}$ | $24.63 \pm 1.23^{a}$ |
| Ash, % | $4.64 \pm 0.03^{a}$ | $4.40 \pm 0.01^{b}$ | $3.93 \pm 0.02^{c}$ | $3.83 \pm 0.01^{d}$ |
| Salt, % | $2.8 \pm 0.1^{a}$ | $2.6 \pm 0.1^{a}$ | $2.3 \pm 0.1^{b}$ | $2.2 \pm 0.1^{b}$ |
| Salt in moisture, % | $6.73 \pm 0.26^{c}$ | $2.6 \pm 0.1^{a}$ | $5.93 \pm 0.36^{c}$ | $2.2 \pm 0.1^{a}$ |
| pH | $5.80 \pm 0.00^{a}$ | $5.90 \pm 0.11^{a}$ | $5.87 \pm 0.05^{b}$ | $5.86 \pm 0.00^{b}$ |

[a-d] Means with different letters within a row are significantly different ($P<0.05$)

### 3.2. Proteolytic processes

The proteolytic processes in the analyzed samples are represented in Table 3.

**Table 3.** Proteolytic processes of Kashkaval from refrigerated and fresh curd

| Component | Kashkaval from refrigerated curd | | Kashkaval from fresh curd | |
|---|---|---|---|---|
| | Beginning of ripening | End of ripening (60 days) | Beginning of ripening | End of ripening (60 days) |
| †TN, mgN/g | $3.82 \pm 0.19^{a}$ | $3.88 \pm 0.20^{a}$ | $3.85 \pm 0.19^{a}$ | $3.86 \pm 0.19^{a}$ |
| WSN, mgN/g | $0.40 \pm 0.02^{a}$ | $0.48 \pm 0.03^{b}$ | $0.32 \pm 0.02^{c}$ | $0.36 \pm 0.02^{a}$ |
| NCN, mgN/g | $0.38 \pm 0.02^{a}$ | $0.46 \pm 0.02^{b}$ | $0.29 \pm 0.02^{c}$ | $0.33 \pm 0.02^{d}$ |
| NPN, mgN/g | $0.28 \pm 0.02^{a}$ | $0.34 \pm 0.02^{b}$ | $0.10 \pm 0.01^{c}$ | $0.18 \pm 0.01^{d}$ |
| WSN/TN, % | $10.47 \pm 0.53^{a}$ | $12.37 \pm 0.62^{b}$ | $8.31 \pm 0.42^{c}$ | $9.33 \pm 0.47^{a}$ |
| NCN/TN, % | $9.82 \pm 0.49^{a}$ | $11.92 \pm 0.60^{b}$ | $7.47 \pm 0.37^{c}$ | $8.42 \pm 0.42^{d}$ |
| NPN/TN, % | $7.20 \pm 0.36^{a}$ | $8.76 \pm 0.44^{c}$ | $2.60 \pm 0.13^{c}$ | $4.66 \pm 0.23^{d}$ |
| Putrescine, mg/kg | 0 | 0 | 0 | $0.73 \pm 0.01^{NS}$ |
| Cadaverine, mg/kg | 0 | 0 | 0 | $1.35 \pm 0.02^{NS}$ |
| Histamine, mg/kg | 0 | 0 | 0 | 0 |
| Tyramine, mg/kg | 0 | $3.70 \pm 0.01^{NS}$ | 0 | $2.13 \pm 0.01^{NS}$ |

$^{a-d}$ Means with different letters within a row are significantly different ($P<0.05$)

$^{NS}$Non Significant (values below 10 mg/kg are below the limit of quantification (LOQ), ie. they are beyond the accuracy of the standard curve); †TN – total nitrogen; WSN/TN - water-soluble nitrogen in total nitrogen; NCN/TN - noncasein nitrogen in total nitrogen; NPN/TN - nonprotein nitrogen in total nitrogen.

It was known that the process of proteolysis started during curd cheddaring (Kozhev, 2006; Kalit *et al.*, 2016). Significant and more pronounced processes of proteolysis in Kashkaval produced from refrigerated curd were observed. This was despite the equal dry matter (water content respectively) of both cheeses and the higher salt in moisture content in the cheese produced from refrigerated curd. Some of the potential explanations could be changes in casein structure during refrigerated storage which made it more easily attacked by proteolytic enzymes. The higher pH of the refrigerated curd which represented a more favorable medium for the activity of these enzymes could be another possible justification. Alichanidis *et al.* (1981) and Picon et al. (2012) reported the same tendency of faster proteolysis for cheese produced from frozen curd. Although the accelerated rate of proteolysis it was found no significant differences in the quantity of biogenic amines accumulated in both cheeses.

### 3.3. Lipolytic processes

The fatty acid composition of obtained cheeses is presented in Table 4.

The fatty acids in Kashkaval cheese were formed not only by the hydrolysis of milk fat, but also by the fermentation of lactose and the desamination of amino acids (Kozhev, 2006).

Manzo *et al*. (2017) found no effect on the cheese fatty acid concentrations after the freezing storage of the curd. Our results confirmed this statement and suggested that the refrigeration of the curd did not affect Kashkaval traditional fatty acid profile described by Ivanova *et al*. (2020). Some individual differences between fatty acids were observed but not for the total amount of saturated and unsaturated fatty acids. This was probably due to the different raw materials for the preparation of two type of cheese.

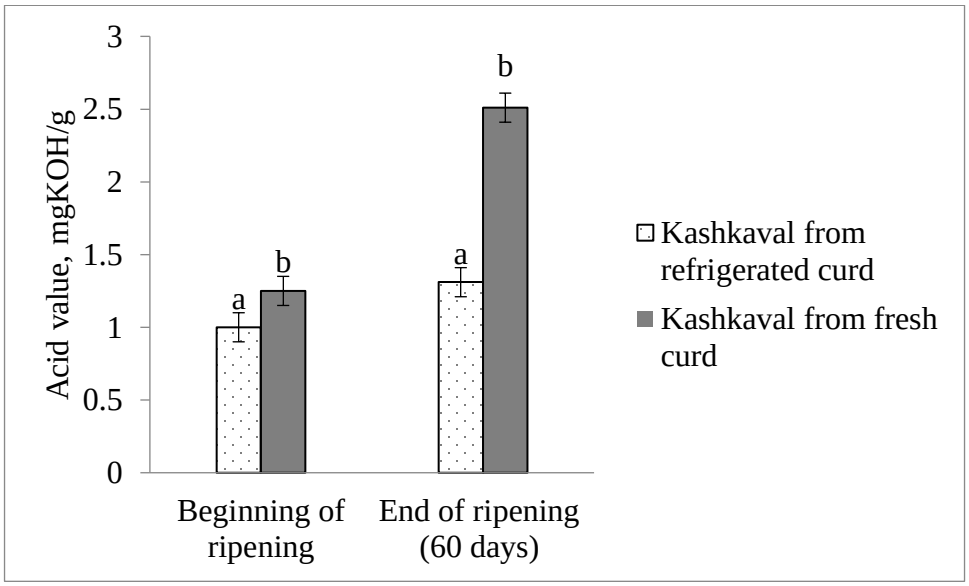
The lipolysis and oxidation of milk fat are presented in Figure 1 (A) and (B).

**Table 4.** Fatty acid profiles of Kashkaval from refrigerated and fresh curd

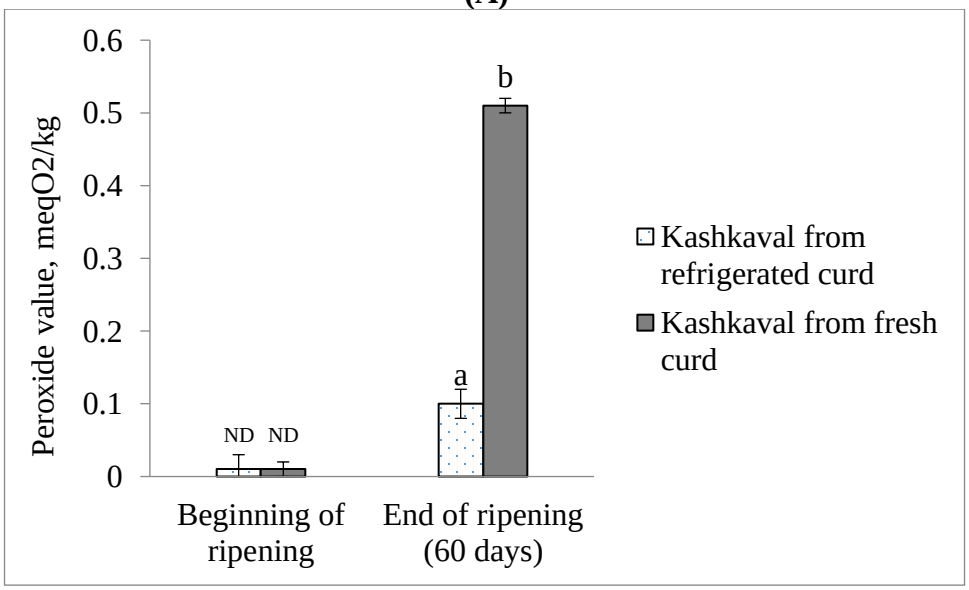
| Fatty acid, g/100 g extracted fat | Kashkaval from refrigerated curd | | Kashkaval from fresh curd | |
|---|---|---|---|---|
| | Beginning of ripening | End of ripening (60 days) | Beginning of ripening | End of ripening (60 days) |
| C4:0 | $1.22 \pm 0.05^{a}$ | $0.87 \pm 0.02^{b}$ | $0.91 \pm 0.01^{b}$ | $1.04 \pm 0.02^{c}$ |
| C6:0 | $1.32 \pm 0.0^{4a}$ | $0.99 \pm 0.03^{b}$ | $0.98 \pm 0.02^{b}$ | $1.00 \pm 0.04^{b}$ |
| C8:0 | $3.60 \pm 0.09^{a}$ | $2.89 \pm 0.09^{a}$ | $2.61 \pm 0.07^{b}$ | $2.66 \pm 0.08^{b}$ |
| C10:0 | $4.80 \pm 0.08^{a}$ | $4.22 \pm 0.08^{b}$ | $3.30 \pm 0.06^{c}$ | $3.28 \pm 0.04^{c}$ |
| C11:0 | $0.19 \pm 0.02^{a}$ | $0.20 \pm 0.01^{a}$ | $0.09 \pm 0.00^{b}$ | $0.13 \pm 0.05^{c}$ |
| C12:0 | $13.49 \pm 0.93^{a}$ | $12.86 \pm 1.10^{a}$ | $11.98 \pm 0.90^{a}$ | $11.68 \pm 0.99^{a}$ |
| C13:0 | $0.56 \pm 0.08^{a}$ | $0.47 \pm 0.07^{a}$ | $0.71 \pm 0.05^{b}$ | $0.73 \pm 0.06^{b}$ |
| C14:0 | $2.22 \pm 0.15^{a}$ | $2.35 \pm 0.08^{a}$ | $2.24 \pm 0.07^{a}$ | $2.28 \pm 0.09^{a}$ |
| C14:1 | $0.21 \pm 0.01^{a}$ | $0.19 \pm 0.05^{a}$ | $0.44 \pm 0.09^{b}$ | $0.34 \pm 0.06^{b}$ |
| C16:0 | $36.57 \pm 1.00^{a}$ | $34.16 \pm 1.10^{b}$ | $32.45 \pm 1.11^{c}$ | $30.20 \pm 1.17^{d}$ |
| C16:1 | $2.20 \pm 0.07^{a}$ | $2.13 \pm 0.08^{a}$ | $2.05 \pm 0.06^{a}$ | $1.60 \pm 0.10^{b}$ |
| C17:0 | $0.73 \pm 0.02^{a}$ | $0.57 \pm 0.02^{b}$ | $0.92 \pm 0.03^{c}$ | $0.89 \pm 0.02^{c}$ |
| C17:1 | $0.34 \pm 0.01^{a}$ | $0.13 \pm 0.01^{b}$ | $0.26 \pm 0.02^{c}$ | $0.23 \pm 0.03^{c}$ |
| C18:0 | $10.58 \pm 1.00^{a}$ | $10.96 \pm 0.09^{a}$ | $13.74 \pm 1.12^{b}$ | $13.01 \pm 0.90^{b}$ |
| C18:1 | $19.4 \pm 1.10^{a}$ | $20.94 \pm 1.30^{a}$ | $23.33 \pm 1.20^{b}$ | $21.75 \pm 1.13^{a}$ |
| C18:2 | $1.69 \pm 0.04^{a}$ | $2.79 \pm 0.04^{b}$ | $2.35 \pm 0.05^{c}$ | $5.16 \pm 0.07^{d}$ |
| C18:3 | ND | $1.68 \pm 0.04^{a}$ | ND | $1.82 \pm 0.05^{b}$ |
| C20:0 | $0.53 \pm 0.01^{a}$ | $0.45 \pm 0.02^{b}$ | $0.79 \pm 0.02^{c}$ | $0.76 \pm 0.03^{c}$ |
| C20:1 | $0.35 \pm 0.01^{a}$ | $1.17 \pm 0.01^{b}$ | $0.85 \pm 0.04^{c}$ | $1.45 \pm 0.05^{d}$ |
| Saturated fatty acids | $75.81 \pm 3.57^{a}$ | $70.99 \pm 3.14^{a}$ | $70.72 \pm 3.90^{a}$ | $67.66 \pm 3.48^{a}$ |
| Unsaturated fatty acids | $24.19 \pm 2.11^{a}$ | $29.03 \pm 2.80^{a}$ | $29.28 \pm 2.91^{a}$ | $32.35 \pm 2.48^{a}$ |

$^{a-d}$ Means with different letters within a row are significantly different ($p<0.05$)

$^{ND}$ Not Detected




**(A)**




**(B)**

[a-b] Means with different letters within columns are significantly different ($p<0.05$)
[ND] Not Detected

**Figure 1.** Acid (A) and peroxide (B) values of Kashkaval milk fat extracted from refrigerated and fresh curd

Lipolytic processes were significantly less pronounced than proteolytic processes. This was due to the starter culture which was known to produce more thermoresistant enzymes with proteolytic activity than lipolytic during cheddaring of the curd and not during ripening (Kozhev, 2006). At Figure 1 (A) it can be seen that the lipolysis was more pronounced in the Kashkaval obtained from fresh curd which could be explained by the lower salt content in this product and the more active lactic acid process which took place in the fresh curd. This was inconsistent with the results obtained from Alonso *et al*. (2012) who found that cheese containing frozen curd from pasteurized ewe milk had concentrations of free fatty acids

similar to those of the control sample. Probably these differences were due to the type of raw material used (sheep's milk), as well as some technological factors - used starter culture, processed curd, etc. The oxidation processes presented in Figure 1 (B) were not very marked in both cheeses. They were more pronounced in the cheese from fresh curd which correlated to the higher lipolysis established in this sample. Similar results were reported by Picon *et al*. (2012) who established lower levels of free fatty acids in the cheeses produced from frozen goat curd.

## 4. Conclusions

The obtained results showed that refrigerated curd, so called "Cagliata" cheese, could be successfully used for the production of Kashkaval cheese or similar Pasta filata-type cheeses. However when making cheese from refrigerated curd, it must be taken into account the fact that the cheese produced from this raw material reached earlier the stage of full breakdown of macropeptides to peptides of low molecular weight and aminoacids which was not the case of the control sample where protein was partially hydrolyzed for the same ripening period. Its casein water-bounding ability was more pronounced compared to the control sample. The effects of refrigerated curd application in the production of Kashkaval cheese on the microbiological and sensory profile as well as on its rheological properties during ripening is yet to be evaluated.

Our results demonstrated that the production of cheese from refrigerated curd was possible with some minor changes in their composition but retaining quality characteristics. The use of chilled curd for the production of Kashkaval or similar cheese is an opportunity to achieve significantly lower cost compared to the production of cheese obtained from raw cow's milk in countries where the unit price is higher.

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# PHYSICOCHEMICAL, SENSORY AND MICROBIAL ASSESSMENT OF NEWLY FORMULATED AND FORTIFIED YOGURT

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**ABSTRACT**

Yogurt is an ideal source of gut microbes. Newly formulated six yogurt samples apart from control were prepared using 10 % and 20 % of coconut milk as partial substitute of milk; followed by incorporation of date molasses (10 %, 15 % and 20 %). Samples were analyzed for physicochemical properties such as moisture, ash, fat, protein, total solids (TS), total soluble solids (TSS), pH, titratable acidity, total phenolic contents (TPC) along with microbial and sensory properties. pH, acidity and microbiological characteristics were examined on the 1st and 7th day (refrigeration at 4ºC). A significant ($p<0.05$) decrease in moisture content (ranged-69.06% to 76.12%) in enriched samples was observed in comparison with control (80.33%). Significant ($p<0.05$) positive effect of treatment in TS, TSS, fat, protein, ash and TPCs of yogurt samples had been noticed. pH and acidity for each treatment level were not significant ($p<0.05$) in paired comparison (1st day and 7th day) but the significant effect of treatments in pH and acidity ($p<0.05$) was marked. The total coliform count was observed as nil in control and experimental yogurt at 1st and 7th day (storage at 4ºC). For all developed samples, total viable bacteria count at 7th day was significantly higher than that of at 1st day (p-value < 0.05). However, these results were significantly lower than in controlled sample. Yogurt formulated using 10% coconut milk followed by 20% date molasses revealed significant higher value for taste, flavor and overall acceptability ($p<0.05$) but color and texture change were not significant ($p<0.05$)

## 1. Introduction

Food which contains components such as specific minerals, vitamins, fatty acids or dietary fibers that aid specific functions in the body in a targeted way so as to have positive effects on health can be said to be a functional food (Roberfroid, 2000). Those foods are developed for good health or to lessen the risk of diseases. Foods with added phytochemicals and those that can support beneficial microbial cultures of interest are within this kind (Ndife and Abbo, 2009). Yogurt is one of the widely used health beneficial fermented dairy product that can be produced by lactic fermentation of milk with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Living microorganisms such as lactic acid bacteria (LAB), *streptococci*, *bifidobacteria* or their combinations, from the starter cultures are accountable for the quality of the product (Amerinasab *et al.,* 2015). Yogurts are varied in different regions of the world and types would be as full-fat, low-fat, non-fat, flavored, probiotic, frozen and drink yogurt (Fiszman *et al.*, 1999). People who are reasonably lactose intolerant can appreciate yogurt without unfriendly effects, as the lactose in milk precursor has been converted to lactic acid by the bacterial culture (Heyman, 2000). It also has medical uses because of the probiotic properties, in serving out on gastro intestinal situations and

in inhibiting antibiotic-associated diarrhea (Mazahreh and Ershidat, 2009). Yogurt was thought to encourage good gum health, supports the absorption of calcium, thus avoiding osteoporosis because of the probiotic effect of lactic acid in yogurt (Kerry *et al.,* 2001). Very recently incorporation of natural food additives and other substances that are supportive to health is highly promising. Development of such products was carried out by adding plant-based flavored syrups or concentrates to cultured milk (Gonzalez *et al.,* 2011).

Coconut milk which has high fat content is usually used in curries and other bakery products as a thickener. It is rich in nutrients such as vitamins (C, E) and minerals like iron, calcium, potassium, magnesium, and zinc and is a good source of fiber (Seow and Gwee, 1997). It has health benefits such as anti-carcinogenic, anti-microbial, anti-bacterial, and anti-viral. A most important constituent of it is saturated fat, lauric acid which is present in mother's milk and has been associated in promoting brain development (Belewu and Belewu, 2007). Lauric acid is also supportive in enhancing the immune system and keeping the elasticity of the blood vessels. Consumption of this milk is hardly accompanying with allergenic responses. Other benefits of coconut milk include: it helps in digestion, nourishes skin and has cooling properties (Sethi *et al.,* 2016). But the presence of saturated fats limits its consumption though it has other health benefits.

Yogurt formulated with fruit or vegetable mixtures provides improved nutritive functions and sensory attributes (Cakmakci *et al.,* 2014). Furthermore, such health promoted and highly preferred dairy products with date molasses or date liquid sugar was also reported (Amerinasab *et al.,* 2015). In Bangladesh, the sap of silver date palm, *Phoenix sylvestris* is tapped to be consumed fresh, or processed into either syrup or the jaggery known as "gur". It contains up to 50% sucrose, up to 20% invert sugars, and up to 20% moisture, with the remainder, made up of other insoluble matter, such as wood ash, proteins, and bagasse fibers. The harvested syrup (collected in clay pots suspended from notches cut in the trunk) is boiled down to get different stabilities ranging from liquid to solid. Most Bengali confectioners use such date molasses in making sweets during the winter months. The undying popularity of the molasses has also found in the literature and culture of the country of Bangladesh and Indian state of West Bengal (Banerji, 2012). In this vein, it was attempted to develop yogurt with different combinations of coconut milk and date palm molasses and to determine the effect of different concentrations of the value additions on their physicochemical, sensory, and microbial characteristics.

## 2. Materials and methods

The research was conducted in the Laboratories of the Department of Nutrition and Food Engineering, Daffodil International University, Dhaka, Bangladesh.

### 2.1. Materials

#### *2.1.1. Chemicals*

All required chemicals and solvents were collected from Sigma-Aldrich Chemical Co. (USA).

#### *2.1.2. Samples*

Fresh matured coconut and pasteurized cow milk were procured from the local market of Dhaka city. Freeze-dried starter culture of *L. bulgaricus* and *S. thermophilus* (CH1, Chr. Hansen's Dairy Cultures, Hoersholm, Denmark) were also purchased from a vendor in Dhaka city, Bangladesh. Date palm molasses without soda or any other stuff was collected from Pantapara village in Jessore's Khajura area, Bangladesh.

### 2.2. Methods

#### *2.2.1. Coconut milk preparation*

The de-husked coconuts were cracked open into halves. The split nuts were de-shelled, brown skin was removed and then washed. Then it was blended using an electric blender (Sanyo SM-B12M) with deionized water. Resultant was then sieved through a vibrating screen filter machine (SY 450) to get coconut milk and analyzed (moisture 70.02 %, fat 17.9 %, protein 2.30 % and ash 0.70 %) followed by refrigeration for further use.

### *2.2.2. Yogurt preparation*

Date palm molasses used was standardized as ash 1.9%, moisture 39%, fat 0.3% and protein 0.62%. Milk and coconut milk (CM) were blended in 90:10 and 80:20 ratios and the resultants (milk with coconut milk 10% and 20%) were further blended with 10%, 15% and 20% date palm molasses (DM) to obtain six samples. Samples were labeled as CM-DM (10-10), CM-DM (10-15), CM-DM (10-20), CM-DM (20-10), CM-DM (20-15) and CM-DM (20-20). One sample with cow milk without coconut milk and date palm molasses was considered as control (C). All samples were heated to 85ºC for 15 min followed by rapid cooling to 45ºC. Culture (0.5 g) was then inoculated to each with gentle mixing. The inoculated samples were transferred to 100 ml plastic cups and incubated at 45°C for 4 hours to allow fermentation, hence rapid production of lactic-acid by the inoculated bacteria resulting in complete coagulation of the milk. All samples were stored in the refrigerator at 4°C for subsequent analysis.

### *2.2.3. Physico-chemical analysis*

According to the Association of Official Analytical Chemists methods (AOAC 1999), percentage of moisture was determined by oven drying method to a constant weight. Total mineral content by dry ashing procedure using muffle furnace (Maurice and Marshall, 2010), percentage of crude protein by Kjeldahl nitrogen considering factor as 6.25 (Lynch and Barbano, 1999) and fat content by using Garber method (Kleyn, 2001) were determined. All these analyses were stated as grams per 100 g of fresh weight.

Total phenolics were determined by a spectrophotometric method using Folin – Ciocalteu reagent. 1.0 mL of sample solution extract in water containing 1.0 mg of sample was pipetted out into a flask. 46 mL of distilled water was then added to it followed by the addition of 1 mL of Folin Ciocalteu reagent with thorough mixing. 3.0 mL of 2 % sodium carbonate was added to the resultant after 3 min of mixing. Then it was incubated for 2 hours at ambient temperature and absorbance was measured at 760 nm using a UV – visible spectrophotometer (T-80, double beam). Concentrations were expressed as micrograms of Gallic acid equivalents (GAE) per 100 mg of fresh weight by using the following linear equation obtained from the calibration curve:

$A = 0.0166C + 0.0542$, $R^2 = 0.9994$, (A is the absorbance and C is concentration as GAE μg/mg).

Titratable acidity, pH and total soluble solids (TSS) were also determined. Total soluble solids (TSS) were determined by an ‘‘Atago, Japan’’ hand refractometer at 20° C. The pH value was determined using a Hanna (HI 8424) pH meter. Direct titration by sodium hydroxide (0.1 M) to a pH value 8.1 using a pH meter was used to measure titratable acidity (T.A) which was expressed as percent of Lactic acid (Jacobs, 1999). Total solids (TS) were calculated by taking into consideration the moisture content as TS = 100 – Moisture content.

### *2.2.4. Microbiological tests*

Yogurt samples were studied by using the plate count agar for the total viable bacteria count and MacConkey agar for the coliform count. The colonies were counted and the result was expressed as colony-forming unit per ml (CFU/ml) (APHA 1978).

### *2.2.5. Organoleptic scoring*

Sensory evaluation of yoghurt samples prepared with changed concentrations of coconut milk and date palm molasses was carried out with a panel of 10 members including graduate students and staff of the Nutrition and Food Engineering Department, Faculty of Allied Health Sciences, Daffodil International University who were accustomed to the typical attributes of samples, on 9-point hedonic scale for different parameters such as color, flavor, taste, texture and overall acceptability according to the suggested method (Ihekoronye and Ngoddy, 1985). Assessors were in sensory booths with proper lighting and requested to indicate on a questionnaire whether they can make comment between samples with regard to these parameters. Each sample was presented thrice, and the samples were presented in random order. This evaluation was based on hedonic 9-point structured scale, in which 9

corresponded to most liked and 1 to most disliked.

***2.2.6. Ethical Issues***

Sensory data was collected by providing prior knowledge to the assessors about the objectives of the study. An informed written consent was signed from the sensory evaluators before the test and was ensured that the products were made in the laboratory with maintaining proper hygiene and the materials used in preparing the products were free from any sort of health risk. The study received approval from the Research Ethical Committee of Faculty of Allied Health Sciences, Daffodil International University (Ref. No. FAHSREC/DIU/2019/1003).

***2.2.7. Statistical Analysis***

All analytical experiments were repeated in triplicates and the results reported as a mean of the values found with standard deviation. The obtained data were statistically analyzed by means of statistical tools such as one-way analysis of variance (ANOVA), Tukey's HSD test and Paired T-test with considering 5% significance level using R (Version 3.4.3, RStudio version: 1.1.383) software and Microsoft excel 2007 (version 12.0.4518.1014).

## 3. Results and discussions

### 3.1. Physico-Chemical characteristics

Considered physicochemical characteristics for the developed samples are depicted in Table 1 and Table 2. Moisture percentage of incorporated samples ranged from 69.06 to 76.12 whereas the value for control was 80.33. Significant ($p<0.05$) difference in moisture with different treatments was observed. This could happen due to varied compositions of milk substitutions in samples. Enriched samples had lower moisture percentage than the control ($p<0.05$) and yogurt samples with 10-20 and 20-20 treatments had the lowest moisture ($p<0.05$). The value decreased with an increased percentage of coconut milk and date palm molasses, as obtained (Gad *et al.,* 2010). However, less water content means more total solid content in yogurt and ensures more storage stability.

The total solids increased in samples developed with coconut-milk and date palm molasses had a significant effect of treatment on TS ($p<0.05$). Samples with 20-20 and 10-20 treatments had significantly higher values of TS than samples with other treatments ($p<0.05$). Increase in total solids may also have a positive impact on the texture of the resultant product reported (Abd El-Tawab, 2009).

As the total solids reflect samples dry matter content, samples developed with a high weight percentage of coconut milk and date palm molasses had the highest soluble solids content ranged from 11.32 % to 17.51 %. Significantly ($p<0.05$) highest TSS value in yogurt samples of 20-20, 10-20 treatments and significantly lowest TSS value in control can be explained by considering the impact of sugars from coconut milk and date palm molasses incorporation (Belewu *et al.,* 2010; Hamdia and Hamdani, 2016).

Protein content in the developed samples was in the range of 4.21% to 4.92%. Supplemented samples had a significant positive effect of treatment ($p<0.05$) on protein content. Yogurt of 20-20 treatment had the highest protein level ($p<0.05$) whereas control had the lowest protein level ($p<0.05$).

The Fat percentage of incorporated samples varied in between 6.92 to 6.98 whereas the value for control was 5.19. The significantly high fat content of amended samples ($p<0.05$) from control was found but there was no significant change of fat percentage with the variation of date palm molasses and coconut milk. It has been reported that fat % has a positive impact on the sensory and physical attributes of yogurt though it has a negative effect on the shelf life of yogurt (Bille and Keya, 2002; Farinde *et al.*, 2009; Marinescu and Pop, 2009; Saint-Eve *et al.,* 2008)

Ash percentage of yogurt without coconut milk and date palm molasses was 0.51 but the value for the developed samples was increased and it was in the range of 0.61 to 0.90. Significant effect of treatment on ash content ($p<0.05$) with the highest ash content in samples of 10-20 and 20-20 treatments were observed while the control had the lowest ash content

($p<0.05$). Ash content significantly ($p<0.05$) increased as percentage of date molasses increased. The increased ash percentage of the enriched samples can be attributed from the incorporation of coconut milk and date palm molasses as coconut milk and date palm molasses are of high mineral content (Imele and Atemnkeng, 2001; Marinescu and Pop, 2009). More ash content in the samples is in line with the results from coconut incorporated yogurts (Ndife *et al.*, 2014).

**Table 1.** Physicochemical attributes of developed samples as a function of coconut milk (CM) and date molasses (DM) content *

| Attributes | Treatment (CM-DM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control** | **10-10** | **10-15** | **10-20** | **20-10** | **20-15** | **20-20** |
| **Moisture** | 80.33 ±0.17$^{a}$ | 76.12 ±0.23$^{b}$ | 73.19 ±0.18$^{d}$ | 69.16 ±0.31$^{e}$ | 75.25 ±0.07$^{c}$ | 73.10 ±0.20$^{d}$ | 69.06 ±0.10$^{e}$ |
| **Protein** | 3.81 ±0.27$^{e}$ | 4.21 ±0.14$^{d}$ | 4.26 ±0.13$^{c}$ | 4.48 ±0.35$^{b}$ | 4.76 ±0.11$^{b}$ | 4.84 ±0.16$^{b}$ | 4.92 ±0.25$^{a}$ |
| **Fat** | 5.19 ±0.19$^{b}$ | 6.92 ±0.29$^{a}$ | 6.95 ±0.25$^{a}$ | 6.97 ±0.28$^{a}$ | 6.96 ±0.23$^{a}$ | 6.92 ±0.32$^{a}$ | 6.98 ±0.25$^{a}$ |
| **Ash** | 0.627 ±0.193$^{c}$ | 0.627 ±0.193$^{b}$ | 0.657 ±0.04$^{b}$ | 0.903 ±0.042$^{a}$ | 0.613 ±0.176$^{b}$ | 0.66 ±0.061$^{b}$ | 0.86 ±0.154$^{a}$ |
| **TSS** | 7.73 ±0.03$^{f}$ | 11.32 ±0.03$^{e}$ | 14.59 ±0.04$^{c}$ | 17.42 ±0.1$^{a}$ | 11.52 ±0.04$^{d}$ | 15.06 ±0.08$^{b}$ | 17.51 ±0.02$^{a}$ |
| **TS** | 19.67 ±0.17$^{e}$ | 23.88 ±0.23$^{d}$ | 26.81 ±0.18$^{b}$ | 30.84 ±0.31$^{a}$ | 24.75 ±0.07$^{c}$ | 26.9 ±0.2$^{b}$ | 30.94 ±0.1$^{a}$ |
| **TPC** | 0.04 ±0.003$^{d}$ | 1.09 ±0.002$^{c}$ | 1.762 ±0.002$^{b}$ | 2.05 ±0.003$^{a}$ | 1.095 ±0.004$^{c}$ | 1.763 ±0.003$^{b}$ | 2.05 ±0.002$^{a}$ |

*Values in the same columns followed by different letters (a, b, c, d, e) are significantly different ($p<0.05$)

**Table 2.** pH and acidity of newly formulated samples as a function of coconut milk (CM) and date molasses (DM) content *

| Attributes | Treatment (CM-DM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control** | **10-10** | **10-15** | **10-20** | **20-10** | **20-15** | **20-20** |
| **pH** | 4.412 ±0.022$^{d}$ | 4.412 ±0.022$^{c}$ | 4.452 ±0.017$^{c}$ | 4.513 ±0.022$^{b}$ | 4.528 ±0.026$^{b}$ | 4.528 ±0.021$^{b}$ | 4.602 ±0.018$^{a}$ |
| **Acidity** | 0.672 ±0.015$^{a}$ | 0.65 ± 0.02$^{b}$ | 0.625 ± 0.024$^{c}$ | 0.595 ± 0.021$^{d}$ | 0.63 ± 0.021$^{c}$ | 0.608 ± 0.025$^{d}$ | 0.605 ±0.018$^{d}$ |

*Values in the same columns followed by different letters (a, b, c, d, e) are significantly different ($p<0.05$)

Significant difference between the paired data (1$^{st}$ day and 7$^{th}$ day) for both pH and acidity for each treatment level was not observed. pH and titratable acidity values of the produced yogurts are depicted in Table 2. Yogurt without coconut milk and date palm molasses were ascertained as pH 4.4. Food Standard code for pH of yogurt was reported as a maximum of 4.5 to avoid the growth of any pathogenic organisms (Donkor *et al.*, 2006). pH value of amended samples was in between 4.41 and 4.60. Sample of 20-20 treatment had significantly different pH ($p<0.05$) followed by the value for a sample of 10-20 treatment ($p<0.05$). pH value of enriched samples would be lower than that of control as there was the lower possibility of the production of lactic acid from milk sugar lactose due to the presence of lower proportion of milk in enriched

samples. However, such an increase of pH is in line with the addition of mulberry juice to yogurt which led to the higher pH value of the product (Celik and Bakirci, 2003).

Titratable acidity ranged from 0.60% to 0.65% in the incorporated samples though control had value 0.67%. Such lower acidity due to the incorporation of coconut milk and date palm molasses is also in line with the reverse change of pH and titratable acidity. Enriched samples showed a significant effect of treatments on acidity ($p<0.05$). In particular, significantly lower value for a sample of 10-20 treatment was detected.

Total phenolic contents in the developed samples were found more than the plain yogurt (0.04 μg GAE/ mg of yogurt) and it was ranged 1.09 to 2.05 μg GAE/ mg of fresh weight. Treatment had a significant effect ($p<0.05$) and TPCs were significantly higher in samples amended as 10-20 and 20-20 ($p<0.05$). Products developed with coconut milk had positive significant change from control but there was no significant increase of the TPCs of the products developed with a variation of the proportion of coconut milk by 10% to 20%. As the proportion of date molasses in the yogurt samples was increased, TPCs were significantly increased. Phenolic contents are plant secondary metabolites and are key components of human diets for their antioxidant properties.

### 3.2. Organoleptic characteristics

Mean score given by the considered panelists for each sensory attributes of all developed yogurts are in short in Table 3 and Fig.1. Control yogurt had the lowest score (5.9±1.1) and (6.3±1.16), while samples with treatment had the higher score (ranged 6.0±1.25 to 7.4±1.26) and (ranged 6.5±0.71 to7.2±1.4) for color and texture respectively. The statistical analysis showed that there were no significant differences ($p<0.05$) among the control and treated yogurt samples in the color and texture observed. Sample of 10-20 treatment had highest scores of 8.1±0.88 and 7.9±0.99 for both taste and flavor, while control sample had the lowest scores of 5.9±0.99 and 5.9±0.74 for taste and flavor respectively.

**Table 3.** Sensory traits of the developed yogurts as a function of coconut milk (CM) & Date molasses (DM) content *

| Sensory Attributes | Treatment (CM-DM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control** | **10-10** | **10-15** | **10-20** | **20-10** | **20-15** | **20-20** |
| **Color** | 5.9 ±1.1$^{a}$ | 6.0 ±1.25$^{a}$ | 6.7 ±1.57$^{a}$ | 7.4 ±1.26$^{a}$ | 6.7 ±1.42$^{a}$ | 7.0 ±2.0$^{a}$ | 6.2 ±2.57$^{a}$ |
| **Taste** | 5.9 ±0.99$^{c}$ | 6.7 ±0.82$^{b}$ | 6.8 ±0.92$^{b}$ | 8.1 ±0.88$^{a}$ | 6.7 ±1.34$^{b}$ | 6.9 ±1.97$^{b}$ | 6.8 ±1.87$^{b}$ |
| **Flavor** | 5.9 ±0.74$^{c}$ | 6.5 ±0.71$^{c}$ | 6.8 ±1.03$^{b}$ | 7.9 ±0.99$^{a}$ | 6.4 ±0.52$^{c}$ | 6.5 ±0.97$^{c}$ | 6.9 ±1.73$^{b}$ |
| **Texture** | 6.3 ±1.16$^{a}$ | 6.5 ±0.71$^{a}$ | 6.6 ±0.7$^{a}$ | 7.2 ±1.4$^{a}$ | 6.5 ±1.27$^{a}$ | 6.7 ±1.83$^{a}$ | 7.1 ±1.6$^{a}$ |
| **Overall acceptability** | 6.5 ±0.53$^{C}$ | 6.7 ±0.68$^{b}$ | 6.9 ±0.74$^{b}$ | 7.5 ±0.97$^{a}$ | 6.4 ±0.7$^{c}$ | 6.8 ±0.79$^{b}$ | 7.3 ±1.06$^{b}$ |

*Values in the same columns followed by different letters (a, b, c) are significantly different ($p<0.05$)

**Table 4.** Microbiological characteristics of yogurt samples *

| Treatment (CM-DM) | TVC (CFU/ml) | | TCC (CFU/ml) | |
|---|---|---|---|---|
| | 1st day | 7th day | 1st day | 7th day |
| **Control** | $8.2\times10^5$ $\pm 0.42426\times10^{5\,a}$ | $9\times10^5$ $\pm 0.84852\times10^{5\,a}$ | Nil | Nil |
| **10-10** | $5.85\times10^5$ $\pm 0.35355\times10^{5\,b}$ | $7.25\times10^5$ $\pm 0.49497\times10^{5\,b}$ | Nil | Nil |
| **10-15** | $5.65\times10^5$ $\pm 1.20208\times10^{5\,b}$ | $6.4\times10^5$ $\pm 0.70710\times10^{5\,b}$ | Nil | Nil |
| **10-20** | $5.4\times10^5$ $\pm 0.70710\times10^{5\,b}$ | $5.75\times10^5$ $\pm 1.06066\times10^{5\,c}$ | Nil | Nil |
| **20-10** | $6.2\times10^5$ $\pm 0.42426\times10^{5\,b}$ | $6.55\times10^5$ $\pm 0.49497\times10^{5\,b}$ | Nil | Nil |
| **20-15** | $4.8\times10^5$ $\pm 0.14142\times10^{5\,c}$ | $6.1\times10^5$ $\pm 0.14142\times10^{5\,c}$ | Nil | Nil |
| **20-20** | $5\times10^5$ $\pm 0.70710\times10^{5\,c}$ | $5.75\times10^5$ $\pm 0.35355\times10^{5\,c}$ | Nil | Nil |

*TVC-Total viable bacteria count; TCC- Total coliform count; Data are mean values of duplicate determinations ± standard deviation; Values in the same columns followed by different letters (a,b,c ) are significantly different ($p<0.05$)

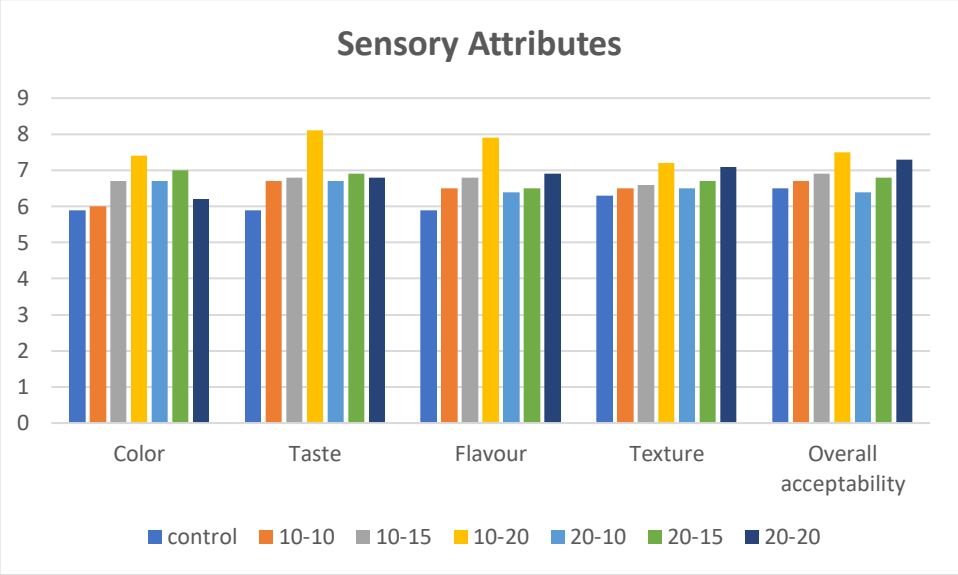


**Figure 1.** Graphical presentation of the mean of sensory attributes of developed samples as a function of coconut milk (CM) & Date molasses (DM) content

The high taste and flavor values could be due to increased oil content and sweetness from coconut milk and date palm molasses. Enriched yogurt supplemented with 20-20 treatment had lowest overall acceptability rating (6.4±0.7) whereas yogurt with 10-20 treatment had highest value (7.5±0.97) for the same. Yogurts supplemented with coconut-cake and date liquid syrup was reported as better samples with the high score of sensory attributes such as texture, aroma, flavor, sweetness and overall acceptability (Amerinasab *et al.*, 2015; Ndife *et al.*, 2014). However, product with 10-20 treatment revealed significant higher value for taste, flavor and overall acceptability ($p<0.05$).

### 3.3. Microbial assessment

Microbial characteristics by considering TVC and TCC at 1st and 7th day of processing (stored at 4ºC) were shown in Table 4. No growth of coliform bacteria (TCC) indicates that samples were free from contamination during production and storage. A significant difference in the paired data (1st day and 7th day) for TVC in control and each treatment level was observed. TVC at 7th day was significantly higher than TVC at 1st day (p-value < 0.05). Significant (p-value < 0.05) lower growth of total viable bacteria in all the developed samples than the control was observed. This can be explained by taking into account the antimicrobial effect of date palm molasses as the antimicrobial properties of date palm were noticed (Hamdia and Al-Hamdani, 2016). Such degeneration in the viable bacterial count may arise due to increased acidity of enriched products (Abd El-Tawab, 2009). However, the total viable bacterial load of the samples was acceptable as the standard of microbial status is $<1x10^6$ CFU/ml (El Bakri and Zubeir, 2009).

## 4. Conclusions

Nowadays, fermented dairy product consumption is growing particularly for its brilliant nutritious and remedial properties. Coconut milk and date molasses as natural and nutritive improver would be healthier supplements for yogurt. Results showed that moisture content of enriched yogurt particularly samples with treatment 10-20 and 20-20 are of low value where TS, TSS, fat, protein, ash, and TPCs of those yogurt samples were found as high value than the control sample. pH and acidity of supplemented samples were found significantly different from the control sample. Organoleptic scoring of the sample with 10-20 treatment is high in terms of taste, flavor, and overall acceptability, but the color and texture of the samples were not shown as significantly different. The total viable bacterial count of formulated samples was of lower value than that of yogurt without treatment. It can be concluded from the study that the yogurt prepared by incorporation of 10 percent coconut milk followed by 20 percent date molasses boosted physicochemical, organoleptic, and nutritive characteristics along with the presence of reasonable total viable bacterial growth in the product. In all considerations, this enriched yogurt would be an alternative functional product for its upgraded characteristics. Due to the short period of time, the sensory evaluation was carried out with a smaller number of assessors. More study is needed for other microbial characteristics and to find out antioxidant properties of the newly formulated yogurt.

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# INHIBITION OF LIPID PEROXIDATION AND RADICAL SCAVENGING ACTIVITY OF SYNTHESIZED CURCUMIN AND BISDEMETHOXYCURCUMIN IN FOOD SYSTEMS

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**ABSTRACT**

In this study, curcumin and bisdemethoxycurcumin (BDMC) were synthesized using a patented procedure and tested *in vitro* for inhibition of lipid peroxidation and for radical scavenging activities. At the same 0.14 mM concentration, the order of the inhibitory effect on lipid peroxidation was 2,6-di-tert-butyl-4-methylphenol (BHT) > curcumin > BDMC (97% > 89.7% > 73.4%, respectively). Curcumin also showed activities in scavenging hydrogen peroxide and DPPH radicals stronger than BDMC due to the presence of two methoxy groups in the curcumin molecule. However, BDMC showed higher $ABTS^{\bullet+}$ cationic radical scavenging activity. Curcumin was then chosen to be used and tested for antioxidant effects in two food systems. At the same molar concentration, curcumin is about 25% less effective than BHT in inhibiting crude fish oil peroxidation. Starch films containing curcumin showed DPPH scavenging activities lower than those of free curcumin due to the protecting effect of gelatinized starch and the slow release of curcumin from the film.

## 1. Introduction

Lipid oxidation is one of the major problems in the food industry because it produces substances that deteriorate product quality and adversely affect the colour and nutrition of lipid-containing products. Moreover, the oxidation of cell membrane lipids is very destructive to human health (Shahidi and Zhong 2010). Among the methods for controlling lipid oxidation, the use of antioxidants is effective, convenient and economical. Antioxidants are also used for health promotion due to their ability to protect the body against oxidative stresses and damages. Traditional antioxidants in the food industry, such as BHA (butylated hydroxyanisole) or BHT (2,6-di-tert-butyl-4-methylphenol), have been used since the 1940s. However, recent studies showed that they may cause adverse effects on human health and the environment (Leclercq, Arcella, and Turrini 2000; Yang et al. 2018; Wang et al. 2019; Ito, Fukushima, and Tsuda 1985).

Curcumin and other curcuminoids isolated from turmeric are classified as multipotent antioxidants, because besides antioxidant activity, these compounds have shown antibacterial, antiviral, anti-inflammatory, antitumor, and many other helpful biological activities (Amalraj et al. 2017). The *in vivo* and *in vitro* properties of curcumin have been tested on several systems. Curcumin was proved to have anti-inflammatory, antioxidant properties and many other therapeutic effects (Hewlings and Kalman 2017).

Compared with curcumin, bisdemethoxycurcumin (BDMC) is less

abundant in turmeric and thus was less extensively investigated. Moreover, few studies compared the biological activities of these two curcuminoids in the same conditions (Jayaprakasha, Rao, and Sakariah 2006; Venkatesan and Rao 2000). However, the demand for BDMC production is increasing due to the discovery of its new biological and pharmaceutical activities (Ramezani, Hatamipour, and Sahebkar 2018; Kim, Park, and Kim 2001; Jin et al. 2020). This demand and the difficulties in isolating BDMC from turmeric remind the importance of the synthetic approach.

In this study, both curcumin and BDMC were synthesized by a patented procedure and tested for antioxidant and radical scavenging activities *in vitro*. After a comparison of the *in vitro* results, curcumin was chosen to be used and tested for antioxidant effects in two food systems (crude fish oil and starch film).

## 2. Materials and methods

### 2.1.Materials

Tributyl borate, n-butylamine, vanillin, 4-hydroxybenzaldehyde, 2,4-pentanedione, boric oxide, isopropanol, absolute ethanol, sodium dihydrogenphosphate ($NaH_2PO_4$), glycerol were purchased from Xilong Scientific (China); linoleic acid, iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$), potassium thiocyanate (KSCN), hydrogen peroxide ($H_2O_2$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 35% hydrochloric acid (HCl), ammonium persulfate ($(NH_4)_2S_2O_8$) from MilliporeSigma (USA). Yellowtail fish and corn starch were purchased from a local supermarket in Ho Chi Minh City.

### 2.2. Methods

#### *2.2.1.Synthesis of curcumin and BDMC*

Curcumin was synthesized following a procedure adapted from a patent with some modifications (Krackov and Bellis 1997). Boron oxide (4.66 g; 67.9 mmol) and 2,4-pentanedione (7.3 mL; 70.1 mmol) were placed into a 100 mL round-bottomed flask under constant magnetic stirring. After 15 min, tributyl borate (27.3 mL; 140 mmol), isopropanol solvent (10 mL) and vanillin (21.3 g; 140 mmol) were subsequently added after every 5 min. The mixture was heated to 60 $^oC$ and kept for 5 min. The reaction was triggered by slow drop-wise addition of *n*-butylamine (2.13 g; 29.1 mmol) into the reaction mixture for 1 h. After 3 h, the reaction mixture was poured into 1 L of 5% aqueous acetic acid solution at 60 $^oC$ to hydrolyse the curcuminoid-boron oxide complex. The product appeared to be a viscous liquid oil but turned to a red-orange solid after 2 h of continuous stirring. The solid product was filtered and recrystallized 2 times in 75% aqueous ethanol, and then dried at 70 $^oC$ for 2 h.

To synthesize BDMC, the same procedure was conducted, but vanillin was replaced by 4-hydroxybenzaldehyde (17.1 g; 140 mmol).

#### *2.2.2.Characterization of curcumin and BDMC*

The synthesized curcumin and BDMC were dissolved separately in absolute ethanol and scanned from 320 to 1100 nm using a UV-vis spectrophotometer (UH5300, Hitachi, Japan). Their melting points were determined by using an MP55 Melting Point System (Mettler Toledo, USA).

#### *2.2.3.Inhibition of linoleic acid peroxidation assay*

The ferric thiocyanate method was used to determine the lipid peroxidation inhibition by curcumin and BDMC (Jayaprakasha, Singh, and Sakariah 2001). Solutions of each curcuminoid with different concentrations (10 - 50µg/mL) in 2.5 mL of a phosphate buffer (0.04 M; pH 7.0) were added to 2.5 mL of an aqueous emulsion of linoleic acid. Each 5 mL of the linoleic emulsion contained 15µL of Tween-20 and 15µL of linoleic acid in the phosphate buffer (0.04 M; pH 7.0). The emulsion with curcuminoid was incubated at 37 $^oC$ in the dark. After predetermined internals of time, 0.1 mL of the sample was mixed with distilled water (9.7 mL), KSCN solution (0.1mL; 30%) and fresh 20 mM $FeSO_4$ in 3.5% HCl solution (0.1 mL). After

exactly 5 min of incubation in the dark, the absorbance of the solution was measured at 500 nm. The same procedure was conducted for the control sample, which was the linoleic emulsion mixed with an equivalent amount of the phosphate buffer instead of the curcuminoid solution.

Inhibition percentage of curcuminoid on lipid peroxidation was calculated as followed:

$$Peroxidation\ inhibition(\%) = (1 - \frac{A_C}{A_o}) \times 100 \quad (1)$$

where $A_C$ is the absorbance of the sample containing curcuminoid after reacting with ferric thiocyanate reagent when the absorbance of the control reached maximum; $A_o$ is the maximum absorbance of the control after reacting with ferric thiocyanate reagent.

### *2.2.4.Hydrogen peroxide scavenging activity*

Hydrogen peroxide scavenging activity was conducted according to a published study (Ruch, Cheng, and Klaunig 1989). Each curcuminoid solution (15 µg/mL; 3.4mL) in phosphate buffer (0.1 M; pH 7.4) was added to a $H_2O_2$ solution (43 mM; 0.6 mL). After 1 h of incubation in the dark, the absorbance at 230 nm of the mixed solution was measured. The control was the 43 mM $H_2O_2$ solution.

The $H_2O_2$ scavenging activity was calculated as followed:

$$H_2O_2\ scavenging(\%) = (1 - \frac{A_C}{A_o}) \times 100 \quad (2)$$

where $A_C$ is the absorbance of the $H_2O_2$ solution containing curcuminoid; $A_o$ is the absorbance of the $H_2O_2$ control sample (without curcuminoids).

### *2.2.5.DPPH radical scavenging capacity assay*

DPPH scavenging activities of the curcuminoids were evaluated according to a published method (Ak and Gülçin 2008). An ethanolic solution of DPPH (0.1 mM; 1 mL) was added to an ethanolic solution of a curcuminoid (10 - 50µg/mL). After 30 min of incubation in the dark, the absorbance at 517 nm of the solution was measured.

DPPH scavenging activity was calculated as followed:

$$DPPH\ scavenging(\%) = (1 - \frac{A_C}{A_o}) \times 100 \quad (3)$$

where $A_C$ is the absorbance of the DPPH solution containing curcuminoid; $A_o$ is the absorbance of the DPPH control sample (without curcuminoids).

### *2.2.6.$ABTS^{\bullet+}$ cationic radical scavenging activity*

The cationic radical scavenging activity was determined according to an improved decolourization method (Re et al. 1999). $ABTS^{\bullet+}$ was generated by mixing an aqueous ABTS solution (2 mM; 5 mL) with an ammonium persulfate solution (2 mM $(NH_4)_2S_2O_8$; 5 mL) and subsequent incubation in the dark for 4 h at room temperature. The $ABTS^{\bullet+}$ solution was then diluted to an absorbance of 0.750 ± 0.025 at 734 nm in a phosphate buffer (0.1 M; pH 7.4). After that, 2.9 mL of the diluted $ABTS^{\bullet+}$ was added to 0.1 mL of ethanolic solution of curcuminoid (10-50 µg/mL). The absorbance at 734 nm was measured after 30 min. The $ABTS^{\bullet+}$ scavenging ability was calculated as followed:

$$ABTS^{\square+}\ scavenging\ (\%) = (1 - \frac{A_C}{A_o}) \times 100 \quad (4)$$

where $A_C$ is the absorbance of the $ABTS^{\bullet+}$ solution containing curcuminoid; $A_o$ is the absorbance of the $ABTS^{\bullet+}$ control sample (without curcuminoids).

### *2.2.7.Testing of oxidation inhibition on fish oil*

Crude fish oil was extracted and purified from yellowtail catfish fat according to a procedure described elsewhere (List 2009). An ethanolic solution of curcumin was added into 10 mL of the oil at different concentrations (1.0 – 5.0 µg/mL). After thorough mixing, the oil was poured into a Petri dish and kept in a drying oven for 24 h at 60 $^oC$ to accelerate the oil oxidation (Pan et al. 2007). After this oxidation period, the oil was testest for peroxide content by the ferric thiocyanate described above.

### *2.2.8.Starch films incorporated with curcumin and DPPH scavenging test*

To incorporate curcumin into starch films, a procedure adapted from another study was used (Nawab et al. 2017). Corn starch (2 g) was suspended in 40 mL of distilled water. An ethanolic curcumin solution was added to the suspension to reach a predetermined curcumin/starch mass ratio (0-5%). Glycerol (0.6 g) as a plasticizer was then added and the mixture was heated under stirring at 90 ºC for 30 min. After that, the mixture was poured into a Petri dish and left for drying at room temperature for 48 h.

To evaluate the antioxidant activity of the composite film, 25 mg of the film was dissolved in 3 mL of distilled water at 55 ºC (Moradi et al. 2012). The radical scavenging activity of the starch – curcumin suspension was determined by the DPPH method. The suspension (0.5 mL) was added to 2.5mL of 0.6 mM DPPH ethanolic solution. After 30 min of incubation in the dark, the absorbance at 517 nm of the mixture was measured. The control of this test was a starch film without curcumin (Bitencourt et al. 2014).

## 3. Results and discussions

### 3.1. The curcuminoids synthesis

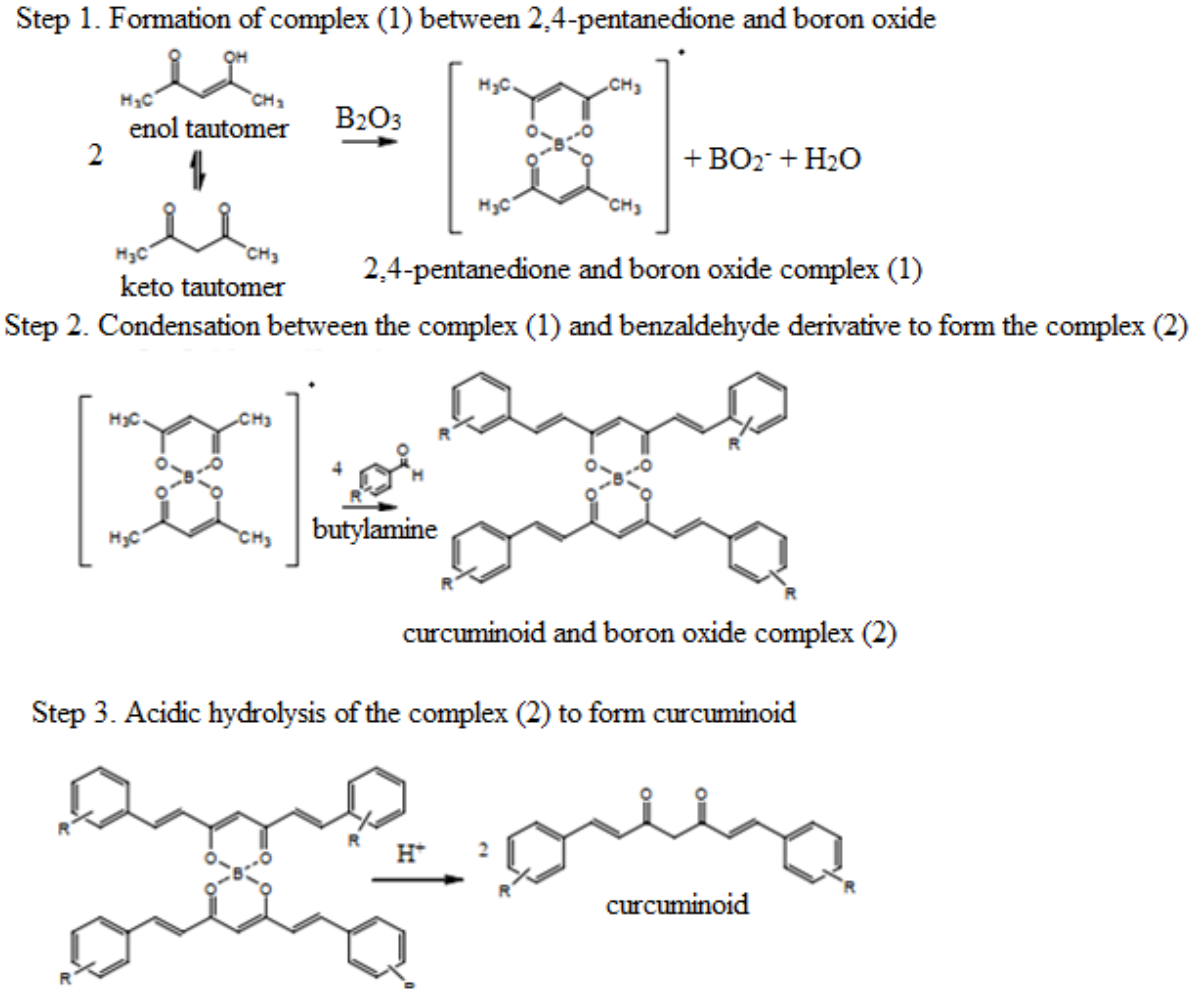


**Figure 1.** Mechanism of the synthesis of curcuminoids.

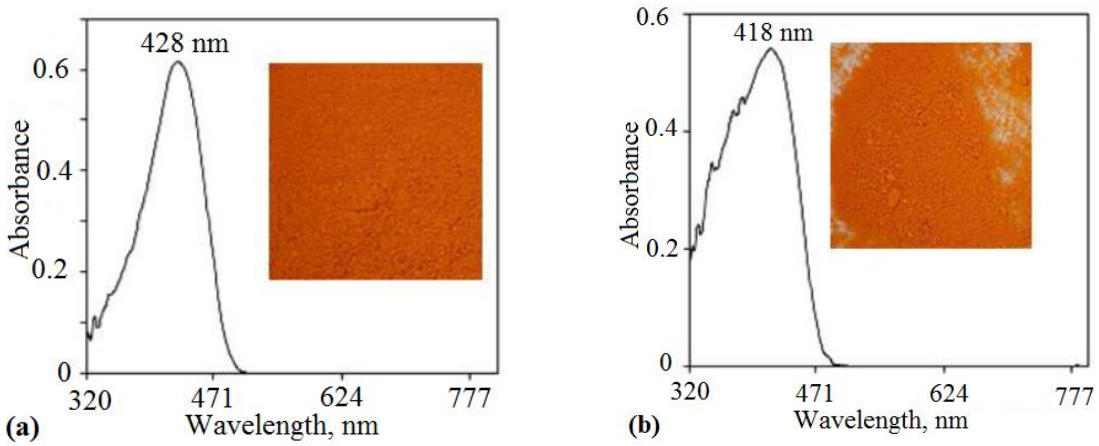


**Figure 2.** UV-vis spectra and the appearance of synthesized curcumin (a) and BDMC (b).

The first step in the synthesis of curcuminoids (Figure 1) is the formation of the complex (1) between 2,4-pentanedione and boron oxide to prevent the Knoevenagel reaction of the methylene group in the 2,4-pentanedione molecule. In the second step, aldol condensations between this complex (1) and a benzaldehyde derivative (vanillin for curcumin or 4-hydroxybenzaldehyde for BDMC) take place twice in the presence of n-butylamine catalyst to form the complex (2). The third step is the acidic hydrolysis of the complex (3) to produce the free curcuminoid (Handler et al. 2007).

The synthesized curcumin was a red-orange powder (melting point 179 $^{o}$C – 182 $^{o}$C) with a maximum absorbance at 428 nm in 95% ethanol (Figure 2a). Meanwhile, the synthesized BDMC was orange-yellow powder (melting point 215 – 220 $^{o}$C) with a maximum absorbance at 418 nm in 95% ethanol (Figure 2b). These physicochemical properties of curcumin and BDMC are in accordance with the literature values. The range of melting points indicates that the synthesized compounds were of high purity (Péret-Almeida et al. 2005).

### 3.2. Inhibition activity toward lipid peroxidation

Lipid oxidation begins with the attack of oxygen on the double bonds the fatty acid fragments to form peroxyls and peroxides (Shahidi and Zhong 2010). The amount of peroxyls and peroxides during the oxidation of linoleic acid is determined in this study by the ferric thiocyanate method. During the analysis, $Fe^{2+}$ ions are oxidized by the peroxides to form $Fe^{3+}$ ions, which in turn react with $SCN^{-}$ ions to form red complex ions $Fe(SCN)_3$, with a maximum absorbance at 500 nm. Therefore, a higher value of absorbance at 500 nm indicates a higher extent of fatty acid peroxidation (Mihaljević, Katušin-Ražem, and Ražem 1996).

Figures 3a and 3b demonstrate that increasing the concentration of any curcuminoid deceased the extent of lipid peroxidation, and curcumin shows a stronger inhibitory effect. This effect is because the curcuminoids in neutral solutions (pH = 7.0) exist predominantly in the keto form and can act as strong hydrogen donors, thus preventing the formation of radicals participating in the lipid peroxidation process (Subramani et al. 2017).

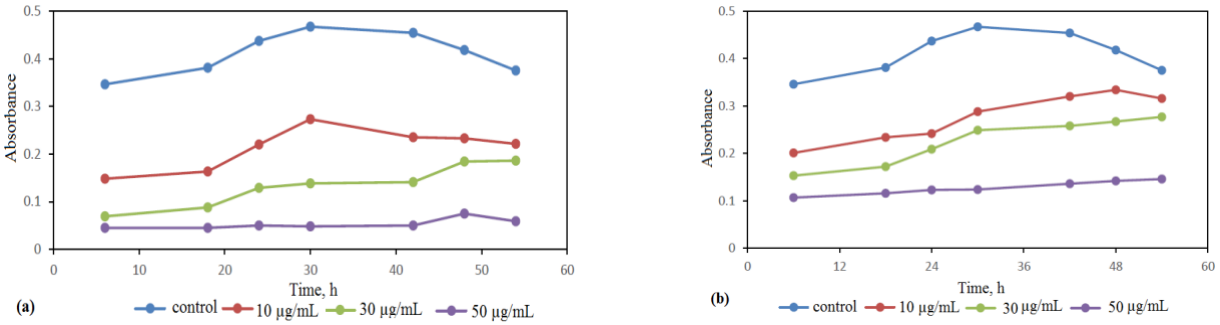


**Figure 3.** Effects of curcumin (a) and BDMC (b) concentrations on the peroxide levels (expressed in absorbance after reaction with $Fe^{2+}/SCN^-$ in ferric thiocyanate assay) of linoleic emulsions.

The relative inhibitory effect of the curcuminoids was compared with that of BHT (butylated hydroxytoluene), a commercial antioxidant widely used in the food industry (Figure 4).

At almost the same molarity, the order of inhibitory effects of the studied compounds was: BHT 0.14 mM > curcumin (50 µg/mL = 0.14 mM) > BDMC (50 µg/mL = 0.16 mM) (97% > 89.7% > 73.4%, respectively). This result indicates that curcuminoids can replace BHT in antioxidant applications with comparable activity. This lower antioxidant activity can be compensated with higher used amounts and the multiple biological activities of the curcuminoids (Jayaprakasha, Rao, and Sakariah 2006).

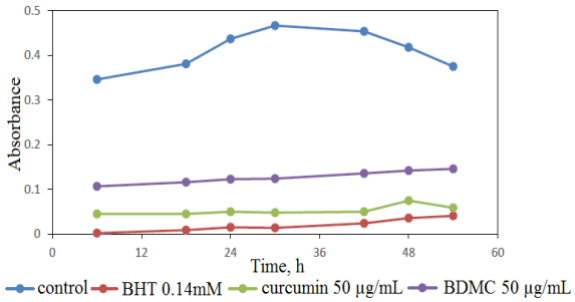


**Figure 4.** Inhibitory effects of curcumin, BDMC and BHT with similar molarities toward linoleic acid peroxidation.

### 3.3. Hydrogen peroxide scavenging ability

It is widely accepted that lipid oxidation is a radical chain reaction with hydrogen peroxide as an important intermediate. Moreover, $H_2O_2$ is very toxic in vivo and must be rapidly eliminated from the cells (Halliwell, Clement, and Long 2000). The ability of curcuminoids to eliminate $H_2O_2$ can break the reaction chain of lipid oxidation and protect human health. The $H_2O_2$ scavenging activities of the curcuminoids and BHT at the same concentration of 15 µg/mL are shown in Table 1, which are similar to the results in another study (Ak and Gülçin 2008). The results indicate that the curcuminoids have $H_2O_2$ scavenging activity higher than BHT. Although curcumin and BDMC showed no significant difference in $H_2O_2$ scavenging activities at a confidence level of 95%, these values are significantly different at a confidence level of 90%. Therefore, curcumin is more likely to be more active than BDMC in $H_2O_2$ scavenging, possibly due to the presence of a methoxy electron-donor group on each benzene ring

**Table 1.** Hydrogen peroxide scavenging activities (mean ± standard deviation) of the curcuminoid and BHT at the concentration of 15µg/mL.

| | Curcumin | BDMC | BHT |
|---|---|---|---|
| $H_2O_2$ scavenging (%) | $27.9 \pm 4.1^{a}$ | $23.7 \pm 3.9^{ab}$ | $16.5 \pm 3.7^{b}$ |

*Different letters a and b show statistically different means (p=0.05).*

### 3.4. DPPH• radical scavenging activity

Free radicals play important roles in lipid oxidation and high amounts of radicals adversely affect human health. Lipophilic curcuminoids can scavenge different forms of free radicals, such as reactive oxygen species and reactive nitrogen species, therefore can be considered as chain-breaking antioxidants (Menon and Sudheer 2007). The radical scavenging activities of the curcuminoids and BHT were tested on the DPPH• radical (Figure 5).

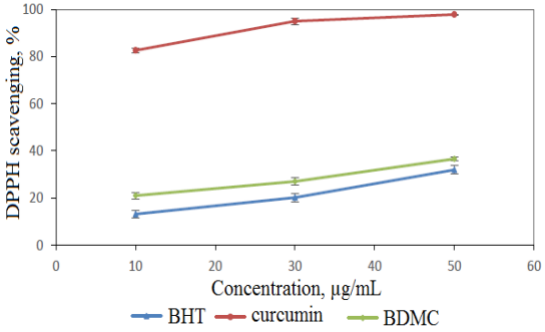


**Figure 5.** DPPH scavenging activities of BHT, curcumin and BDMC at different concentrations.

The phenolic –OH groups of curcuminoids have been shown to play essential roles in free radical scavenging reactions (Priyadarsini et al. 2003). Therefore, as in the case of $H_2O_2$ scavenging activity, the methoxy group on the benzene ring of curcumin enhances the stability of the phenoxy radicals formed during the radical scavenging. As a result, curcumin has a DPPH radical scavenging ability significantly higher than BDMC and BHT (Ak and Gülçin 2008).

### 3.5. ABTS•+ cationic radical scavenging activity

ABTS•+ is the most popular cationic radical used in evaluating antioxidant activities of pure compounds, solutions and beverages. ABTS•+ radicals are water-soluble, and the reactions with ABTS•+ radicals involve electron-transfer, apart from the H-atom transfer in reactions with DPPH radicals (Kaviarasan et al. 2007). In the presence of antioxidants, the coloured ABTS•+ radical cation is reduced to the colourless ABTS molecule. The decolourization extent is related to the antioxidant activity.

The results in Figure 6 show that the ABTS•+ scavenging activities of curcumin, BDMC and BHT are not very different, as in the DPPH scavenging assay.

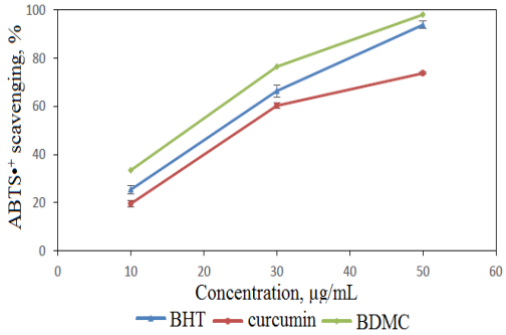


**Figure 6.** ABTS•+ scavenging activity of BHT, curcumin and BDMC at different concentrations.

In contrast with other types of antioxidant activities, ABTS•+ scavenging activity of curcumin is lower than BDMC, which is in accordance with another study, where synthesized curcuminoids with a methoxy, ethoxy, methyl or tert-butyl group in the ortho-position to the hydroxyl group showed lower ABTS•+ scavenging activity, compared with BDMC (Venkatesan and Rao 2000). This result, which is in contrast with the result in DPPH scavenging activity, can be explained by the fact the ortho-methoxy group forms intramolecular hydrogen bonds with the phenoxyl hydrogen and thus increases its bond dissociation energy (Kajiyama and Ohkatsu 2001).

### 3.6. Applications in some food systems

#### *3.6.1. Inhibition of fish oil oxidation*

Fish oil is an industrial product with high nutritional values thanks to the high content of polyunsaturated fatty acids, such as eicosapentaenoic acid (C20:5, EPA), docosapentaenoic acid (C22:5, DPA) and docosahexaenoic acid (C22:6, DHA) (Valenzuela, Sanhueza, and de la Barra 2012). The high contents of these polyunsaturated fatty acids make fish oil sensitive to oxidation with the formation of rancidity products. In this study, due to its higher inhibition activity toward linoleic acid peroxidation, curcumin was chosen as a candidate for BHT alternative to protect fish oils.

Figure 7 shows that at the same concentrations and the same accelerated oxidative conditions, the inhibition effect of BHT is about 25% higher than that of curcumin.

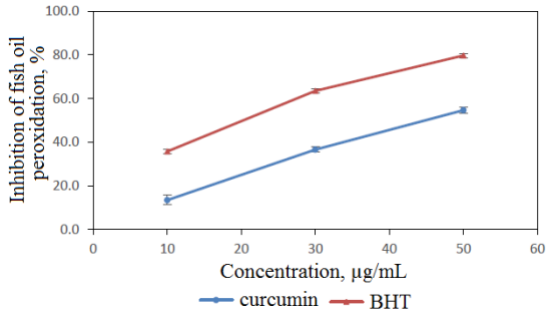


**Figure 7.** Inhibition of fish oil peroxidation by curcumin and BHT at different concentrations.

Although curcumin is less active than BHT, it can be used with much higher amounts to reach the same inhibiting effect as BHT. From several human studies, curcumin induced no toxicities at dosages up to 8 g/day in phase I clinical trials (Hsieh 2001; Hsu and Cheng 2007). Moreover, using curcumin can impart the oil with many other biological activities, including antibacterial, antiviral, anti-inflammation, anticancer effects of curcumin, which are beneficial to human health (Hewlings and Kalman 2017).

#### *3.6.2. Radical scavenging activity of starch-curcumin composite films*

Incorporating curcumin into edible starch films can produce packaging materials and new food with helpful functional properties. Figure 8 shows that increasing the amount of curcumin in the starch films make them more yellow and less transparent. At the studied range of contents, curcumin was uniformly dispersed in the films.

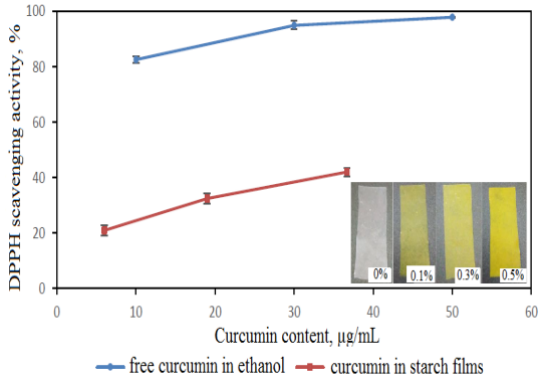


**Figure 8.** DPPH scavenging ability of free curcumin (blue line) and curcumin in starch films (red line). Inset: the appearance of starch films with different curcumin contents.

The red line in Figure 8 shows that the DPPH scavenging activity of the starch – curcumin films increases with the curcumin content. However, these values were significantly lower than the DPPH scavenging activities of free curcumin. It is because the starch molecules surrounding curcumin were a barrier hindering the diffusion of curcumin and DPPH, thus slow down the reaction rate between these molecules. This means that incorporating curcumin into starch films can protect it from adverse environments. Moreover, this curcumin – starch composite film, if incorporated with flavours, can be used as a multifunctional food or multifunctional packaging material (Mujtaba et al. 2019).

## 4. Conclusions

Relatively pure curcumin and BDMC were synthesized from 2,4-pentanedione and benzaldehyde derivatives. This synthetic approach is more convenient and environmentally benign than the extraction and

isolation of these compounds from turmeric. Curcumin shows higher antioxidant and radical scavenging activities, but lower activity in $ABTS^{\bullet+}$ scavenging. Curcumin has high potential to replace BHT as an oil antioxidant and to be a multifunctional ingredient in active and intelligent packaging.

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# CATERING OF CHILDREN WITH SPECIAL DIETARY NEEDS IN SCHOOL CANTEENS

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**ABSTRACT**

The occurrence of diagnosed food intolerances and food allergies shows an increasing trend worldwide and the most affected age group is schoolchildren. School catering services are not always prepared for fulfilling the strict requirements of special diets. The main goal of the paper is to summarize the main food intolerances, allergies and diets and to show the situation of dietary food based on an empirical research representing 44 secondary schools in Hungary, where the existence of special diets, the number of consumers with special dietary needs and the appropriateness of conditions were analyzed. 4.3% of children of the sample required for special diets, but in 20% of all cases, diets were requested without appropriate documents. Only 3 kitchens complied fully the conditions of special dietary needs, an important problem was non-appropriate labelling of meals and cross-contamination. The increasing need for special diets generates additional tasks and responsibilities for school kitchens and food providers by providing special technologies, processes and conditions required for preparing dietary meals, and present research findings call attention for these requirements. In the future stage of the research the role of technical background and the knowledge of employees related to the special diets are to be explored.

## 1. Introduction

Food allergies and food intolerances are well known and frequently diagnosed diseases of our times. In many countries labelling of food allergens are determined by official regulations and acts. In the European Union EC Regulation No. 1169/2011 (EC, 2011) while in the United States the Food Allergen Labeling and Consumer Protection Act of 2004 (FDA, 2004) describes the regulations related to the main allergens. The major allergens are the following: cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk (including lactose), tree nuts, celery, mustard, sesame seeds, sulphur dioxide and sulphites, lupin and molluscs (EC, 2011). According to the summary of FARRP (Food Allergy Research and Resource Program of Institute of Agriculture and Natural Resources at the University of Nebraska-Lincoln), these main allergens are labelled all across the world. FARRP analyses different countries in North and South America, Europe (EU countries, Iceland, Liechtenstein, Norway, Switzerland, Ukraine), Turkey and Arab States of the Gulf, Australia and New

Zealand, China, Japan, Korea, India, Malaysia, Thailand, and South Africa. Gluten/wheat, crustaceans, egg, milk, peanut, tree nuts are labelled in all these countries, soybean, fish is not labelled in Japan, sulphur dioxide/sulphites is not labelled in China, Japan and South Africa. EU and other European countries, Arab States of the Gulf, Canada, Australia, New Zealand and Kora are those countries which label the most allergens in food (FARRP, 2019).

In the past few years, the number of food product withdrawals due to missing or incorrect labelling was higher than the withdrawal of food products due to the existence of pathogens (Spotz, 2018). Food intolerances are frequent in childhood, so providing appropriate food for children with special dietary needs represent an important task for school catering services.

To provide appropriate food for schoolchildren at school is an important task, as students spend most of their days at school and they need the required nutrients in order to maintain their good health status, wellbeing and proper school performance (Kiss *et al*., 2019). In many countries of the European Union, the measures for improving the school catering services are outlined by school food programs. In some countries, these programs are compulsory, but other countries the proposed measures are only suggestions. It should be noted that only seven national school food programs (Northern Ireland, Wales, Hungary, Ireland, Spain, Sweden, Germany, Belgium) consider the special needs of children with food allergies and intolerances (EU JRC, 2015). According to Hungarian regulations, for children with special dietary needs at least one meal should be served in the daytime at educational institutions. The need for the special diets shall be justified by an official certification issued by specialist, but only those certifications may be accepted which are issued by doctors specialized in endocrinology, gastroenterology, diabetes or allergology/clinical immunology.

The organization of dietary catering processes brings many challenges for the school caterers and kitchen staff. These challenges are mostly related to organizing activities (food handling, control, training of kitchen staff etc.) which represent additional costs and requires for additional financial sources (Tóth *et al.*, 2017). Special diets of different food allergies are prepared by the total exclusion of particular ingredients, while in case of food intolerances, some ingredients may appear in the prepared meals at a limited amount. An important deficiency of regulations related to labelling (Regulation EC 1169/2011) (EC, 2011) is that it does not deal with the problem of cross-contamination that may appear during the preparation or packaging process and the final product accidentally will be contaminated by harmful ingredients (Popping and Diaz-Amigo, 2018).

For catering services avoiding cross-contamination problems means the most important task of allergen management. According to the Hungarian regulations, the preparation of dietary food and meals shall be performed or supervised by certified experts such as dietary cooks or dietitians. Based on the abovementioned circumstances, the official limits of dietary or 'free from' meals were indicated by literature sources and were implemented into practice. The most important work of this field was conducted by the Australian Allergen Bureau (called Voluntary Incidental Trace Allergen Labelling - VITAL scheme), which limiting factors are spread and widely used in the catering industry (Taylor *et al.*, 2014).

In school catering system, dietary food may be served in two ways. In case of cooking kitchens with appropriate equipment and circumstances, the dietary meals are prepared under the supervision of dietary cooks or dieticians. If this process cannot be managed, then dietary food shall be ordered from an external food provider. Due to the relatively small number of consumers and the wide variety of diets, the dietary meals are packaged individually for the children on special diet.

A key factor of the proper management of special dietary meals is the knowledge and experience of food handlers in the field of different diets and food intolerances, which was

already discussed by different researches [Ajala *et al*., 2010; Lee and Sozen, 2008; Soon, 2018; Dunay *et al.,* 2019).

The objective of our research was to explore and evaluate the main features of special diets, i.e. the number of students with special dietary needs, the conditions for preparing dietary meals and the compliance with the special requirements.

## 2. Materials and methods

### 2.1. Materials

Our research was performed in 44 secondary schools, where 4800 students eat their meals at a daily average. In 5 schools cooking kitchens are working (i.e. the meals are prepared at the institution), the rest of the schools have only serving kitchens, which means that food is prepared at other institutions, only serving of the portions is done at the schools.

### 2.2. Methods

We assessed how many students required for special food, what type of diets they followed and by which documents they justified their dietary requirements. In case of medical documents, we also checked their appropriateness, i.e. the required professional certificates of doctors. In case of religious diets, the statements by the parents were also checked. In the next step, the school kitchens were checked through a food safety checklist with 10 questions, in order to assess the appropriateness of their structure and equipment to meet the requirements of preparing food for special diets. (The questions of the checklist are summarized later, in Table 2.) In this step, we focused on the traceability of dietary meals and the presence and use of equipment and utensils by which cross-contamination may be avoided.

Data processing and statistical analyses were performed by using the IBM SPSS Statistics 22.0 for Windows. Independent two-sample t tests were used to compare means between two groups. Significant differences were considered at $p>0.05$. The equality of group variances was tested by Levene-test.

## 3. Results and discussions

### 3.1. Number of students requiring special diets

From the total 4800 students 208 students required dietary food, which represent 4,3% of the total number of schoolchildren in the sample. There were no claims for special dietary food in ten schools from the 44.

From human health aspects, the special diet categories should be differentiated: diets for children with diabetes mellitus and/or obesity require different preparation and handling methods than food for children with food intolerances or allergies, as in the latter case, cross-contamination represent an additional challenge. The most frequent special diets in the examined sample are summarized by Table 1.

**Table 1.** Types of special diets, number of students with dietary needs and number of affected schools in the surveyed sample

| **Allergens, diets** | **Students with allergies or special diets** | | **Affected schools** | |
|---|---|---|---|---|
| | **Number** | **%** | **Number** | **%** |
| Gluten | 62 | 1,29 | 37 | 84 |
| Milk | 53 | 1,10 | 34 | 77 |
| Lactose | 44 | 0,92 | 30 | 68 |
| Low energy | 34 | 0,71 | 23 | 52 |
| Diabetes | 24 | 0,50 | 17 | 39 |
| Egg | 12 | 0,25 | 12 | 27 |
| Soybeans | 6 | 0,13 | 6 | 14 |
| Nuts | 6 | 0,13 | 6 | 14 |
| Pork | 7 | 0,15 | 6 | 14 |
| Other | 18 | 0,38 | 15 | 34 |

From these data it is seen that the most of the required diets are caused by food intolerances and allergies (gluten, lactose, milk, egg, soybeans and nuts), but the number of diets related to diabetes or obesity (low energy diet) is also significant. Diets due to religious requirements or other issues (e.g. vegetarian) were less significant in the sample. For 39 children, two or more diet types were prescribed,

the most frequent compositions were milk and gluten-free diets (10 children) and milk and egg free diets (15 children).

The medical certificates issued by specialists were also checked in the research, we checked that the documents are in accordance with the legal regulations and are they in harmony with the required diets. It was stated, that in 136 cases the certificates were correct, while in 44 cases were not appropriate. In additional 28 cases, the appropriateness of the certificates could not be assessed. It means that in 20% of all cases the claims for special diets were prepared incorrectly, not in accordance with the existing rules.

### 3.2. Requirements of the preparation of special dietary food/menus

#### *3.2.1. Labelling*

As most kitchens in the examined sample work as serving kitchens (i.e. food is delivered by the food providers from an external location, and only serving is done by the kitchen staff) labelling plays a critical role in the process as the criteria of traceability.

The information flow by labelling was correct in 14 serving kitchens and in 1 cooking kitchen, but there were 3 kitchens (two serving kitchens and one cooking kitchen), where the criteria of labelling were not in compliance with the requirements.

In the checklist, five questions were related to proper information about the given meals (Questions 3-8 in Table 2). Statistical differences were found between serving and cooking kitchens in relation with the information about the name of food, the name of consumer and the time of preparation. In all three cases, the performance of serving kitchens was more correct. This difference is probably because in cooking kitchens there is an opportunity of oral information flow, therefore the risks arising from missing or not correct labelling is lower.

**Table 2.** Checklist questions related to special food and the number of 'yes' answers in the examined 44 schools (in numbers and in %)

| **Questions** | | **All kitchens (n=44)** | | **Serving kitchens (n=39)** | | **Cooking kitchens (n=5)** | |
|---|---|---|---|---|---|---|---|
| | | ***Number*** | ***%*** | ***Number*** | ***%*** | ***Number*** | ***%*** |
| 1 | Does the food arrive at the kitchen at cold temperature? | 29 | *66* | 29 | *74* | 0 | *0* |
| 2 | Does the food arrive at the kitchen in portions? | 41 | *93* | 37 | *95* | 4 | *80* |
| 3 | Is the name of food indicated?* | 32 | *73* | 31 | *79* | 1 | *20* |
| 4 | Is the type of the diet indicated? | 36 | *82* | 33 | *85* | 3 | *60* |
| 5 | Is the name of the consumer indicated?* | 37 | *84* | 35 | *90* | 2 | *40* |
| 6 | Are the circumstances of food storing methods indicated? | 18 | *41* | 17 | *44* | 1 | *20* |
| 7 | Is the time of preparation indicated?* | 32 | *73* | 31 | *79* | 1 | *20* |
| 8 | Are the suggestions for handling and serving of food indicated? | 15 | *34* | 14 | *36* | 1 | *20* |
| 9 | Are there isolated devices available for handling dietary food? | 33 | *75* | 28 | *72* | 5 | *100* |
| 10 | Are there appropriate circumstances to store dietary food in a separated way?* | 7 | *16* | 2 | *5* | 5 | *100* |

* Differences between cooking and serving kitchens are statistically significant

The methods and requirements of storing and handling of food (Questions 6 and 8) were mentioned in relatively few cases, and these requirements were not appropriate in more than the half of the examined kitchens. Surprisingly, in more than 25% of the kitchens trivial information, like the name of the food was not given (Question 3).

### *3.2.2. Cross-contamination*

Cross-contamination is caused most frequently by inappropriate cleaning of the different utensils which are used for both dietary and non-dietary food (Do *et al.,* 2018). According to the strict requirements, the conventional and dietary food and even the meals of different diet types shall be handled separately in time and space during the stages of preparation, storage, delivery and serving. When there is no opportunity for spatial separation, then the separation in time shall have higher attention.

In the checklist, illustrated by Table 2, two questions (Question 9 and 10) were related to cross-contamination. Seven kitchens from the examined sample get 'yes' answers for both questions, including all the 5 cooking kitchens. The result for Question 9 (about isolated devices for handling) were acceptable (above 70%) but the results of Question 10 (circumstances for separated storage of dietary food) were much weaker due to the performance of serving kitchens ('yes' answers represented only 5%). The most frequent problem at serving kitchens was related to separated storage of dietary food. Nevertheless, as in most of the serving kitchens dietary meals are served in individually packed form for the students concerned, these problems did not cause serious risks. In two of serving kitchens, dietary food was delivered in bigger portions in heat storing dishes, but in these locations, the separation of the utensils was correct.

## 3.3. Types of the special diets

In this section, a brief summary of the represented diets is given based on literature sources and the main characteristics of the diets in our survey results are displayed.

### *3.3.1. Gluten-free diet*

In our sample 62 schoolchildren in 37 institutions needed special diet due to gluten intolerance (celiac disease).

In allergen management aspects, the preparation of gluten free meals represents special problems. Gluten is a protein fraction, which is insoluble in water, it is found in wheat, barley, rye and their hybrids, as well as in products derived from these grains. Approximately 1% of the European population suffers from celiac disease, which is the most serious form of gluten intolerance (Lionetti *et al.,* 2015). Presently, the only treatment for this disease is the lifelong diet, where all ingredients and food containing gluten shall not be consumed. After the diagnosis, the specialists prescribe the diet for the patients, and from this point, the patient is responsible for keeping the diet, which represent a huge challenge for them, their families and even for the catering services. Gluten must be totally excluded from the diet (Do *et al.,* 2018). In many countries (e.g. as it is regulated by EU Regulation 828/2014), the gluten content of gluten free meals and food products must be lower than 20 mg/kg (EC, 2014). Food products shall be labelled by 'very low gluten' indication, when their gluten content is lower than 10 mg/kg (Popping and Diaz-Amigo, 2018).

In public catering systems, the main problem is cross-contamination, as the processes of preparing conventional and gluten-free meals are conducted simultaneously. In the preparation process of gluten-free meals, the separation in time and space must be kept strictly during the preparation, storing and handling phases. Moreover, different and separated utensils (even different cutlery, plates and glasses) shall be used and shall be stored separately, in closed storage site. According to research results, by keeping the strict separation rules the safe preparation of gluten-free meals can be managed successfully (Vincentini *et al.*, 2016).

### *3.3.2. Milk-free diet*

Milk allergy is an adverse immune reaction to one or more proteins in cow's milk, most frequently to casein. In our research sample, 53 children of 34 schools were affected by milk-free diet. Milk allergy could be cured by the total exclusion of milk proteins although milk – from dietetic point of view – is one of the most important nutrient and mineral source, which can be hardly substituted (Di Constanzo and Berni Canani, 2018). The most frequent reason of product withdrawals due to allergenic content is not labelled milk content (Bucchini *et al.,* 2016). Many food products contain hidden milk and milk derivatives, so the risk of contamination with milk protein is very high (Do *et al.,* 2018), therefore, a strict separation should be secured in the handling process of milk-free food and meals.

### *3.3.3. Lactose-free diet*

A common problem in public catering is that many food handlers confuse the meaning of lactose-free diet and milk-free diet (Di Constanzo and Berni Canani, 2018). Lactose intolerance is such a condition, when the patient is not able to digest lactose (a sugar found in dairy products). The problem refers to the lack of lactase enzyme, but the symptoms may occur temporarily, even due to adverse drug reactions or reactions for other bowel diseases. In technological aspects, the preparation of lactose-free food is easier than in case of milk-free meals, as lactose-free ingredients may be used in lactose-free diet and not only plant-based milk substitutes are allowed to use.

In our research sample 44 children kept lactose-free diet in 30 school kitchens. Although the proper regulation of lactose-free products is still not defined properly, according to suggestions 100 g meal shall contain maximum 10 mg lactose in this diet (Suri *et al.,* 2019).

### *3.3.4. Low energy diet*

In many countries of the world the number of obese people is growing (Lobstein *et al.,* 2015), in the USA the proportion of obese children of 2-19 age group is 18,5% (Hales *et al.,* 2017). This trend is reflected in our sample as well, as quite a big number, 34 schoolchildren needed low energy diet.

Low energy diet is prescribed for overweight or obese children. The main characteristics of this diet are reduced fat and carbohydrate content. The Hungarian school catering practice does not prescribe defined caloric values for the served portions, but the meals are prepared without sugar, with low-fat meats and reduced amount of fats. In technology aspects, low energy diet does not represent any problems, as the meals and ingredients are not sensitive for cross-contamination.

### *3.3.5. Diabetes diet*

The insulin dependent diabetes mellitus (IDDM), also known as type 1 or childhood diabetes showed an increasing tendency until the early 2000s between 2,8-3,9%, but since then this growth rate has slowed down (Patterson *et al.,* 2019). Formerly, type 2 (non-insulin dependent) diabetes was considered as a disease of adult people, but nowadays, the occurrence of this diabetes type is increasing among children (Candler *et al.,* 2018). Children with diabetes mellitus must control their carbohydrate (CH) intake strictly and doctors define the maximum CH amount of each meals.

In our sample, 24 diabetic children were found, and all of their official documents were proper. It is probably due to the strict treatment of diabetic disease, as it is not a food intolerance or food allergy, and the therapy of the diet shall be prescribed by specialists.

### *3.3.6. Egg-free diet*

Egg-free diet and preparing such meals is an important issue in public catering, mainly for the younger age groups, as babies and infants represent the mostly endangered age groups and the frequency of egg allergy decreases with age. In the examined schools, only 12 children needed egg-free diet. Egg-free diet affects about 0.5-2,5% of children and young people worldwide, but according to research results it shows an increasing tendency (Österlund *et al.,* 2019).

Egg is a popular and versatile ingredient of different meals in many cultures therefore it is not easy to exclude from meal preparation

processes. In case of egg-free diets, avoiding cross-contamination is very important step. The minimum limit of egg-free meals is represented by egg-protein content (0,03 mg/kg) according to the VITAL scheme (Taylor *et al.*, 2014).

### *3.3.7. Soybean-free diet*

Soybean allergy is one of the most frequent childhood food allergy which affects 0,4% of children, but in most cases it children outgrow this allergy by the age of 10 (Savage *et al.*, 2010). Soybean is an important food ingredient and a popular meat substitute, moreover many meat products contain soybean as ingredient, and therefore it is not easy to prepare soybean-free meals. According to the VITAL scheme the required limit for soybean-free food is 1 mg/kg (Taylor *et al.*, 2014).

### *3.3.8. Peanut-free diet*

Peanut allergy affects 0,6-1,0% of the population in well-developed countries, it is one of the most frequent causes of severe allergy attacks. Peanut allergy requires the highest attention as a tiny amount of its protein may cause life-threatening symptoms. Peanut allergy is not restricted to childhood age. Most of the registered anaphylactic shocks are caused by peanut or other nuts (Al-Muhsen *et al.*, 2003).

Due to these facts, from the kitchen technology aspects, the management of peanut allergy is the most complicated task. Peanut-free food and meals are very sensitive to cross-contamination. In general, due to the high risk, children with severe peanut allergy do not require for school catering services. Some schools in the United States have launched peanut-free regulations, but as the regulations are not unified, the efficient performance of these programs cannot be evaluated properly (Stukus, 2017).

### *3.3.9. Tree nut-free diet*

Tree-nut allergy affects 0,1-4,3% of world population (Weinberger and Sicherer, 2018). Nuts show very strong cross-reactions with each other and most of allergic persons are sensitive for more types of nuts. Tree-nut allergy – together with peanut allergy – may cause severe, potentially fatal, allergic reaction. From treatment aspects, the different types of nuts are considered similarly (Dantzer and Wood, 2019) although generally there are differences of sensitivity between individuals, and not all nuts should be excluded from the individual diets.

### *3.3.10. Other special diets*

Nowadays, in school catering, the role of other diets such as diets due to religious reasons or reasoned by personal eating habits is increasing. In our sample, pork-free menu was required by 7 students, while 2 students claimed for vegetarian food. This phenomenon is regulated in different ways in different countries. In some places, the requests for religious or habitual diets are fully considered (EU JRC, 2015). In Hungary, it is compulsory to serve dietary food prescribed by specialists because of health problems and claims reasoned by religious requirements are also may be considered, but it is not mandatory to prepare food according to habitual diets based upon personal needs. In the latter case, the institutions (or food providers) prepare dietary food voluntarily.

## 4. Conclusions

Providing food for children with special dietary needs (food intolerances and allergies, eating prohibitions) put an extra load on the school catering services. Rules and regulations of school catering are different in different countries.

In Hungary, the regulation system is relatively strict, special dietary food and meals shall be justified by official documents issued by specialists. In the Hungarian school catering system, the existence of official health documents should be checked at the first claim, and shall be submitted by the parents towards the food provider at the beginning of each the school year, or in case of any changes. By this process, the food providers get the relevant information, for making proper food management decisions. In one hand, the exclusion of particular ingredients may cause nutrient deficiencies in the diet, which shall be compensated, and on the other hand, the preparation of special dietary food may bring

extra costs and extra conditions for the food providers, which need preliminary preparations.

Our research results, which were drawn based on a wide sample get insight into the present situation of dietary food provision in Hungary. In the upcoming years, the number of children requiring for special food may show an increasing tendency, therefore the knowledge and experience level of kitchen staff at school catering services should be raised and developed. Food handlers shall be trained and educated for these special challenges, either in physical or personal aspects. It is very important that besides the installation of special equipment and utensils for preparing appropriate dietary food, the special knowledge and experiences of food handlers/kitchen staff about the main characteristics of the diets, labelling information and cross-contamination problems during preparation and serving stages shall also be developed through target-oriented trainings.

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# INFLUENCE OF EDIBLE COATING ON SHELF LIFE AND QUALITY OF SWEET CHERRY

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**ABSTRACT**

Edible coatings are environment friendly materials for extension the shelf-life and preservation the nutritional value of fruits. In the present research, two different sweet cherry cultivars were treated with chitosan, chitosan Ca-lactate (multicomponent, mono-layer coating) and chitosan alginate (polyelectrolyte complex – bi-layer coating). Immersing technology was used to apply the coatings, ones for the mono-component, monolayer chitosan and for the multicomponent, monolayer chitosan Ca-lactate and twice for the polyelectrolyte complex – bilayer chitosan alginate. The fruit quality of control and coated samples was evaluated about refractometric, colorimetric, textural, antioxidant activity (DPPH), acidity, microbiological and sensory parameters during 21 days refrigerated storage. The coatings delayed the decay of the sweet cherry varieties in different scale. There were differences in browning, in texture changing, in sensory parameters and microbiological contamination as well. The chitosan based edible coatings extended the shelf-life period of sweet cherry varieties.

## 1. Introduction

A relatively new practice for maintaining the quality of fresh cherries after harvest is their packaging with edible coatings and films (Vargas et al, 2009). There is information in the literature on the use of edible chitosan coatings as a means of extending the shelf life of cherries. Chaovanalikit and Wrolstad (2004) reported improved quality (based on texture, dry matter, and acidity) for 60 days for cherries coated with a chitosan film stored at low temperature and high relative humidity (98%). The most detailed study on the parameters of cherries packaged in edible coatings was carried out by Aday et al. (2010). Edible coatings were prepared from 3% chitosan (low viscosity) in acetic acid solution with added glycerol as a plasticizer. The cherries are stored at a temperature of 20 °C and a relative humidity of 55%. During 11 days of storage, the authors reported a greatly reduced level of oxygen and an increased level of $CO_2$ for packaged cherries, ie. chitosan exhibits membrane properties and creates a modified atmosphere at the surface of the fruits. They also reported decreases of the breathing rate. The weight loss on the 9th day for the coated samples was 25%, against 49% for the control. Weight retention, ie. the preservation of the water content in the cherries is directly related to the better appearance and the preserved texture. There is also a tendency for a slower change in pH, dry content and acidity. These results indicate the good barrier properties of chitosan coatings and their ability to extend the shelf life of packaged cherries. In order to optimize edible packages of chitosan, in our earlier research water-soluble chitosan was used in lower

concentrations. When applying a 1% solution of chitosan in combination with alginate and calcium lactate, the shelf life of packaged cherries can be extended to 21-25 days. The respiration is reduced and the influence of the barrier properties of the packaging is less pronounced. The extension of the shelf life depends not only on the composition of the coating, but also on the cherry's variety.

Sweet cherries (Prunus avium L.) and sour cherries (Prunus cerasus) are among the most important commercial species of Prunus fruit trees, which are grown in continental climates. The cherry is the main structural species in Bulgaria (Malchev & Zhivondov, 2016). Production of sour cherry in Bulgaria, is relatively limited. Cherries are highly perishable, non-climatic fruits. The destruction of cherries occurs with rapid weight loss, discoloration, softening (Bernalte et al., 2003). Their shelf life is shortened in case of loss of hardness, change in stem color, drying and they are not suitable for long storage. Maintaining lower fruit temperatures immediately after harvest leads to harder fruits with reduced rot and greener stems (Schick & Toivonen, 2002). In the context of the significant changes in environmental conditions caused by climate change, it is essential that crops are well adapted to warmer winter and spring temperatures and to more extreme climatic phenomena, such as variable spring frosts and hot summer. Cherry and sour cherry fruits are highly sensitive to quality loss during the short ripening period and after harvest, during transport or storage (Zhivondov et al., 2003). Fruit strength was defined as the amount of healthy fruit left over during storage. Fungal diseases (*Botrytis cinerea*, *Rhizopus stolonifer*, *Colletotrichum gloeosporioides* and *Alternaria alternata*) are one of the main visible causes of loss of production during storage of fresh fruit (Bautista-Baños et al., 2003). Production rot processes can be reduced to some extent by minimizing mechanical damage during harvesting. The use of varieties with natural resistance to certain diseases, as well as the storage of products under optimal conditions, contributes to keeping the fruit for fresh consumption for a longer time. As a natural polymer with pronounced bacteriostatic and antifungal properties, chitosan limits diseases that occur in orchards and those that result from the storage of fresh fruit (Li & Yu, 2001).

In this study, the Regina variety and the Elite 17-37 Tsvetina hybrid were used. Pure chitosan, chitosan in combination with calcium lactate and chitosan in combination with alginate were applied for packaging.

## 2. Materials and methods

### 2.1. Materials

In the present study, a traditional cv. Regina and a new Elite 17-37 cv. Tsvetina hybrid were treated (Figure 1). The fruits were obtained in optimal maturated stage, from the Fruit Growing Institute, Plovdiv, Bulgaria.

(**a**) cv 'Regina' (**b**) cv. Tsvetina

**Figure 1.** Studied sweat cherry cultivars

Low molecular weight water-soluble chitosan (deacetylation rate: ≥90%, molecular weight 1.6 kD and viscosity 100 mPa.s) was used for edible coatings. Chitosan was purchased from Lyphar Biotech Co., LTD, China. The food grade Ca-lactate and the sodium alginate was bought from Sigma Aldrich, Bulgaria.

### 2.2. Sample preparation

Three types of edible coatings based on water-soluble chitosan were used in the experimental series: Pure chitosan solution containing 1% water-soluble chitosan and distilled water. A multicomponent system containing 1% chitosan and 1% Ca lactate. The coating is obtained by immersing the fruit once in the multicomponent solution. As a result, a single-layer coating is formed on the fruit. Two-layers coating of polyelectrolyte complex of 1% chitosan and 1% alginate. The coating is

obtained by sequential immersion in solutions of chitosan and alginate. As a result, a layer of chitosan is first formed on the fruit, and then a second layer of alginate. After treatment, the experimental series of cherries were stored in a refrigerator at 4 °C for 3 weeks. The treated and untreated (control) fruits were analyzed during storage according to their physical, physicochemical, microbiological and sensory parameters. The end of storage was determined according to the number of remaining healthy samples.

### 2.3. Physical parameters

Quantitative changes (rate of non-decayed or browned fruits, weight and volume loss), and refractometric dry matter (Brix and refractive index) were reported on the first day and then once a week. The firmness of healthy fruits was determined by puncture test, on the same days as the other physical parameters.

### 2.4. Physicochemical parameters

These parameters were tested three times (1st 11th and 21st days) during the period for fruit pulp prepared from healthy samples.

#### *2.4.1. The color of the fruit pulps*

The color of the fruit pulp was detected by a "Colorgard 2000" colorimeter. The parameters are reported according to the CIE Lab system, where: L - illumination (L = 0 - black, L = 100 - white), + a - red color, -a - green color, + b - yellow color, -b - blue color (ASTM D2244-16, 2016). The color coordinates of each sample are the arithmetic mean of several measured coordinates. Saturation and color tone are the parameters that characterize the quality of color in the so-called physiological visual system and are related to the visual perception of color.

The color saturation C is calculated by the formula:

$$C = \sqrt{a^2 + b^2} \quad (1)$$

The hue angle (h° value) shows the change in the color of fruit pulp, with a lower h° indicating more severe browning.

$$h = arctan\left(\frac{b}{a}\right) \quad (2)$$

The color differences between the individual samples are determined by the values ΔL, Δa and Δb, and ΔE is a generalized parameter of the final color difference. The difference in color change ΔE is determined by the formula (Atarés & Chiralt, 2016):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (3)$$

Where:

$\Delta L = L_i - L_0$

$\Delta a = a_i - a_0$

$\Delta b = b_i - b_0$

"0" - etalon

"i" – probe

The parameter ΔE can be used to determine the change of fruit color (browning) during the storage time, as $L_0$, $a_0$, $b_0$ are the coordinates of fresh fruits $L_i$, $a_i$, $b_i$ are the coordinates of stored fruits. The browning index (BI) used as a parameter of brown intensity is calculated by the following formula (Kumar et al., 2018):

$$BI = [100 \cdot (x - 0.31)]/0.172 \quad (4)$$

Where

$$x = \frac{(a + 1.75 \cdot L)}{(5.646 \cdot L + a - 3.012 \cdot b)} \quad (5)$$

#### *2.4.2. Anti-oxidant activity*

One of the methods for determining antioxidant activity is the DPPH test (Arnao et al., 2001). The method is based on measuring the decrease in the concentration of free radicals under the influence of the test sample. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used as a source of free radicals. Measurement of the decrease in concentration is performed by determining the decrease in specific absorption at a wavelength of 515 nm. The results of the analysis can be presented as a percentage decrease in the concentration of free radicals in a solution containing the measured sample compared to a solution with the same amount of distilled water added. If a comparison is sought not only in one series but between different series or different methods of determination, the results are presented as trolox equivalents (TE), the reaction system being calibrated by adding exact amounts of the standard antioxidant Trolox and the results are presented in units of

TE (trolox equivalent). An UV/VIS spectrometer "Thermo EVOLUTION 201" was used in these experiments

***2.4.3. Active acidity (pH)***

The pH values of the cherries were detected using a pH meter (Milwaukee Model MW1 02-FOOD, USA), with temperature compensation.

***2.4.4. Determination of titratable acidity***

Titratable acidity was determined as described by Zhang et al. (2016). The titratable acidity is expressed as the percentage of malic acid (%).

**2.5. Microbiological parameters**

In parallel with the physicochemical tests on microbiological criteria for foodstuffs, according to COMMISSION REGULATION (EC) № 2073/2005 of 15 November 2005 (EFSA 2010), the total microbial count (BS EN ISO 4833-1: 2013), the total amount of molds and yeasts (BS ISO 21527-2: 2011), Enterobacteriaceae (BS ISO 21528-2: 2017), Escherichia coli (BS ISO 16649-2: 2014), Sulfite reducing and clostridia (BS EN 26461-2: 2004) were determined for one third of the healthy samples.

**2.6. Sensory analysis**

Consumer test, with a 9-point scale for quality assessment was done. The whole cherry fruits were evaluated by thirty volunteers between 20–50 years old, who liked and eat cherries frequently. A total of 12 samples from 4 varieties coated or uncoated, labelled with 3 digits numbers were randomly provided to the panellists. The evaluated samples for each session comprised four samples, one coated with Chitosan (1%), one with Chitosan (1%) & Calcium Lactate (1%) and one with Chitosan (1%) & Alginate (1%), and non-coated control. A glass containing potable water and pieces of non-salted cracker were provided to panellists for eliminating the residual taste between samples. Appearance, shape&size, color, fruit taste, aroma, firmness and browning around the stone were the evaluated attributes by the panellists. Each attribute was scored on a structured 9 points scale for quality evaluation labelled from “absolutely no quality” (1) to “extremely good quality” (9) (Wichchukit & O'Mahony, 2015).

**2.7. Statistical analysis**

Statistical analysis was performed using the statistical package Statistica. A multifactor ANOVA with posterior Multiple Range Test was used to find significant differences (p=0.05) among variety, storage time and edible coating on the sensory evaluation profile and in the analysis of the data from the other experiments as well.

**3. Results and discussions**

**3.1. Quantitative changes**

Relative number of healthy fruits: The percentage of healthy fruit changes during the full storage time. The fastest changes are observed in non-packaged fruit (to 35-40%). In both cultivars, the smallest loss can be reported when packed with chitosan (to ~77 %). The effect of packages with chitosan and calcium lactate and chitosan and alginate is almost the same (to 65-70%).

Weight and volume losses are expressed as the ratio between the weight or volume of the current day of storage and the first day (100%). As a result of volume losses, the fruits wrinkle. The losses are greatest in the control samples. The smallest volume losses were found in the cv. Tsvetina, packed with chitosan alginate (to about 73.6%), followed by a package of pure chitosan by 71.5%, a package of chitosan and calcium lactate by 70% and the control by 64%. In the cv. Regina, the losses are smaller. The lowest loss was observed in chitosan-calcium lactate packaging to 82%, followed by chitosan-packing 79%, chitosan-alginate packaging 73.5% and control by 72%. Weight loss is associated with water loss (dehydration) and loss of respiration. The differences in weight loss are smaller. For the cv. Tsvetina the weight loss of the packaged fruits is to about 77-78%, and for the control - to 70%. For the cv. Regina, the loss in fruits packed with chitosan and chitosan calcium lactate is to 83-85%, for fruits packed in chitosan alginate is 80% and for non-packed fruits is 76%.

### 3.2. Refractometric dry matter content

During the storage time for all tested samples (packaged and unpackaged) the refractive index and the refractometric dry matter content (Brix) are constantly increasing, and the growth of the change is different depending on the composition of the package and the cultivar. These changes are the result of drying (weight and volume losses). The increase may be due to the breakdown of starch to sugars, to a decrease in respiration rate and the conversion of sugars to $CO_2$ and $H_2O$ (Ghasemnezhad et al., 2011), to the hydrolysis of cell wall polysaccharides (Comabella & Lara, 2013) and to an increase of the dry content due to water loss (Petriccione et al., 2015).

The fastest changes are in the control samples (Table 1).

**Table 1.** Refractive index and refractometric dry content content (Brix) of sweet cherries

| Cult. | Treat-ment | Day | Refractive index | Brix | Cult. | Refractive index | Brix |
|---|---|---|---|---|---|---|---|
| Tsvetina | Control. | 1 | 1.3656±0.0021$^{a}$ | 21.26±1.29$^{a}$ | Regina | 1.3587±0.0044$^{a}$ | 16.80±2.86$^{a}$ |
| | | 8 | 1.3686±0.0022$^{b}$ | 22.81±1.30$^{ab}$ | | 1.3596±0.0026$^{ab}$ | 17.36±1.60$^{ab}$ |
| | | 15 | 1.3748±0.0021$^{c}$ | 26.26±1.29$^{c}$ | | 1.3646±0.0020$^{c}$ | 20.37±1.22$^{c}$ |
| | | 21 | 1.3794±0.0028$^{cd}$ | 28.73±1.27$^{d}$ | | 1.3653±0.0035$^{cd}$ | 20.92±1.92$^{cd}$ |
| | Ch. | 1 | 1.3653±0.0029$^{a}$ | 20.81±1.77$^{a}$ | | 1.3586±0.0026$^{a}$ | 16.76±1.57$^{a}$ |
| | | 8 | 1.3653±0.0025$^{a}$ | 20.85±1.47$^{a}$ | | 1.3593±0.0035$^{ab}$ | 17.18±2.10$^{ab}$ |
| | | 15 | 1.3685±0.0027$^{b}$ | 22.71±1.54$^{b}$ | | 1.3624±0.0021$^{c}$ | 19.10±1.32$^{c}$ |
| | | 21 | 1.3705±0.0050$^{bc}$ | 23.86±2.86$^{bc}$ | | 1.3662±0.0049$^{d}$ | 19.36±2.92$^{d}$ |
| | Ch+CaL | 1 | 1.3632±0.0027$^{a}$ | 19.56±1.50$^{a}$ | | 1.3579±0.0041$^{a}$ | 16.36±2.49$^{b}$ |
| | | 8 | 1.3667±0.0032$^{b}$ | 21.56±1.87$^{b}$ | | 1.3623±0.0058$^{b}$ | 18.94±3.64$^{a}$ |
| | | 15 | 1.3704±0.0022$^{bc}$ | 24.01±1.30$^{c}$ | | 1.3636±0.0014$^{bc}$ | 19.75±0.81$^{bc}$ |
| | | 21 | 1.3828±0.0039$^{c}$ | 25.82±2.29$^{c}$ | | 1.3632±0.0030$^{bc}$ | 19.52±1.80$^{bc}$ |
| | Ch+Alg | 1 | 1.3638±0.0034$^{a}$ | 19.93±2.02$^{a}$ | | 1.3561±0.0029$^{a}$ | 15.26±1.73$^{a}$ |
| | | 8 | 1.3653±0.0038$^{ab}$ | 20.78±2.24$^{ab}$ | | 1.3560±0.0032$^{a}$ | 16.10±2.02$^{a}$ |
| | | 15 | 1.3658±0.0037$^{ab}$ | 21.06±2.20$^{bc}$ | | 1.3618±0.0032$^{b}$ | 18.73±1.99$^{b}$ |
| | | 21 | 1.3667±0.0012$^{b}$ | 21.74±0.75$^{c}$ | | 1.3679±0.0058$^{c}$ | 19.33±3.45$^{c}$ |

$^{a, b, c, d}$: the different letters mean a significant difference between the values. (p=0.05)

In confirmation of expectations, the packaging reduces the rate of change and preserves the hydration of the fruit. This result is due to the ability of edible packaging to modify the internal atmosphere (Martínez-Romero et al., 2006). The slowest increase is observed for the cv. Tsvetina, packed with chitosan-alginate, where the studied parameters are almost constant. The weakest is the effect of packaging in the cv. Tsvetina, packaged in a multi-component coating of chitosan and calcium lactate. The increase in performance is slower in the cv. Regina, most likely because this cultivar contains less dry content at the beginning of storage. Cv. Regina with chitosan packaging responds slowest in dry content increasing. The results are consistent with published literature data (Mali & Grossmann, 2003; Serrano et al., 2005).

### 3.3. Texture changes

Sweet cherries are crunchy fruits. When analyzing the texture of crunchy fruit, the most appropriate to use is a destructive test. The parameters of the puncture test are shown in Table 2. From the parameters determined in this test the reducing of the yield stress indicates the softening of the fruit. The rate of change in texture depends on the variety and the packaging. Non-coated fruit (control) changes the fastest. The slowest change in the cv. Tsvetina is observed in the package chitosan-calcium lactate, and in this cultivar the change is the smallest in the package chitosan-alginate.

**Table 2.** Texture parameters of the sweet cherries during the storage period

| Cult. | Treat. | Day | Yield point | | | | Rupture point | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Stress, kPa | Relative deformation | Young modulus, kPa | Deformation work, kPa | Stress, kPa | Relative deformation | Deformation work, kPa |
| Tsvetina | Cont. | 1 | 286.8±40.8 | 0.164±0.029 | 1324.0±223.0 | 16.8±3.4 | 378.4± 75.1 | 0.290±0.024 | 71.0± 9.3 |
| | | 7 | 221.4±56.0 | 0.215±0.052 | 1192.7±249.2 | 27.1±5.2 | 587.5±115.0 | 0.340±0.070 | 95.1±10.7 |
| | | 14 | 211.7±32.7 | 0.230±0.040 | 1097.3±201.9 | 15.4±1.6 | 387.4± 66.9 | 0.436±0.086 | 67.9± 6.9 |
| | | 21 | 161.3±26.1 | 0.247±0.016 | 753.7±157.1 | 14.5±1.9 | 569.6±136.8 | 0.439±0.057 | 81.8±10.0 |
| | Ch. | 1 | 290.7+40.6 | 0.158±0.026 | 1392.8±246.3 | 16.2±3.2 | 360.0± 63.5 | 0.273±0.048 | 50.6± 6.8 |
| | | 7 | 277.8+68.4 | 0.244±0.053 | 1346.3±236.4 | 31.0±6.0 | 742.3±146.6 | 0.398±0.079 | 87.0±13.8 |
| | | 14 | 257.8+42.3 | 0.280±0.053 | 1130.7±198.9 | 19.1±3.3 | 580.2±102.9 | 0.434±0.081 | 86.8±11.1 |
| | | 21 | 217.4+12.5 | 0.296±0.052 | 859.1±126.5 | 18.7±3.0 | 602.4± 77.8 | 0.452±0.042 | 70.9± 7.2 |
| | Ch+CaL | 1 | 279.1±50.1 | 0.159±0.030 | 1604.5±227.3 | 20.9±3.8 | 409.4± 51.8 | 0.280±0.030 | 59.9± 6.3 |
| | | 7 | 268.3±49.1 | 0.202±0.035 | 1404.7±247.1 | 19.1±3.7 | 631.1±121.4 | 0.360±0.066 | 65.6±10.8 |
| | | 14 | 254.3±56.1 | 0.279±0.084 | 1227.4±290.9 | 24.3±3.5 | 631.8±108.6 | 0.417±0.063 | 84.1±13.4 |
| | | 21 | 214.3±82.5 | 0.379±0.052 | 777.5±153.8 | 14.6±1.9 | 370.7± 71.2 | 0.439±0.076 | 61.4± 7.7 |
| | Ch+Alg | 1 | 295.7±39.8 | 0.155±0.019 | 1515.3±192.6 | 17.5±3.2 | 367.6± 51.1 | 0.263±0.032 | 51.4± 8.3 |
| | | 7 | 252.8±54.0 | 0.224±0.042 | 1410.5±264.5 | 27.4±5.0 | 631.3±121.4 | 0.381±0.071 | 71.3±10.3 |
| | | 14 | 236.5±48.5 | 0.239±0.063 | 1190.2±206.3 | 21.5±2.4 | 409.7± 35.5 | 0.413±0.077 | 61.7± 5.8 |
| | | 21 | 184.1±28.1 | 0.283±0.041 | 757.0±124.8 | 18.2±3.7 | 445.1± 61.6 | 0.440±0.054 | 76.8± 8.8 |
| Regina | Cont | 1 | 176.2±21.9 | 0.123±0.026 | 972.5±172.7 | 7.1±1.5 | 565.0± 53.2 | 0.335±0.059 | 50.0± 6.5 |
| | | 7 | 137.9±26.5 | 0.161±0.058 | 859.3±171.1 | 17.6±3.2 | 422.3± 62.0 | 0.429±0.055 | 47.5± 4.5 |
| | | 14 | 133.2±31.9 | 0.163±0.033 | 785.3±208.8 | 10.5±1.7 | 415.7± 46.0 | 0.434±0.065 | 42.4± 4.9 |
| | | 21 | 114.2±22.0 | 0.183±0.027 | 692.4± 96.3 | 8.1±1.5 | 274.7±104.9 | 0.469±0.025 | 87.9±10.2 |
| | Ch. | 1 | 176.1±16.3 | 0.164±0.039 | 905.3±165.8 | 9.8±2.2 | 406.7± 53.6 | 0.361±0.077 | 45.7± 6.4 |
| | | 7 | 149.6±24.4 | 0.175±0.024 | 893.1±180.5 | 6.9±1.2 | 347.1± 66.4 | 0.402±0.083 | 60.1±11.8 |
| | | 14 | 135.3±29.1 | 0.196±0.039 | 853.2±130.9 | 10.4±2.2 | 329.2± 51.0 | 0.414±0.076 | 33.1± 3.2 |
| | | 21 | 124.3±31.7 | 0.231±0.047 | 707.6±148.5 | 9.6±2.0 | 282.7± 67.3 | 0.465±0.067 | 61.3±11.0 |
| | Ch+CaL | 1 | 179.0±17.5 | 0.122±0.017 | 880.6±137.2 | 6.6±1.0 | 397.9± 57.4 | 0.363±0.072 | 50.3± 9.5 |
| | | 7 | 150.1±20.4 | 0.133±0.023 | 876.0±153.5 | 7.6±1.4 | 373.2± 72.1 | 0.425±0.069 | 63.1±10.2 |
| | | 14 | 113.8±28.4 | 0.157±0.033 | 819.6±150.2 | 8.7±1.8 | 367.2± 52.5 | 0.439±0.077 | 37.2± 4.4 |
| | | 21 | 104.2±24.8 | 0.163±0.034 | 686.4±116.9 | 9.9±2.6 | 292.5± 86.8 | 0.454±0.056 | 63.1± 8.7 |
| | Ch+Alg | 1 | 176.1±23.0 | 0.114±0.024 | 1062.4±206.9 | 7.2±1.5 | 307.5± 60.5 | 0.389±0.076 | 56.2± 8.4 |
| | | 7 | 145.4±24.8 | 0.120±0.025 | 978.9±175.1 | 6.0±1.2 | 352.0± 68.4 | 0.400±0.073 | 66.0±11.1 |
| | | 14 | 127.8±25.5 | 0.161±0.030 | 821.6±157.9 | 5.6±0.5 | 316.6± 50.7 | 0.415±0.081 | 25.9± 3.7 |
| | | 21 | 125.5±24.8 | 0.278±0.057 | 680.4±108.6 | 15.7±3.5 | 317.5± 63.3 | 0.485±0.079 | 74.5± 9.6 |

During the 3rd week, the hardness of all samples decreases at a different rate. The best preservation of the value of crunchiness is observed when applying a pure chitosan coating in both sweet cherry cultivars (figure 2).

The decrease in hardness can be explained by the delayed degradation of insoluble protopectins to the more soluble pectic acid and pectin.

During fruit ripening, depolymerization or shortening of the chain length of pectin substances occurs with an increase in pectinesterase and polygalactronase activity. Low oxygen concentrations and high carbon dioxide content reduce the activity of these enzymes and allow to reduce the hardness of fruits and vegetables during storage (Yaman & Bayoındırlı, 2002). The explanation for the

increase in yield point deformation and rupture point deformation is similar.

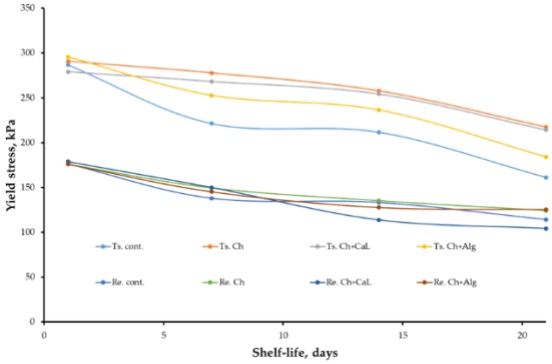


**Figure 2.** Changes in yield stress during storage of whole cherries with different packaging

The scientific literature reports that the softening process in cherries depends on the increase in the activity of polygalacturonase, β-galactosidase and pectin methyl esterase, which are responsible for the loss of fruit quality. The reduction in softening of coated samples can be explained by lower weight losses, reduced rot rate and less susceptibility to mechanical damage (Martínez-Romero et al., 2006).

### 3.4. Physicochemical parameters

The effect of chitosan packaging on antioxidant activity, active acidity (pH) and titratable acidity is shown in Table 3. According to our results, no change in in cherry acidity was observed during the storage (control and packaged cherries). Similar results have also been reported in the literature (Tokatlı, & Demirdöven, 2020). The antioxidant activity of sweet cherries decreases during storage. Its reduction depends on the cultivar of cherries and the composition of the package. For the cv. Tsvetina, the slowest is the reduction of AOA in the chitosan - calcium lactate package, faster in the chitosan-alginate packages and chitosan and the fastest without packaging. For the cv. Regina, the effect of packaging is reversed, the reduction rate is higher for packaged samples compared to the control. The overall reduction in antioxidant capacity (DPPH method) observed in all treatments can be explained by possible aging and breakdown, which often reduces antioxidant capacity (Kawhena et al., 2020).

**Table 3.** Physicochemical parameters during the storage of coated sweet cherries

| Cult. | Treat. | Day | AOA, µmol/L | OTK% | pH | Cult. | AOA, µmol/L | Titratable acidity, % | pH |
|---|---|---|---|---|---|---|---|---|---|
| Tsvetina | Cont. | 1 | 1288.68±12.50$^{c}$ | 0.61±0.012$^{bc}$ | 3.53±0.02$^{a}$ | Regina | 1265.72±30.15$^{bc}$ | 0.69±0.006$^{bc}$ | 3.25±0.02$^{ab}$ |
| | | 11 | 1242.28±26.10$^{ab}$ | 0.60±0.012$^{ab}$ | 3.57±0.02$^{b}$ | | 1242.88±40.70$^{ab}$ | 0.66±0.017$^{ab}$ | 3.22±0.02$^{a}$ |
| | | 21 | 1212.23±10.06$^{a}$ | 0.58±0.012$^{a}$ | 3.59±0.02$^{c}$ | | 1234.10±30.95$^{a}$ | 0.63±0.012$^{a}$ | 3.26±0.02$^{b}$ |
| | Ch. | 1 | 1282.42± 9.19$^{c}$ | 0.59±0.017$^{ab}$ | 3.55±0.03$^{a}$ | | 1263.61± 8.51$^{bc}$ | 0.61±0.012$^{b}$ | 3.43±0.02$^{a}$ |
| | | 11 | 1236.18± 5.94$^{ab}$ | 0.57±0.012$^{ab}$ | 3.59±0.01$^{b}$ | | 1236.26±39.93$^{ab}$ | 0.59±0.006$^{ab}$ | 3.42±0.02$^{a}$ |
| | | 21 | 1208.21±11.52$^{a}$ | 0.56±0.012$^{a}$ | 3.63±0.01$^{c}$ | | 1212.60±28.54$^{a}$ | 0.58±0.012$^{a}$ | 3.44±0.02$^{a}$ |
| | Ch+ CaL | 1 | 1233.23±10.80$^{bc}$ | 0.76±0.006$^{c}$ | 3.52±0.03$^{a}$ | | 1260.26±21.99$^{c}$ | 0.64±0.012$^{bc}$ | 3.34±0.02$^{ab}$ |
| | | 11 | 1211.33± 8.78$^{ab}$ | 0.68±0.017$^{b}$ | 3.51±0.02$^{a}$ | | 1222.16±39.35$^{ab}$ | 0.61±0.006$^{bc}$ | 3.32±0.02$^{a}$ |
| | | 21 | 1193.57±11.13$^{a}$ | 0.64±0.012$^{a}$ | 3.58±0.01$^{b}$ | | 1200.22±13.80$^{a}$ | 0.57±0.012$^{a}$ | 3.37±0.01$^{b}$ |
| | Ch+Alg | 1 | 1246.16± 9.07$^{bc}$ | 0.78±0.012$^{c}$ | 3.56±0.02$^{ab}$ | | 1262.70±18.08$^{c}$ | 0.63±0.012$^{ab}$ | 3.32±0.02$^{ab}$ |
| | | 11 | 1221.44±17.24$^{ab}$ | 0.65±0.006$^{ab}$ | 3.53±0.02$^{a}$ | | 1224.44±35.82$^{cb}$ | 0.62±0.012$^{ab}$ | 3.31±0.02$^{a}$ |
| | | 21 | 1205.14± 8.95$^{a}$ | 0.62±0.012$^{a}$ | 3.57±0.02$^{b}$ | | 1206.09±10.67$^{c}$ | 0.59±0.012$^{a}$ | 3.35±0.02$^{bc}$ |

$^{a, b, c, d}$: the different letters mean a significant difference between the values. (p=0.05)

### 3.5. Colour parameters

The colour parametrs of the tested samples are presented in Table 4. Colour is an important factor for consumers when choosing food. Usually, the acceptability of processed fruits and vegetables increases when their colours are close to the original fresh and unprocessed. It is generally accepted that the most important parameter determining the acceptability of sweet cherries by consumers is the bright red colour (Crisosto et al., 2003). Changes in colour during ripening and storage of cherries are related to the content of anthocyanins (Serrano et al, 2005). The value of the illuminance parameter shows an increasing trend in the cv.

Tsvetina (with and without packaging), ie. the fruits become lighter, and only in the case of cherries covered with chitosan the values are approximately the same. The L parameter for the cv. Regina shows a decrease for all samples (the fruits become darker). The red colour index (a) increases during storage. This parameter shows the ripening of the fruit. The increase is less in packaged fruits. A smaller increase means a delay in over ripening and the intensity of respiration. A similar change in sensory quality was obtained by Martínez-Romero et al. (2006) and Tokatlı and Demirdöven (2020) for the packaging of cherries with aloe vera extracts and chitosan. The value of b becomes extremely high at the end of storage, decreasing only for cherries with a package of chitosan.

This indicator is probably related to the deterioration of the samples. Coated fruits show less and slower reduction in C than the control. The best preservation of the saturation value is observed with pure chitosan coating in both varieties of cherry.

**Table 4.** Colour parameters of the sweet cherries during the storage period.

| Cult. | Treat. | Day | L | a | b | C | BI |
|---|---|---|---|---|---|---|---|
| Tsvetina | Cont. | 1 | 15.02±0.98$^{a}$ | 12.47±4.88$^{a}$ | 0.57±0.10$^{ab}$ | 12.48±4.88$^{a}$ | 56.18+4.70$^{a}$ |
| | | 11 | 15.43±1.02$^{ab}$ | 15.25±3.91$^{ab}$ | 0.16±0.13$^{a}$ | 15.25±3.91$^{ab}$ | 62.16+8.86$^{ab}$ |
| | | 21 | 18.45±1.71$^{c}$ | 23.58±0.68$^{b}$ | 4.43±2.65$^{c}$ | 24.08±1.12$^{c}$ | 106.00+9.05$^{c}$ |
| | Ch. | 1 | 15.01±1.17$^{a}$ | 14.82±2.75$^{a}$ | 0.11±0.08$^{a}$ | 14.82±2.75$^{a}$ | 61.97+8.53$^{a}$ |
| | | 11 | 15.15±0.54$^{ab}$ | 14.85±2.40$^{a}$ | 0.45±0.12$^{b}$ | 14.86±2.40$^{a}$ | 64.04+7.03$^{a}$ |
| | | 21 | 14.61±1.65$^{a}$ | 16.29±2.95$^{ab}$ | 0.57±0.09$^{b}$ | 16.30±2.95$^{ab}$ | 72.07+5.61$^{ab}$ |
| | Ch+CaL | 1 | 15.40±2.19$^{a}$ | 13.12±1.18$^{a}$ | 0.16±0.04$^{a}$ | 13.12±1.18$^{a}$ | 55.29+8.60$^{a}$ |
| | | 11 | 14.91±1.96$^{a}$ | 15.14±3.20$^{a}$ | 0.79±0.79$^{ab}$ | 15.17±3.24$^{ab}$ | 68.11+9.26$^{ab}$ |
| | | 21 | 17.22±2.90$^{ab}$ | 18.89±5.72$^{a}$ | 2.96±1.60$^{c}$ | 19.14±5.88$^{bc}$ | 85.9+10.38$^{bc}$ |
| | Ch+Alg | 1 | 16.01±0.98$^{a}$ | 14.56±0.76$^{a}$ | 0.94±0.07$^{a}$ | 14.59±0.75$^{a}$ | 63.95+1.96$^{a}$ |
| | | 11 | 16.56±0.84$^{ab}$ | 14.95±2.11$^{a}$ | 1.36±0.25$^{ab}$ | 15.01±2.12$^{a}$ | 68.08+6.44$^{a}$ |
| | | 21 | 18.78±0.77$^{c}$ | 21.83±1.20$^{b}$ | 3.35±0.26$^{c}$ | 22.10±1.22$^{b}$ | 92.34+6.95$^{b}$ |
| Regina | Cont. | 1 | 15.83±0.41$^{b}$ | 9.15±1.59$^{a}$ | 0.30±0.03$^{a}$ | 9.15±1.59$^{a}$ | 40.61+6.52$^{a}$ |
| | | 11 | 12.48±3.21$^{a}$ | 11.83±5.37$^{b}$ | 1.40±0.20$^{b}$ | 11.91±5.36$^{ab}$ | 77.38+5.86$^{b}$ |
| | | 21 | 11.22±3.08$^{a}$ | 13.91±3.84$^{b}$ | 0.88±0.18$^{ab}$ | 13.93±3.84$^{b}$ | 87.95+8.91$^{bc}$ |
| | Ch. | 1 | 14.36±3.22$^{b}$ | 8.34±2.03$^{a}$ | 0.68±0.26$^{ab}$ | 8.36±2.04$^{a}$ | 46.13+7.72$^{a}$ |
| | | 11 | 13.48±0.94$^{ab}$ | 8.83±2.22$^{a}$ | 0.53±0.13$^{ab}$ | 8.85±2.22$^{a}$ | 47.36+8.53$^{a}$ |
| | | 21 | 12.77±2.97$^{a}$ | 9.46±3.97$^{a}$ | 0.25±0.06$^{a}$ | 9.46±3.97$^{ab}$ | 52.77+7.62$^{ab}$ |
| | Ch+CaL | 1 | 13.87±1.33$^{a}$ | 6.81±0.93$^{a}$ | 1.46±0.54$^{b}$ | 6.98±1.00$^{a}$ | 46.00+8.02$^{a}$ |
| | | 11 | 14.69±1.29$^{b}$ | 10.21±4.48$^{ab}$ | 1.10±0.39$^{ab}$ | 10.27±4.49$^{ab}$ | 53.74+9.04$^{ab}$ |
| | | 21 | 13.56±1.01$^{a}$ | 11.47±3.31$^{b}$ | 0.51±0.13$^{a}$ | 11.49±3.30$^{b}$ | 58.31+6.52$^{ab}$ |
| | Ch+Alg | 1 | 15.49±1.62$^{b}$ | 6.45±1.15$^{a}$ | 1.44±0.31$^{b}$ | 6.61±1.19$^{a}$ | 39.74+8.26$^{a}$ |
| | | 11 | 15.15±2.46$^{b}$ | 7.22±1.37$^{ab}$ | 1.25±0.13$^{ab}$ | 7.32±1.37$^{ab}$ | 42.68+8.78$^{a}$ |
| | | 21 | 11.61±1.00$^{a}$ | 8.47±0.39$^{bc}$ | 0.71±0.21$^{a}$ | 8.50±0.39$^{b}$ | 54.29+7.54$^{ab}$ |

$^{a, b, c, d}$: the different letters mean a significant difference between the values during storage. (p=0.05)

The browning of the fruit increases during storage at most in the control samples (figure 3). Packaging reduces the rate of change. The increase is the smallest in cherries with chitosan packaging in both varieties.

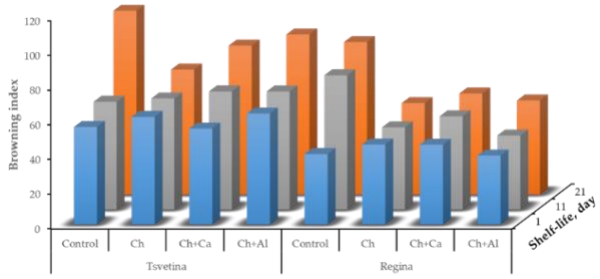


**Figure 3.** Browning index during storage of whole cherries with different packaging

### 3.6. Microbiological parameters

The samples remained safe without pathogens, but with increasing microbiological contamination (Table 5). Chitosan coating reduced the growth of fungi and other microbial contaminants on the surface of cherries for both varieties.

Antimicrobial effect of chitosan is due to the fact that the positively charged amino groups of chitosan bind to the negatively charged carboxyl groups on the bacterial cell membrane, thus changing the charge distribution on the cell surface, which leads to impaired membrane stability (Rabea et al., 2003; Dutta et al., 2009, Moreira et al., 2015).

**Table 5.** Microbiological parameters of the sweet cherries during the storage period

| Cult. | Treat | Day | TMC | Molds and yeasts | Entero-bacteriaceae | Escherichia coli | Sulfitreducing and clostridia |
|---|---|---|---|---|---|---|---|
| | | | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g |
| Tsvetina | Cont. | 1 | 10800 | 4200 | < 10 | < 10 | < 10 |
| | | 11 | 78000 | 26500 | < 10 | < 10 | < 10 |
| | | 21 | 210000 | 34590 | < 10 | < 10 | < 10 |
| | Ch | 1 | 10 | < 10 | < 10 | < 10 | < 10 |
| | | 11 | 30 | 10 | < 10 | < 10 | < 10 |
| | | 21 | 830 | 80 | < 10 | < 10 | < 10 |
| | Ch+Ca | 1 | 70 | 40 | < 10 | < 10 | < 10 |
| | | 11 | 1510 | 880 | < 10 | < 10 | < 10 |
| | | 21 | 4100 | 3700 | < 10 | < 10 | < 10 |
| | Ch+Al | 1 | 1210 | 600 | < 10 | < 10 | < 10 |
| | | 11 | 6200 | 3100 | < 10 | < 10 | < 10 |
| | | 21 | 15000 | 7300 | < 10 | < 10 | < 10 |
| Regina | Cont. | 1 | 3700 | 2680 | < 10 | < 10 | < 10 |
| | | 11 | 12500 | 5600 | < 10 | < 10 | < 10 |
| | | 21 | 59000 | 21400 | < 10 | < 10 | < 10 |
| | Ch | 1 | < 10 | < 10 | < 10 | < 10 | < 10 |
| | | 11 | 10 | < 10 | < 10 | < 10 | < 10 |
| | | 21 | 150 | < 10 | < 10 | < 10 | < 10 |
| | Ch+Ca | 1 | 300 | 120 | < 10 | < 10 | < 10 |
| | | 11 | 310 | 190 | < 10 | < 10 | < 10 |
| | | 21 | 5400 | 1600 | < 10 | < 10 | < 10 |
| | Ch+Al | 1 | 1800 | 780 | < 10 | < 10 | < 10 |
| | | 11 | 7400 | 830 | < 10 | < 10 | < 10 |
| | | 21 | 16200 | 10400 | < 10 | < 10 | < 10 |

The antimicrobial activity of multi-component packages is lower, but they also significantly reduce contamination compared to controls by the end of the storage period. A very similar result was reported in the Tokatlı and Demirdöven (2020) study for cherries.

### 3.7. The results of sensory analysis

On the first day, the points were approximately the same for packaged and unpackaged cherries in both varieties, only the chitosan-calcium lactate coated cherries received significantly smaller points (figure 4).

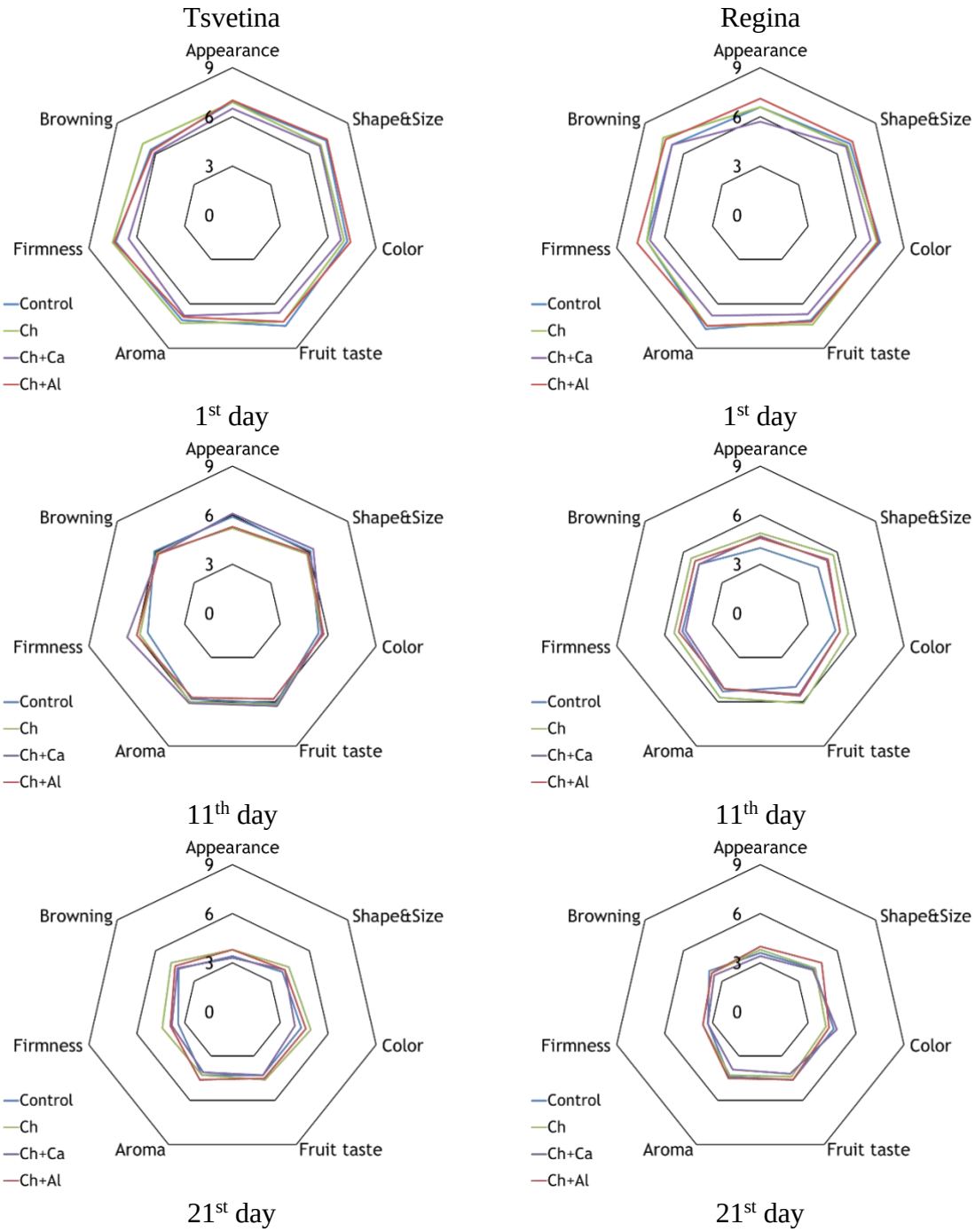


**Figure 4.** The results of the sensory analysis.

After 11 days of storage, the cv Tsvetina shows higher points compared to the cv Regina. On this day, the highest points are given to fruits packed with chitosan - calcium lactate.

The fruits with this packaging have retained their quality up to 90% of the original. The reason is the best preservation of the texture. The control and the other two packages show similar quality, about 80%. On the 11th day, the control has the least points in the Regina variety (66%). The fruits, coated in chitosan received the highest points (77%). Fruits packed with chitosan - calcium lactate and chitosan alginate received lower points (about 70%). At the end of storage, both varieties demonstrated low storage quality (55 to 60%). A similar change in sensory quality was obtained by Martínez-Romero et al. (2006) for the packaging of cherries with aloe vera extract.

## 4. Conclusions

Based on all the studied parameters, the Tsvetina variety retains better quality compared to the Regina variety.

Our experimental series provide information on the shelf life of coated cherries. It is no more than 21 days, about 3 weeks.

The samples remained safe without pathogens, but with increasing microbiological contamination. The smallest microbiological contamination is observed with pure chitosan coating. The other two coatings show a shorter extension of the storage period.

The pure chitosan coating showed the longest preservation of quality and safety between the applied treatments.

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# IDENTIFICATION AND CONTROL OF BLACK COLOUR SPECK FUNGAL FORMATION IN VIRGIN COCONUT OIL

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**ABSTRACT**

A critical issue in virgin coconut oil (VCO) industry is fungal contaminations which leads to black colour speck formation. This study was designed to distinguish the type of fungal growth and to determine the remedial actions. VCO was extracted by cold press method and subjected to eight treatments. Efficacy of the treatments were evaluated in terms of changes in microbial properties [yeast and mould count (YMC) and aerobic plate count(APC)], physicochemical properties [moisture and volatile matter% at 105˚C (MV), specific gravity at 30˚C (SG), saponification value(SV), iodine value(IV), peroxide value(PV), acid value (AV), relative fatty acid profile (RFAP) by gas chromatography and free radical scavenging activity (DPPH assay)] along with a non-treated sample. The results revealed presence of *Aspergillus* sp. as the black colour speck in VCO and among those treatments the combination (X2) where VCO was subjected to 65˚C, 253.7nm UV radiation for 60 seconds was identified as the best because it gave a null YMC, 15CFU/mL in APC, 0.12±0.01 in MV %, 0.9194±0.00 in SG and it was within the APCC standards. Further, IV, SV, PV and AV were obtained as 5.52±0.37mg/g, 262.55±0.16mg KOH/g, 2.96±0.02 Meq/kg and 0.14±0.04mg/g respectively. The X2 sample showed a higher lauric acid percentage (50.489±0.011) compared to the non-treated (NT) sample (49.646±0.001). A lower $EC_{50}$ value was noted in X2 (3.27±0.01mg/L) compared to NT (3.27±0.01 mg/L) sample. Evidently, the present results suggest that combination of heat, UV radiation with time has a significant influence on retarding the black speck formation in VCO.

## 1. Introduction

VCO is a popular edible oil consumed around the world. It is the purest form of oil extracted from coconut (*Cocos nucifera* Linn.) kernel with unique colourless (water clear) to pale yellowish brown and viscous nature (Dayrit *et al.*, 2007; APCC, 2009). Coconut oil is comprised of a significant level of saturated fatty acids which are in low molecular weight including lauric acid. The chemical properties of VCO increases its potential for use in both edible and non-edible applications (Marina *et al.*, 2009). The unique characteristics of this oil include bland flavor, pleasant odor (fresh natural coconut scent), taste free of rancidity, having a narrow range of melting temperature, improved digestibility with absorbability, and higher potential for foam retention (Che and Mariana, 2006). It is made from the fresh coconut meat (matured kernel 12 months old from pollination) subjected to mechanical press "with or without heat application, which does not lead to alteration of the nature of the oil". (Bawalan and Chapman, 2006; APCC, 2009).

The APCC (Asian and Pacific Coconut Community) and Codex Alimentarius are the major organizations that establish international standards for commercial coconut oil. Both APCC and Codex standards defines "virgin oil" should be edible for humans in its liquid nature and maybe purely obtained by washing with water, settling, filtering, and centrifuging. Even though Codex standards do not have specific standards for VCO, APCC has initialed standards for VCO (Alimentarius, 1999; APCC, 2009; Dayrit *et al*., 2007).

VCO is extracted by wet process directly from coconut milk under controlled temperature conditions and has more beneficial effects over coconut oil because it preserves beneficial constituents (Nevin and Rajamohan, 2004; Marina *et al*., 2009a). It is comprised of natural vitamins resulting in impaired oxidation (hydrolytic and atmospheric). This high antioxidant potential results in lower acid value and peroxide value of coconut oil. VCO has a characteristic fresh coconut aroma (Mansor *el al*., 2012) with a number of health benefits. One of the main issues encountered in VCO industry in Sri Lanka is the "black colour" sediment observed in the bottom of the containers stored under low temperature. It leads to poor consumer perception, retailer un-satisfaction and market returns from the export market thereby declining the demand. Therefore, it is critical and essential for the local VCO manufacturers as well as the exporters to overcome the issue of black speck formation. This study was carried out to distinguish the type of microorganism and to retard the growth by designing treatments changing the heat at cold pressing process (temperature is maintained less than 60˚C) while exposing to UV radiation and changing the time combinations (Table 1). Consequently, physicochemical properties, fatty acid profile, and antioxidant properties of VCO were determined in the given treatments and the best treatment was identified. Furthermore, there have been no previously reported studies conducted in this area locally or internationally. According to authors' knowledge, this is the first time, a research study of this manner has been conducted. In addition, these findings will be beneficial for both commercial scale VCO manufacturers as well as persons who will carry out future studies related to the field of coconut oil and especially, virgin coconut oil.

## 2. Materials and methods

### 2.1. Sample Collection

VCO samples (prepared under cold pressed methods) were collected from a local VCO processor in Kurunegala district, North Western province, Sri Lanka.

### 2.2. Microbial Tests

Initially the microbial species were identified through slide culture technique (Woo *et al*., 2011). Dilution plating method was implemented to evaluate microbial count in VCO samples (Table 1). Both treated and non–treated VCO samples were analysed through total plate count, yeast and mould count using standard plate count agar following AOAC methods (AOAC, 1995).

### 2.3. Physicochemical Tests

Moisture and volatile matter percentage was determined according to AOAC method (AOAC, 1997), specific gravity was obtained by American Oil Chemists' Society (AOCS) official method (Cc 10a-25, 1999) with slight modifications, saponification value was obtained via AOCS official method (Cd 3-25, 1999), iodine value (Cd 1-25, 1999), peroxide value (Cd 8b, 1999) and acid value (3a-63, 1999) were obtained by AOCS official methods (AOCS,1990).

Relative fatty acid profile was obtained through gas chromatography model-7890 A (GC), equipped with mass spectrometer model-5975 C (MS) inert XL EI/CI MSD with triple-axis detector by preparation of fatty acid methyl esters in oil sample following the programme described in Munasinghe and Wansapala (2015)

### 2.4. Antioxidant Activity

The free radical scavenging activity of the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) was reported according to the procedure

followed by Marina and others (2009b). This assay was conducted for all VCO samples separately (treated samples and non-treated sample (Table 1). The inhibition activity percentage was calculated as $[(A_0 - A_1) / A_0] \times 100$, where the absorbance of the control (containing no sample extract) is denoted by $A_0$ and absorbance of the extract is given by $A_1$. The effective concentration ($EC_{50}$ (mg/mL)) value was obtained depending on the quantity of VCO extracts required to reduce the initial DPPH radical concentration by 50%.All the physicochemical and microbiological tests were carried out to non-treated and treated samples and compared with the values of VCO standards. For the antioxidant study, results were evaluated by one-way analysis of variance (ANOVA) followed by Tukey pairwise comparison test. All the tested samples were triplicated to obtain results and statistical analysis were done by Minitab 17 software.

## 3.Results and discussions

### 3.1. Microbial Identification

The microorganism was identified as *Aspergillus* sp. according to its colony morphology (Figure 3) results obtained through slide culture technique (Figure 2). Initially a white colour colony appeared. Later it became black in colour showing a "salt and pepper appearance" (Figure 1). This was resulted from "darkly pigmented conidia born in large numbers on conidiophores and reverse turning pale yellow" (Debets *et al*., 1990). VCO samples obtained from single UV or heat treatment (T1, T2, UV1, and UV2) and non-treated (NT) sample gave positive results for black specks. Negative results were produced for both heat and UV combinations applied samples (X1, X2, X3 and X4).

Non-treated sample showed highest number of CFU/mL for both TPC (155) and YMC (45) which exceeds the APCC (Asian and Pacific Coconut Community) standard limits (<100 CFU/mL for TPC and <10 CFU/mL for YMC). Inability to meet this standard shows the lack of quality and unsafe product which has higher potential to cause health hazards (Uthpala and Navaratne, 2019; Uthpala *et al*., 2021; BFAD, 2004). There was a gradual decrement of microbial load with heat application (Table 2). The implementation of UV technology in the food industry is an alternative to chemical sterilization and it minimizes microbial population (González *et al.*, 2007). Heat and UV combined treatments were absent with fungal growth in PDA media while NT sample noted to have higher amount. Green and others (2007) had reported the impact of UV irradiation over *Aspergillus flavus* and *Aspergillus fumigatus*. In their study, the exposure time of UV radiation had significant impact on disinfectant potential of the above-mentioned fungus (Green *et al.*, 2004). Moreover, Rotem and Aust (1991) had shown that *Aspergillus niger* is highly sensitive to UV and sunlight, even though *Aspergillus* sp. is resistant to high temperatures in dark conditions (Rotem & Aust, 1991).

The results showed the YMC could be reduced to some extent by application of heat and UV combined treatments.

**Table 1**. Description of treatments and symbols

| Treatment No | Symbol | Description |
|---|---|---|
| *1* | NT | Non-treated oil sample |
| *2* | T1 | Oil sample is heated up to 65˚C for 60 seconds |
| *3* | T2 | Oil sample is heated up to 85˚C for 60 seconds |
| *4* | UV1 | Oil sample is exposed to 253.7nm UV radiation for 30 seconds |
| *5* | UV2 | Oil sample is exposed to 253.7nm UV radiation for 60 seconds |
| *6* | X1 | Oil sample is heated up to 65˚C for 60 seconds and exposed to 253.7nm UV radiation for 30 seconds |
| *7* | X2 | Oil sample is heated up to 65˚C for 60 seconds and exposed to 253.7nm UV radiation for 60 seconds |
| *8* | X3 | Oil sample is heated up to 85˚C for 60 seconds and exposed to 253.7 nm UV radiation for 30 seconds |
| *9* | X4 | Oil sample is heated up to 85˚C for 60 seconds and exposed to 253.7 nm UV radiation for 60 seconds |

T1, T2, UV1, and UV2 are single treatments. X1, X2, X3 and X4 are combined treatments.

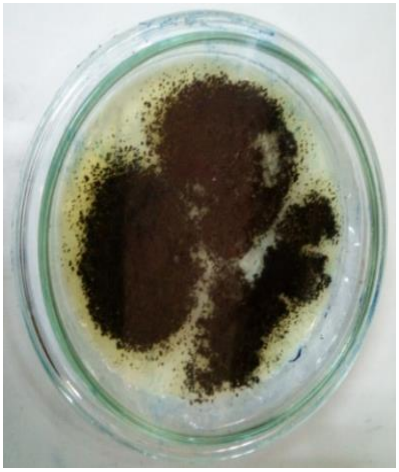

**Figure 1.**
Fungal growth after a week from inoculation

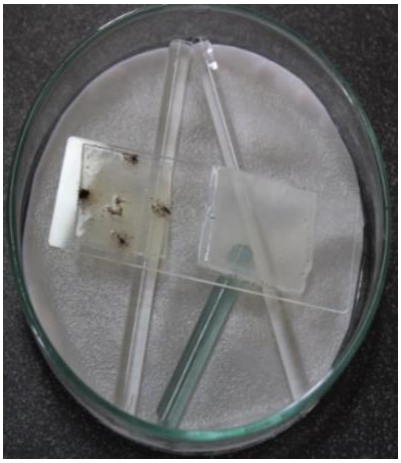

**Figure 2.**
Slide culture observations after 48 hours.

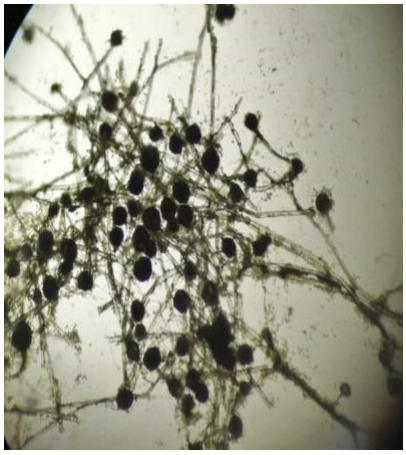

**Figure 3.**
Microscopic view of the fungus in slide culture under the power of 10x40.

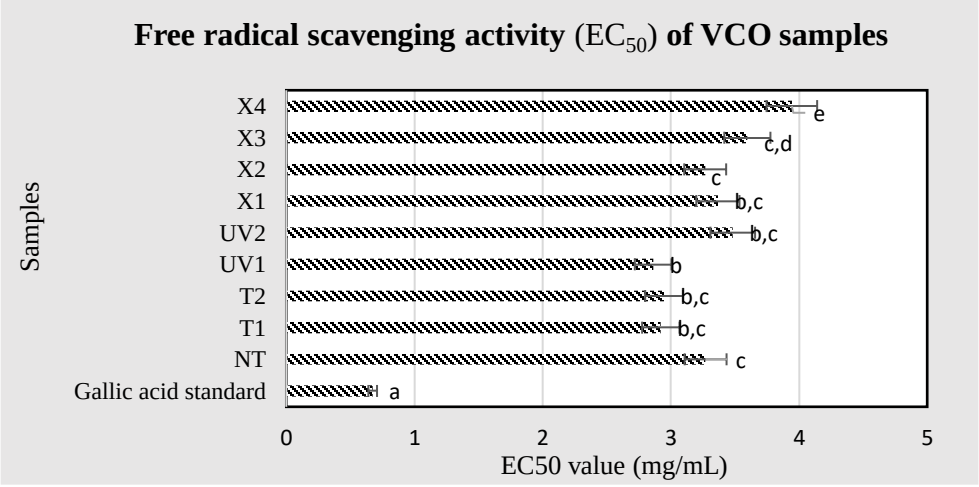


**Figure 4**.
Antioxidant activity ($EC_{50}$ values) of VCO standard, non-treated sample and treated samples. Each value represents the mean and standard deviations of triplicate determinations.
a,b,c,d,c,d Values with different superscripts are significantly different at 0.05 level.

**Table 2**. Changes of microbiological count, physicochemical properties of treated VCO samples compared with the non-treated sample and APCC standard

| **Sample**<br>**Parameter** | **Non-Treated (NT)** | **Treatment 1 (T1)** | **Treatment 2 (T2)** | **Treatment 3 (UV1)** | **Treatment 4 (UV2)** | **Combined Treatment 1 (X1)** | **Combined Treatment 2 (X2)** | **Combined Treatment 3 (X3)** | **Combined Treatment 4 (X4)** | **APCC standards** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Microbial Properties** | | | | | | | | | | |
| **Total Plate Count** (CFU/mL) | 155 | 95 | 85 | 65 | 30 | 20 | 15 | 15 | 15 | <100 |
| **Yeast & Mould Count** (CFU/mL) | 45 | 10 | 2.5 | 7.5 | 5 | Nil | Nil | Nil | Nil | <10 |
| **Physicochemical properties** | | | | | | | | | | |
| **Moisture & volatile %** | 0.08±0.01 | 0.07±0.01 | 0.07±0.01 | 0.09±0.01 | 0.11±0.01 | 0.07±0.01 | 0.12±0.01 | 0.09±0.00 | 0.08±0.01 | 0.2 |
| **Specific gravity** | 0.9188± 0.00 | 0.9200± 0.00 | 0.9197± 0.00 | 0.9197± 0.00 | 0.9198± 0.00 | 0.9197± 0.00 | 0.9194± 0.00 | 0.9196± 0.00 | 0.9194± 0.00 | 0.915 – 0.920 |
| **Saponification value** (mg KOH/g) | 262.13± 0.85 | 262.56± 2.47 | 262.80± 2.00 | 262.31± 0.11 | 262.62± 1.00 | 262.88± 0.58 | 262.55± 0.16 | 262.51± 0.95 | 262.67± 0.11 | 250-260 (min) |
| **Iodine value** (g $I_2$/100g sample) | 6.94±0.42 | 6.42±0.52 | 5.75±0.39 | 6.27±0.38 | 6.82±0.53 | 6.23±0.17 | 5.52±0.37 | 5.35±0.28 | 5.16±0.49 | 4.1-11.0 |
| **Peroxide value** (Meq/kg) | 0.00±0.00 | 1.97±0.00 | 2.96±0.00 | 2.00±0.00 | 2.96±0.02 | 1.98±0.01 | 2.96±0.02 | 3.96±0.02 | 4.94±0.03 | 3.0 (max) |
| **Acid value** (mg KOH/g sample) | 0.00±0.00 | 0.00±0.00 | 0.17±0.00 | 0.06±0.00 | 0.11±0.00 | 0.06±0.00 | 0.14±0.04 | 0.22±0.00 | 0.28±0.00 | 0.40 (max) |

| Relative Fatty Acid Profile (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Carbon number** | **Name** | NT | T1 | T2 | UV1 | UV2 | X1 | X2 | X3 | X4 | APCC standards |
| **C6:0** | hexanoic acid /caproic | 0.296 | 0.300 | 0.479 | 0.469 | 0.432 | 0.182 | 0.220 | 0.070 | 0.222 | 0.10 -0.95 |
| **C8:0** | octanoic acid /caprylic | 6.023 | 5.992 | 7.497 | 7.417 | 7.055 | 4.237 | 4.574 | 1.036 | 3.964 | 4 -10 |
| **C10:0** | decanoic acid /capric | 4.779 | 4.917 | 5.497 | 5.457 | 5.154 | 3.785 | 3.973 | 1.022 | 2.926 | 4 - 8 |
| **C12:0** | dodecanoic acid /**lauric** | 49.646 | 48.817 | 48.393 | 48.945 | 48.213 | 41.591 | 50.489 | 30.571 | 41.925 | 45 -56 |
| **C14:0** | methyl tetradecanoic /myristic | 18.950 | 18.658 | 20.791 | 21.682 | 20.947 | 21.440 | 22.084 | 27.665 | 22.723 | 16 - 21 |
| **C16:0** | hexadecanoic /palmitic | 8.168 | 8.355 | 8.163 | 7.896 | 8.397 | 11.218 | 8.841 | 17.142 | 12.502 | 7.5 -10.2 |
| **C19:0** | 10,13-octadecadienoic acid | NA | 1.066 | NA | NA | NA | NA | NA | NA | NA | NA |
| **C18:2** | 9,12-octadecadienoic acid /linoleic | 0.908 | NA | NA | 0.638 | 0.752 | 1.397 | 0.744 | 1.706 | 1.201 | 0.7 - 2.5 |
| **C18:2** | 9,15 -octadecadienoic acid | NA | NA | 0.747 | NA | NA | NA | NA | NA | NA | NA |
| **C18:1** | Cis-13-octadecadienoic acid | NA | NA | NA | NA | 5.417 | NA | NA | NA | NA | NA |
| **C18:1** | 9-octadecenoic acid /oleic | 6.637 | 7.165 | 5.034 | 4.508 | NA | 9.414 | 5.373 | NA | NA | 4.5 - 10 |
| **C18:1** | 11-octadecenoic acid /vaccenic | NA | NA | NA | NA | NA | NA | NA | 12.477 | 8.770 | NA |
| **C18:0** | octadecanoic acid /stearic | 4.593 | 4.731 | 3.398 | 2.988 | 3.634 | 6.499 | 3.701 | 8.312 | 5.768 | 2 - 10 |
| | **TSFA**[a] | 92.455 | 92.836 | 94.218 | 94.854 | 93.832 | 88.952 | 93.882 | 85.818 | 90.029 | NA |
| | **TUFA**[b] | 7.545 | 7.165 | 5.781 | 5.146 | 6.169 | 10.808 | 6.117 | 14.183 | 9.970 | NA |
| | **S/U**[c] | 12.254 | 12.957 | 16.298 | 18.433 | 15.210 | 8.230 | 15.348 | 6.051 | 9.030 | NA |

Each value represents the mean of triplicated samples, NA-not available

Since VCO is stored in airtight containers it maintains anaerobic conditions. Hence there is a high possibility for the growth of anaerobic microorganisms.

### 3.2. Physicochemical Analysis

Water activity of VCO is very low. Therefore, the freely available moisture content is not enough for the growth and survival of most of the microorganisms. Due to several facts, the moisture content can be increased during the manufacturing process. The MV percentage values obtained from all the treatments are significantly less than or equal to 0.13% (Table 2). Non-treated sample gave 0.08±0.10% moisture and volatile matter value. The high moisture content would initiate hydrolysis reactions (Osawa *et al*., 2007; Lawson, 1985). Therefore, moisture content within the standards ensures safeness of the product.

Specific gravity is a measure of fatty acids available in a unit volume. It is an important parameter for chemical engineering unit operations in the fatty acid industry. All the treated samples showed higher SG values, compared with the non-treated sample. But all samples were within APCC (2009) standards at $30^0$C (0.915–0.920) (APCC, 2009).

Saponification is oil hydrolysis under basic conditions to produce glycerol and the salt of the relative FA (Tan *et al*., 2013). SVs of the samples ranged from 262.20 to 263.46 mg KOH/g of fat which was comparable with the VCO research studies done in Malaysia (Mansor *et al.*, 2012). In addition, SVs of all the samples were aligned with the APCC (Table 2) standards (APCC, 2009). SV implies an idea about characteristics of fatty acids available in oil. Lower amount of acid is liberated per gram of fat hydrolysed of fat containing longer carbon chains. It is a measure to get a rough estimation on the molecular weight or chain length of fatty acids available in oil. The longer the chain of fatty acids the lower the SV. They have relatively fewer number of carboxylic functional groups per unit mass of the fat leading to high molecular weight.

Iodine value (IV) is used to evaluate the degree of unsaturation of an oil. Saturated oils take up no iodine leading to zero IV. The IVs obtained for the VCO samples in this study had an average value range of 5.16 to 6.94 g $I_2$/100 g which is compatible with the APCC (Table 2) standards (APCC, 2009). With both heat and UV treatments the IVs have been reduced. Low IVs reduce the chance of VCO to enhance rancidity (Onyeike and Acheru, 2002; Mansor *et al*., 2012). Since IV is an estimate of overall double bonds in an oil sample, the IVs should be comparable with the relative fatty acid profile results in the absence of other olefinic (double bond containing) compounds (Dayrit *et al.*, 2007).

Double bonds in unsaturated fatty acids can be oxidized and form hydroperoxides which leads to rancidity with time or due to temperature increments (Gunstone, 1996; Dayrit *et al.,* 2007). Codex standards give a peroxide value limit of 15 Meq/kg while APCC specifies 3 Meq/kg for VCO (Alimentarius, 1999; APCC, 2009; Dayrit *et al*., 2007). Even though X3 (3.96±0.02 Meq/kg) and X4 (4.94±0.03 Meq/kg)) treatments exceed the limit of PV compared above APCC specifications, it is compatible with Codex (max 15 Meq/kg) limitations.

Acid value is a quantitative parameter of the free fatty acids (FFA) available in the oil. FFA increment in oil sample indicates hydrolysis of triglycerides which produces glycerol. FFA is a reason for flavors and aroma (Osawa *et al*., 2007). In addition, short chain FFA are volatile and soluble in water with a unique odor. If the oil contains both long chain saturated and unsaturated fatty acids they tend to oxidize and form products (aldehydes, ketones, alcohols, and organic acids) that provide characteristic flavours and aroma (Marina *et al.*, 2009a). All the results obtained in each treatment showed low AVs compared to APCC standard value (0.4 max) (APCC, 2009). Acid values in combined treatment samples are slightly increased compared to the NT sample (Table 2). Degradation of fatty acids due to heat and UV exposure might be the reason because, extrinsic

(light, temperatures and oxygen) and intrinsic (antioxidants, pro-oxidants and water) factors can stimulate lipid oxidation (Kamal, 2006, Barrera *et al*, 2002). Due to chemical or enzymatic mechanisms acid hydrolysis can be initiated. Plant enzymes (lipases) or microbial contaminants are the factors of enzymatic hydrolysis. High levels of FFA are undesirable due to unpleasant mouthfeel and aroma.

The relative fatty acid profile cannot be used to indicate the exact acid value. The ratio of saturation/unsaturation (S/U ratio) had changed significantly with heat and UV applications (Table 2), even main FFA percentages, in most treatments remained within the APCC standard values (APCC, 2009). The treatment X2 was observed with the highest lauric acid percentage (50.49%) while X3 showed the lowest (30%). NT sample had shown 49% lauric acid and single treatments (only heat or UV applied) indicated approximately 48% of lauric acid percentage. Under T1 treatment there is a formation of 10,13-octadecadienoic acid (C19:0), but the amount does not affect the S/U ratio (Table 2).

VCO is predominantly made up of lauric acid (C12:0). Researches have revealed that monolaurin (a monoglyceride form of lauric acid) has antimicrobial properties (Wang *et al*., 1993; Kabara, 1984; Enig, 1998; Mansor *el al*., 2012). The total lauric acid contents obtained for X1, X2 and NT samples are according to the ranges of APCC and Codex (45.10–53.20%) standards (Marina *et a*l, 2009b; Dayrit *et al*., 2007). But lauric acid values for X3 and X4 sample are slightly lower than the above standards. This could be due to application of higher temperature.

Lauric acid (C12) has distinctive biochemical and nutritional properties that are not similar to long-chain saturated fatty acids (SFA). Medium-chain SFA (C6-C12) show different metabolic and physiological properties from long-chain SFA (C14-C18). Lauric acid accounts approximately half of the FA in coconut oil. Further, medium-chain triglycerides account for approximately half of all triacyl glycerides in coconut oil. Hence coconut oil is classified under vegetable oil which has medium-chain FAs (Dayrit *et al.,* 2007)

### 3.3. Antioxidant potential

As $EC_{50}$ value increases the free radical scavenging ability or the antioxidant potential reduces. According to the $EC_{50}$ value, the effectiveness of free radical scavenging potential in descending order was Gallic acid standard > UV1> T1> T2> X2> NT> X1> UV2> X3> X4 (Figure 4). There was a significant difference ($P<0.05$) in the $EC_{50}$ values among the treatments. The lower $E_{50}$ values compared to NT can be considered as positive impact having treatments. NT sample showed 3.27 mg/mL $EC_{50}$ value and T1, T2, UV1 and X2 showed better antioxidant potential compared to NT sample (Figure 4). González and others (2007) have revealed that the exposure to UV radiation can increase the antioxidant potential in food (González *et al*., 2007). Natural antioxidants can control free radicals which can cause pathological effects such as cancer and vascular diseases. Oxidation is a natural reaction in metabolism and often resulted by reactive compounds such as highly reactive hydroxyl radicals, •OH. Antioxidants quench these free radicals. Natural antioxidants help food manufactures to produce stable products with natural ingredients (Ramadan *et al*., 2006).

Cold press method was implemented to obtain VCO in this manufacturing company from where the samples were obtained for the research study. As from this study it was revealed that black colour speck formation is due to fungal contamination of *Aspergillus* sp. The moisture content, oxygen, temperature and oil as a nutritional source are positive factors for this fungal growth.

According to the study X2 (VCO samples heated up to 65˚C, 60 seconds and exposed to 253.7nm UV, 60 seconds) treatment was evaluated to have better performance having treatment in terms of moisture, TPC, YMC, SV, IV and FAP along with the improved antioxidant properties free of black speck formation. Hence, this treatment can be implemented in controlling

black speck formation in virgin coconut industry while improving product quality simultaneously maintaining within the standard APCC specifications.

## 4. Conclusions

Virgin coconut oil samples subjected to heat at 65˚C for 60 seconds and expose to 253.7 nm UV radiations for 60 seconds can be implemented as a remedial action to black speck formation. This treatment can be applied to control black speck formation in the virgin coconut industry while improving product quality whilst maintaining the quality parameters within the standard of APCC.

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# INVESTIGATING THE RHEOLOGICAL AND PHYSICAL BEHAVIORS OF YOGURT DURING STORAGE

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**ABSTRACT**

As a popular fermented dairy product, yogurt enjoys a special status among the consumers and, therefore, its quality is the great interest. Investigating the viscosity, physical properties and color of yogurt in the storage time are the necessary step for improving its quality. The aim of this study is to explore the changes of $L^*$and viscosity of yogurt with the changes of variables like temperature, loading speed, type of yogurt and storage period. These tests have been performed by using of axial test to measured viscosity, as a new method, on two types of yogurts, low-fat (1% fat) and high-fat (4.2% fat). The tests were performed at loading speeds of 40, 60 and 80 *mm*/*min* and temperatures of 4 and 25 ºC. The backward extrusion axial test device and the cyclic method have been employed to determine the viscosity of yogurts. The variance analysis results related to the data of $L^*$and yogurt viscosity became significant at a probability level of 1% for the main effects and some of interaction. By increasing the loading speed, a decreasing trend in viscosity was observed. Both yogurt types had a higher viscosity at the temperature of 4 ºC than at 25 ºC. The storage period didn't have a steady and consistent influence on viscosity. The viscosity of high-fat yogurt was greater than the viscosity of low-fat yogurt at both temperatures of 4 and 25 ºC

## 1.Introduction

Yogurt is one of the most extensively used fermented milk productions, which due to its high nutritional value has a significant effect on human health, and enjoys a special dietary status. In Iran, 833 units are involved in yogurt production and produce about 1.9 million tons of yogurts per year (Karazhyan and Salari, 2012). Yogurt is produced through the fermentation of some milk components by certain microorganisms. From a nutritional standpoint, yogurt is one of the best milk products. Due to its high amounts of calcium, vitamins, minerals, low fat content, and because of its favorable effect on health and longevity, yogurt has become a popular product for general public (Kowalski et al., 2000; Rosemont, 1990). Hence, yogurt is an excellent food product; and in the regions where people do not receive sufficient protein, yogurt can be the best supplementary product. Nowadays, various types of yogurts are produced in the dairy industry, including the low-fat and high-fat, probiotic, drinkable and frozen yogurts (Aqdaei et al., 2010; Fiszman et al, 1999).

In the rheological classification of foodstuff, yogurt is classified among the semisolid materials with thixotropic properties (Khalifeh et al., 2013). In order to control the texture quality and the useful shelf life of foodstuffs and also to design the relevant processing equipment, it is necessary to know the rheological properties of these materials. Fluids such as air and water exhibit simple behaviors which are independent of performed processes on them; while foodstuffs are more complex and their flow behavior may depend on earlier processes that have been carried out on them and the storage period times (Basim and Mohameed, 2006). The rheological properties and the textures of the fermented milk products are influenced by the initial combination of the raw milk, amount of dry material, heat treatment, type of the starting culture, incubation temperature, initial milk viscosity, fermentation kinetics and homogenization, on the other hand the properties of some milk productions like yogurt dependent on fat content, storage temperature, storage time etc. (Hardi and Slacanac, 2000; Jumah et al., 2001). An important issue regarding yogurt quality is the preservation of its nutritional properties as well as its physical, textural and sensory characteristics during the storage period. It has been realized that some changes occur in pH and acidity of yogurt during storage. These changes are closely related to the sensory changes and the fluidity behavior of yogurt (Karsheva et al., 2013). Studying the behavior of yogurt enables producers and marketer to predict the properties of the final products. To be able to estimate the rheological behavior of yogurt, an appropriate rheological behavior has to be selected (Mullineux and Simmons, 2008).

Recently some of researchers are focused on properties of dairy productions that mentioned here: the physical and chemical parameters and the rheological properties of yogurt during storage were investigated by Karsheva et al. (2013). In this research the effects of storage time on the pH, acidity, lactic acid concentration, and number of microorganisms, gel firmness and the rheological parameters of yogurt were measured. Yuguchi et al. (1989) performed cyclic tests on yogurt by using a stretching and pressing apparatus for foodstuffs, and obtained some of the yogurt properties including adhesiveness, firmness and cohesion. The effect of storage time on yogurt fluidity was analyzed and modeled by Basim et al. (2006) by means of a rotational viscometer. The samples were evaluated during 14 days of storage. The apparent viscosity was measured as a function of shear rate.

The effects of hydrostatic pressure on the rheological and textural properties of low-fat probiotic yogurts prepared from different starters were explored and evaluated by Penna et al. (2006). The rheological characteristics of low-fat, high-fat and sheep milk yogurts were investigated by Khalifeh et al. (2013). The chemical, organoleptic and rheological properties of two types of yogurts made from fresh cow milk and dry milk formula during a storage period of 21 days were evaluated by Karazhyan and Salari (2012). The power law model was selected to describe the flow behavior of these samples.

The aim of this research was to determine the effects of factors like storage period, storage temperature, fat content (%) and loading speed on yogurt viscosity and color. The novelty of this study was used of axial test device and backward extrusion technique to measuring viscosity of yogurts.

## 2.Materials and methods

In this study, two types of yogurt samples, low-fat (1% fat) and high-fat (4.2% fat), produced by the Iran's dairy company (*Pegah*) were obtained. The high-fat yogurt produced by Fresh cow milk, pasteurized cream and mixed lactic starter (*Lactobacillus* of *bulgaricus* species, *Esther Ptukukus salivarius* of *thermophilus* species), low fat yogurt produced by Fresh cow milk and the same starter. In order to prevent any type of shaking during transportation, the 1.0 kg samples were carefully packed in special containers. Then the packaged yogurts were transferred to a cold room at a temperature of 4 ºC and with 65% relative humidity. The viscosity of the samples measured by means

of the axial test device (Bbt1-Fro.5th.D14, Made in Germany) equipped with a force gauge (X Force Hp nominal Force: 500 N Capacity) and backward extrusion method in 3 cycle (Fig. 1).

In this experiment, a cylindrical probe with a 40 mm diameter was inserted into a cylinder with a 50 mm diameter and 60 mm height. Cylinder filled with yogurt up to a height of 40 mm. The probe was advanced to a depth of 30 mm (Fig. 1B) and then it was moved in reverse up to a certain height and this cyclical (reciprocating) movement with a course length of 30 mm was terminated in three cycles. The moving speeds of the probe were selected as 40, 60 and 80 mm/min and the penetration depth of the piston into the cylinder was chosen as 30 mm from the yogurt surface. In general, each type of yogurt was tested using combinations of the loading speeds and temperatures of 4 and 25 ºC and with three iterations for each test condition. The samples were evaluated at temperatures of 4 and 25 ºC and on the 1st, 7th, 14th and 21st days of the storage period. Eq.1 was used to determine the apparent viscosity of the yogurt samples with the backward extrusion method.

$$\eta = \frac{P \times (r_c - r_p)^3 \times (r_c + r_p)}{6 \times h \times (r_c^{\,2} + r_p^{\,2}) \times V_P} \qquad (1)$$

Where η is viscosity (Pa.s), P is pressure ($N/m^2$), $r_c$, $r_p$ and h are radius of the container, radius of the piston and height of the piston (mm) respectively, and $V_p$ is piston speed (mm/min).

The colors of the samples were measured by using a portable colorimeter (Model HP-2 made by China) at $L^*$, brightness, ($a^*$) redness-greenness and ($b^*$) blueness-yellowness values as color scales.

The mentioned device was calibrated by standard black and white plates. Before conducting the tests, the yogurt samples were kept at two temperature levels of 4 and 25 ºC.

In this research, data were analyzed by the factorial test in a totally random format, the effects of parameters like yogurt types, temperature levels and penetration speed of the probe on brightness ($L^*$) and viscosity were investigated.

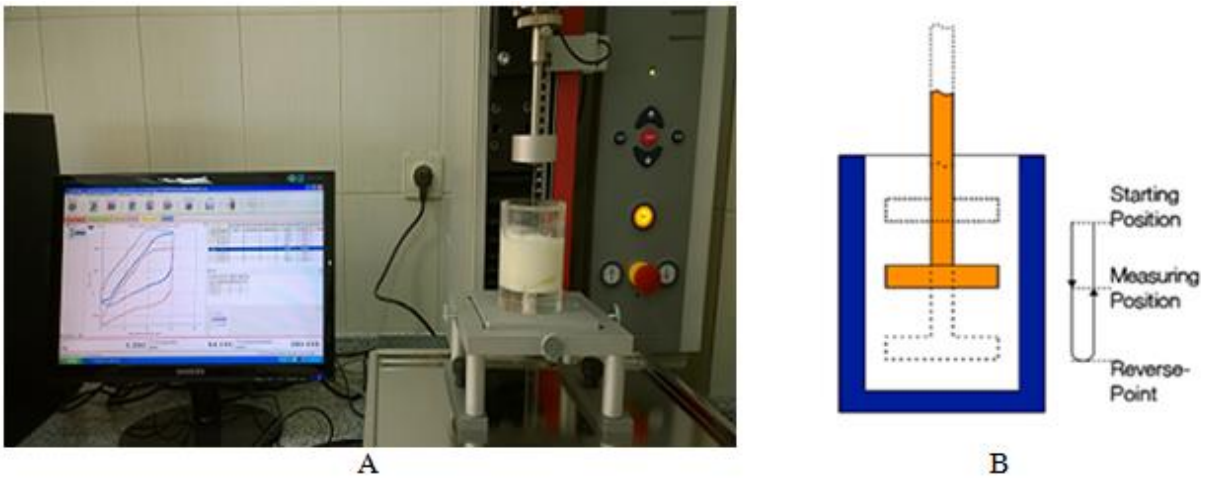


**Figure 1. A-** Universal testing machine, **B-** a schematic of the sample under loading

After determining the properties of each sample, the obtained data were normalized by means of the SPSS (19) software program and provided to determining the error variance. Also, the factorial test was employed to determine the effects of the independent variable on the dependent variables.

## 3.Results and discussions

The variance analysis results for the effects of temperature, loading speed, yogurt type and storage period on $L^*$ and viscosity of yogurt have been presented in Table 1. For $L^*$, the main effects of the yogurt types, temperature and storage duration and some of

the mutual effects became significant at a probability level of 1%. Also, in view of the variance analysis table, yogurt type, temperature, loading speed and storage duration, and mutual effect of yogurt type and pressing speed have significant effects (1%) on yogurt viscosity.

**Table 1.** Analysis of variance (mean square) for effects of temperature, yogurt type, day and loading speed of probe.

| **Source of variations** | **Degree of freedom** | **Sources mean-square** | |
|---|---|---|---|
| | | **Color index ($L^*$)** | **Viscosity** |
| Yogurt Type | 1 | 445.94** | 905.83** |
| Temperature | 1 | 148.72** | 1339.96** |
| Day | 3 | 98.7** | 126.7 |
| Loading speed | 2 | 0 | 4776.09** |
| Type × Temperature | 1 | 165.9** | 25.52 |
| Type × Day | 3 | 404.4** | 47.61 |
| Type × Loading speed | 2 | 0 | 138.8** |
| Temperature × Day | 3 | 284.8** | 38.5 |
| Temperature × Loading speed | 2 | 0 | 24.16 |
| Day × Velocity | 6 | 0 | 102.3 |
| Type × Temperature × Day | 3 | 1384.1 | 71.2 |
| Type × Temperature × Loading speed | 2 | 0** | 8.32 |
| Type × Day × Loading speed | 6 | 0 | 18.61 |
| Significant at 1% of probability levels** | | | |

The force-displacement diagram (high-fat yogurt, 1st day of the storage period and temperature of 25 ºC) obtained with the backward extrusion test by using the cyclic method has been illustrated in Fig. 2, as an example. Table 2 shows the changes in the viscosities of low-fat and high-fat yogurts at temperatures of 4 and 25 ºC throughout the storage period. Viscosity has increased by fat content increasing, so that the maximum amount of viscosity is observed in the high-fat sample at a loading speed of 40 mm/min and the least amount of viscosity was seen in the low-fat sample at a loading speed of 80 mm/min.

**Table 2.** Changes in the viscosities of low-fat and high-fat yogurts during the storage period at temperatures of 4 and 25 ºC.

| Yogurt type | Velocity loading | Viscosity | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 4 | | | | 25 | | | |
| | | 1 | 7 | 14 | 21 | 1 | 7 | 14 | 21 |
| Low fat | 40 | 31.8± 1.2 | 32.3± 1.5 | 34.7± 1.4 | 31.6± 1.75 | 25.8 ± 1.87 | 24.42± 1.58 | 28.22± 1.47 | 26.9± 1.87 |
| | 60 | 28.53± 1.5 | 27.14± 2.5 | 25.6± 0.5 | 21.9 ± 1.57 | 15.8 ± 0.67 | 13.32± 1.45 | 26.85± 1.57 | 18.03± 1.47 |
| | 80 | 19.59± 0.9 | 14.7± 2.1 | 17.9± 1.89 | 17.18± 1.64 | 12.3± 2.15 | 10.9± 1.87 | 12.7± 1.47 | 10.5± 1.32 |
| High fat | 40 | 35.33± 1.42 | 46.2 ± 1.54 | 46.4± 1.87 | 47.7 ± 1.87 | 35.09± 1.57 | 31.05± 1.54 | 33.73± 1.45 | 36.4± 1.54 |
| | 60 | 29.81± 1.2 | 29.3 ± 1.87 | 34.7± 1.57 | 28.8 ± 1.88 | 21.4± 1.54 | 19.42± 1.78 | 21.2± 1.64 | 23.3± 1.34 |
| | 80 | 25.65± 1.17 | 20.5 ± 1.25 | 23.1± 0.84 | 21.8 ± 1.24 | 15.6± 1.24 | 14.83± 1.47 | 16.96± 1.54 | 18.2± 1.54 |

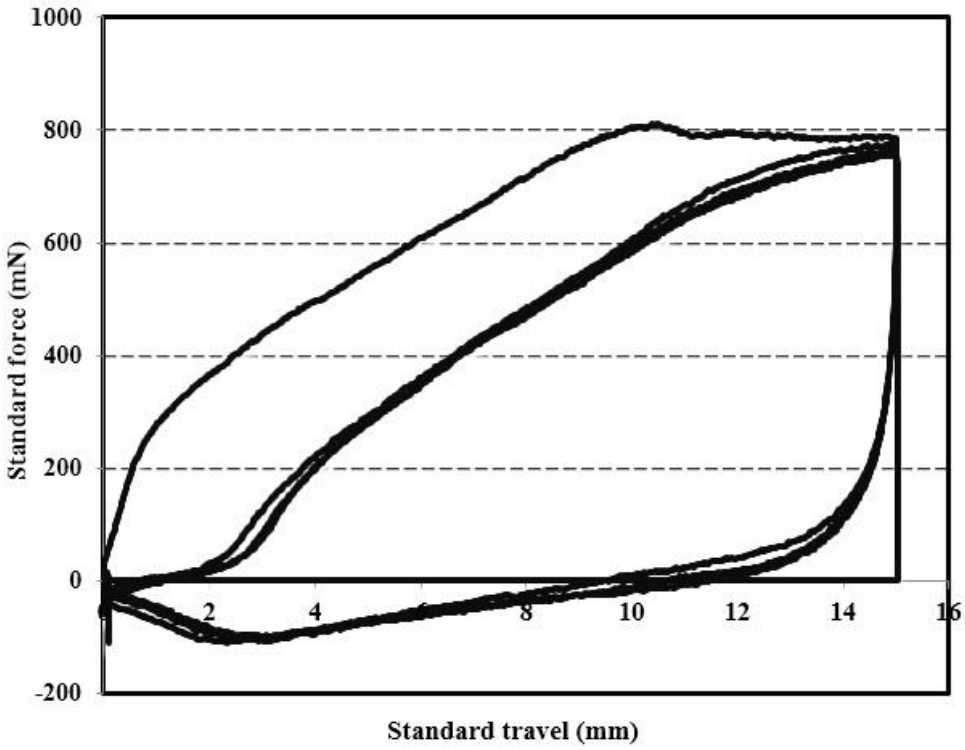


**Figure 2.** Force-displacement (yogurt, day and temperature 25 °c) in the extrusion test beck ward

### 3.1. Viscosity

Viscosity is influenced by numerous factors such as the special constituent compounds of fat and stabilizer, type and quality of components, the processing mechanism of the mixture, concentration and temperature (Rezaei et al., 2011). The statistical analysis results showed that the storage period didn't have a significant effect on viscosity; while the loading speed, temperature and the type of yogurt had a significant effect on viscosity at a probability level of 1% (Table 1). Fig. 3 shows the viscosity-loading speed diagram for the low-fat and high-fat yogurts at the 4 ºC. According to this diagram, yogurt viscosity decreased by increasing the compressing probe speed.

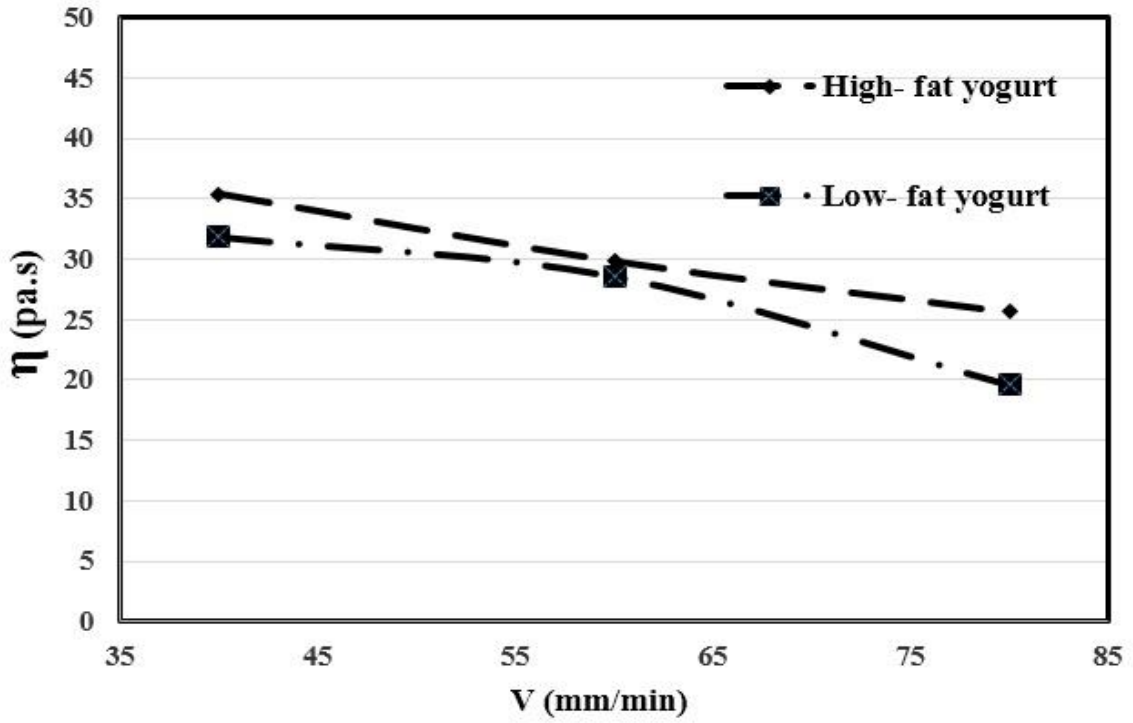


**Figure 3.** Effect of loading speed on the viscosity of yogurt samples at the 4 ºC temperature

The main effects (yogurt type, temperature and loading speed) and the mutual effects (yogurt type × loading speed) became significant on viscosity at a probability level of 1% (table 1); while other mutual effects did not have a significant influence on viscosity at the 1% probability level.

The diagram of experimental values versus predicted values by the regression model have been illustrated in Fig 4. According to this figure, there is an acceptable correlation ($R^2$ = 0.83) between the experimental and predicted values of viscosity. The models for the effects of variables on viscosity have been presented in Table 3. As indicated by the model coefficients, the loading speed and the temperature have a negative effect on viscosity, while temperature is more effective than loading speed; temperature increasing has a higher influence on the reduction of viscosity. The higher fat content has a positive influence on viscosity, and the increasing in fat content raises the viscosity of the samples. Similar results have been reported by Augustin et al. (1999). They reported when fat content increased, the amount of syneresis decreased in the samples, and then textural properties improved. The improvement of textural properties with the increasing of fat content may be due to the increasing in the total amount of dry matter and consequently may make the product firmer and raises its viscosity, because a greater amount of dry matter makes the gel lattice more stable and increases the water bonding capacity.

**Table 3.** Multiple regression equation of the "Viscosity" relation to velocity (V), day (D), temperature (T) and yogurt type (Ty) as independent variables.

| **Model*** | **$R^2$** |
|---|---|
| Viscosity = (-0.266 × V) - ( 0.366× T ) + (12.68 × Ty) – (0.109 × Ty × V) + 37.04 | 0.83 |
| *: Minimum probability threshold P ≤ 0.01 | |

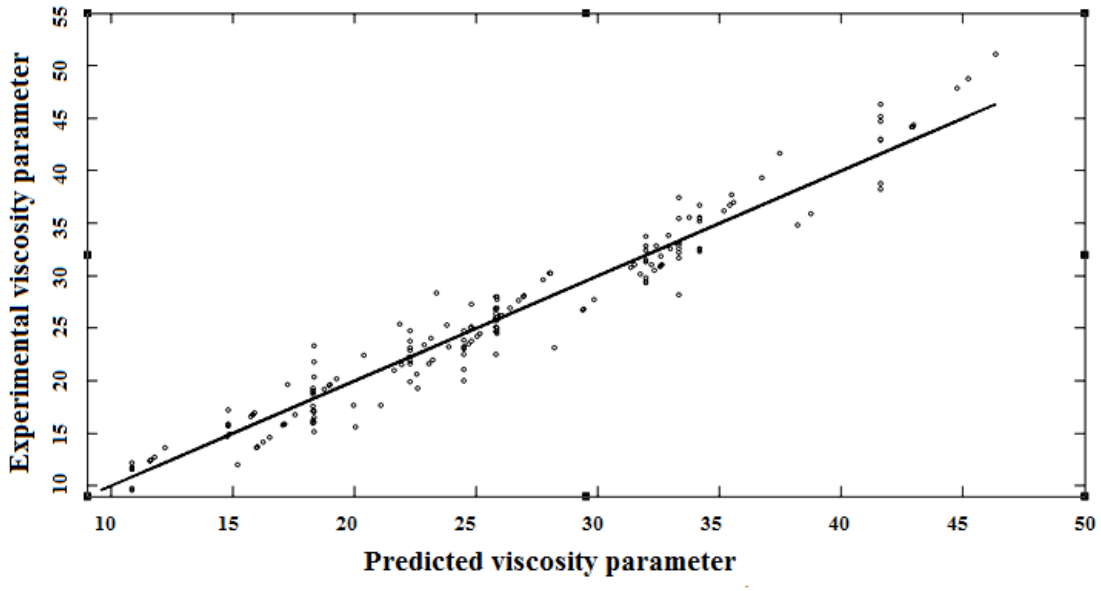


**Figure 4.** The predicted values of the viscosity parameter, "η" (PA.S) versus viscosity parameter experimental

### *3.1.1. Effects of loading speed and yogurt type on the viscosity of yogurt during storage at the 4 ºC temperature*

In view of Table 1, also at storage temperature of 4 ºC, the pressing speed of probe and yogurt type has a significant effect on the samples viscosity at a probability level of 1%. By evaluating the mean values and also consideration of Fig. 5, it is observed that, when the pressing speed of the probe throughout the storage period increased, the viscosities of the high-fat and low-fat yogurts decreased. In view of Fig. 6, at the 4 ºC temperature, when the pressing speed of the probe increased from 40 to 80 mm/min, the amount of low-fat yogurt viscosity decreased by 12.26, 17.6, 16.8 and 14.5%, respectively, for the 1st, 7th, 14th and 21st days of the storage period and the amount of high-fat yogurt viscosity decreased by 9.7, 25.4, 23.28 and 25.94% for the 1th, 7th, 14th and 21th days of the storage period, respectively.

The experimental results indicate that the high-fat samples have a higher viscosity than the low-fat samples. This increasing of viscosity is directly related to the increasing of mucilage concentration, because free water molecules in the samples bonded by the hydrocolloids. Akin et al. (2007) reported an increasing in the viscosity of yogurt and frozen yogurt by increasing in the concentration of inulin (as a stabilizer and fat substitute).

Among all the samples, the highest and lowest viscosities were observed in the high-fat samples at 4 ºC on the 21st day of the storage period and in the low-fat samples at 25 ºC on the 21st day of the storage period, respectively. With the prolongation of the storage period, the viscosities of the samples decreased, but this reduction did not have a consistent trend, which is due to the rearrangement of proteins and the changes occurring in the protein-protein bonds (Sahan et al., 2008). Similar findings were reported on this subject by Karsheva et al. (2013).

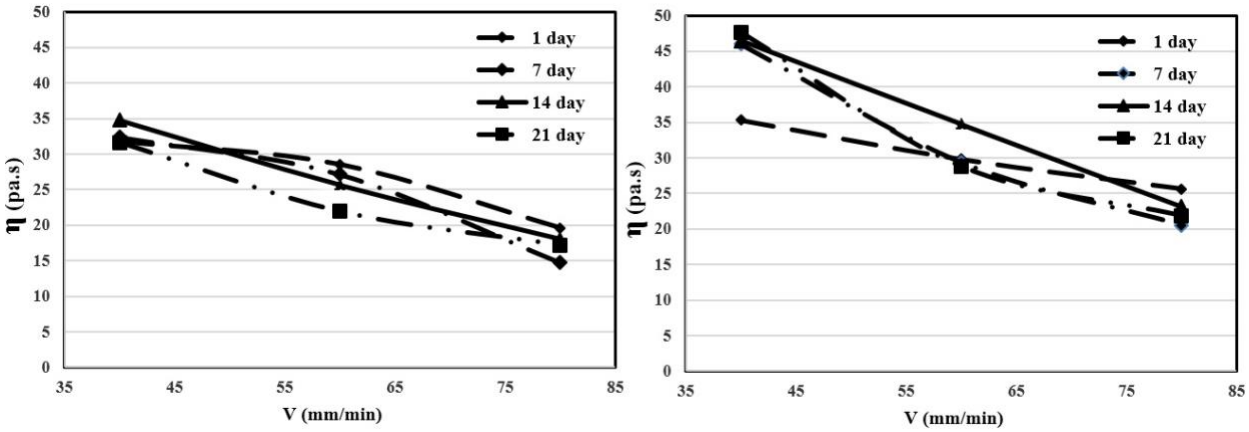


**Figure 5.** Changes in the viscosities of low-fat and high-fat yogurt samples during storage at the 4 ºC temperature

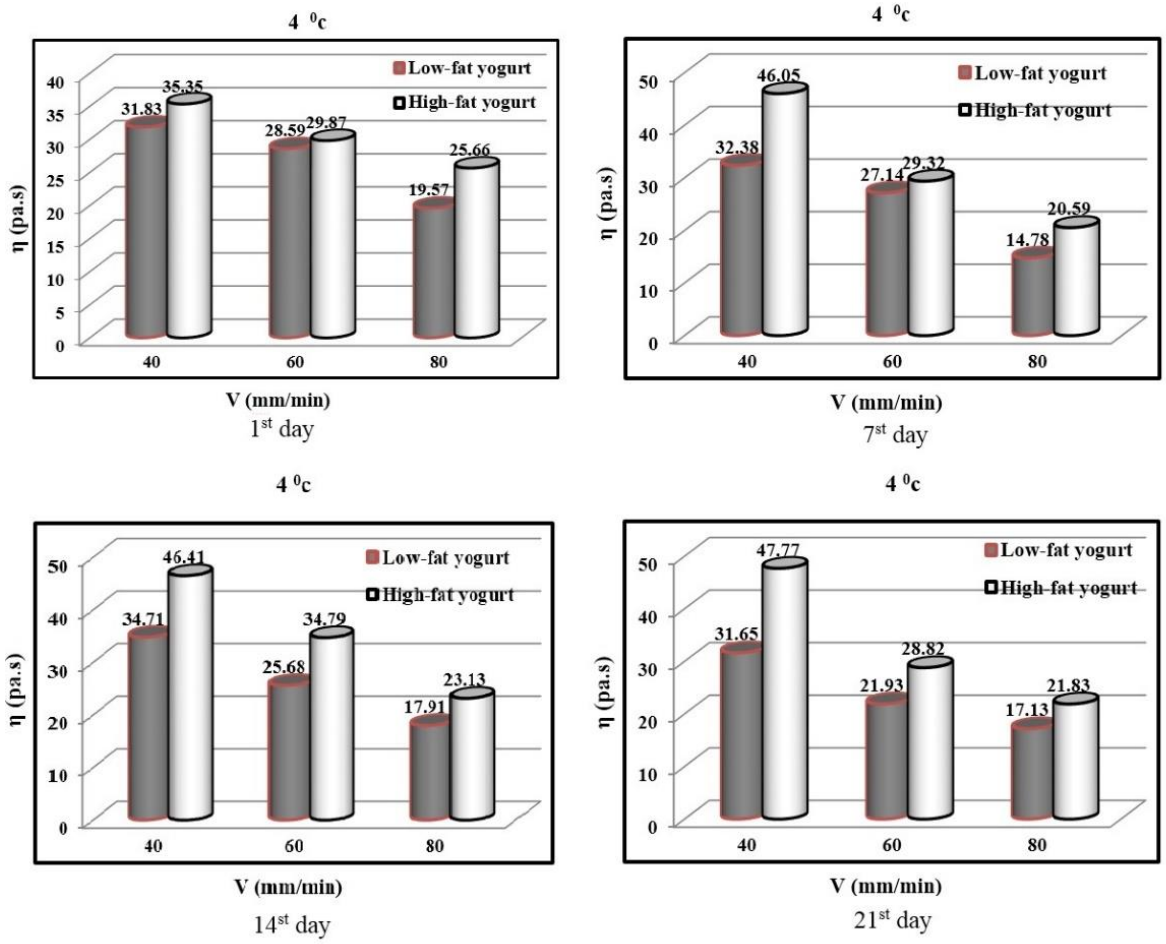


**Figure 6.** Effects of loading speed and yogurt type on the viscosity during storage period at the 4 ºC temperature

### *3.1.2. Effects of loading speed and yogurt type on the viscosity of yogurt during storage at the 25 ºC temperature*

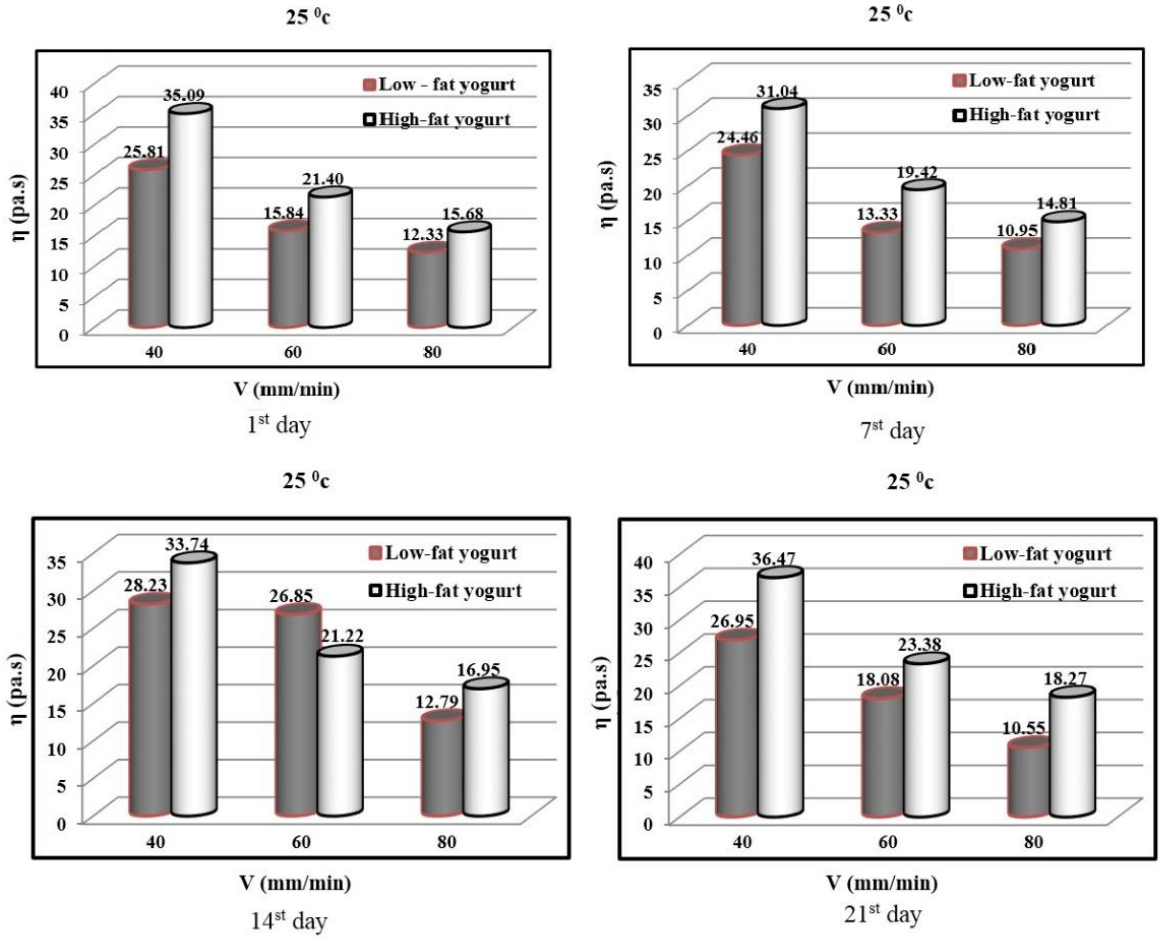


**Figure 7.** Effects of loading speed and yogurt type on the viscosity during storage period at the 25 ºC temperature

With regards to Table 1, at a storage temperature of 25 ºC, the pressing speed of probe and the yogurt type have a significant effect on the viscosity of samples at a probability level of 1%. By comparing the mean values, and in consideration of Fig. 7, it can be concluded that yogurt viscosity decreased with increasing in the pressing speed of the probe. According to Fig. 7, at the 25ºC temperature, with the increasing in pressing speed of the probe from 40 to 80 mm/min, the amount of low-fat yogurt viscosity decreased by 51.48, 13.13, 15.44 and 16.4%, respectively, for the 1st, 7th, 14th and 21st days of the storage period and the amount of high-fat yogurt viscosity decreased by 19.41, 16.23, 16.52 and 18.2% for the 1st, 7th, 14th and 21st days of the storage period, respectively. Viscosity decreased with increasing in the loading speed, which is probably due to the breakup of yogurt structure as a result of loading speed (Fig. 7). At a low loading speed, viscosity is the cause of foodstuff consolidation, while at a high loading speed; the amount of viscosity indicates a product's viscosity at various stages of the process (Morris and Taylor, 1982). The semi-solid texture of yogurt is due to the development of a 3D protein matrix during the fermentation process (Hassan et al., 1996). In both samples, viscosity decreased with the increasing in the loading speed. Therefore, both samples display a softening behavior when the loading speed increased, which could be due to the breakup of the gel structure as a result of different loading speeds (Ahmedna and Goktepe, 2007) and also due to a weakened interaction

between the yogurt's lattice structures (Mohammeed et al., 2004). Similar findings have also been reported by Morris et al. (1982) under shearing conditions.

In general, under all circumstances, measurement viscosity of the high-fat yogurt was more than the viscosity of low-fat yogurt. Mohammeed et al. (2004) reported the same results, that increasing in yogurt total solid matter (fat content) has direct relation with viscosity. They stated that the increasing in the total solid matter of yogurt has definite effects on the increasing of viscosity; so that a sample with a higher amount of solid matter has a greater consolidation coefficient and a lower index (Mohammeed et al., 2004). Increasing the storage temperature of the samples from 4 to 25 ºC under similar conditions caused a dropping in the viscosity of these samples.

### 3.2. Effects of yogurt type, temperature, probe pressing speed, and storage period on L*

**Table 4.** Multiple regression equation of the "Color Index" relation to velocity (V), day (D), temperature (T) and yogurt type (Ty) as independent variables.

| **Model*** | **$R^2$** |
|---|---|
| Color = (0.336 × D) + (0.274 × T) – (7.29 × Ty) – (0.109 × Ty × V) – (0.014 × T × D) – (0.005 × T × V) + (0.003 × Ty × V × D) + 71.45 | 0.67 |
| *: Minimum probability threshold P ≤ 0.01 | |

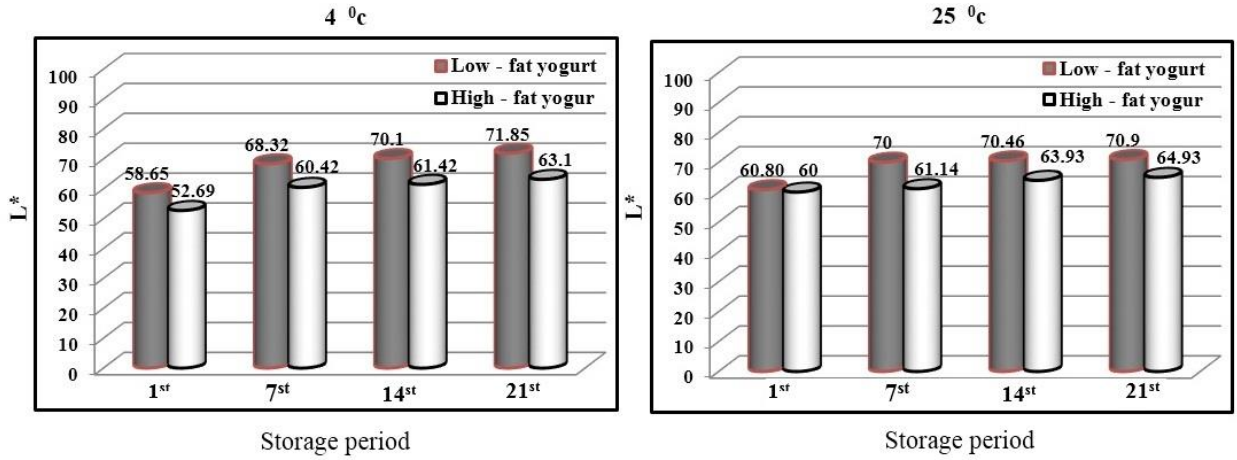


**Figure 8.** Effects of yogurt type, temperature and storage period on L*

Table 1 shows the results of variance analysis for the effects of factors like temperature, pressing speed of probe, yogurt type, and storage duration on L* of yogurt. The main effects (yogurt type, temperature, and storage period) and the mutual effects (yogurt type × temperature, storage period × yogurt type, storage period × temperature and yogurt type × temperature × pressing speed of probe) have significant relationship on the L* at a probability level of 1%.

The presented models for the effects of variables on L* have been shown in table 4. As the model coefficients indicate, temperature and storage time have a positive effect on L*, while storage time has a greater influence on the increasing of L*. The effect of yogurt type is negative; and increasing in the fat percentage caused a reduction in L*. The model coefficients have been listed in Table 4.

By comparing the mean values, and in consideration of Fig. 8, it can be concluded that yogurt L* increased with increasing in the temperature and storage period. According to Fig. 8, at the 4ºC and 25ºC

temperature, with the increasing in storage period from 1st to 21st, the amount of low-fat yogurt L* increased by 22.5% and 16.6%, respectively. According to Fig. 8, at the 4ºC and 25ºC temperature, with the increasing in storage period of from 1st to 21st, the amount of high-fat yogurt L* increased by 19.75% and 8.21%, respectively. As well as L* decreased with the increasing in the fat percentage (Fig. 8). $L^*$ Index indicates the brightness of food samples. The brightness of milk is due to the presence of colloidal particles such as fat corpuscles and Casein micelles and it has a favorable effect on consumer acceptance (Garcia et al., 2005). Then as previously was mentioned, the increasing of fat content reduced the $L^*$ index in yogurt samples. Similar results have also been presented by Garcia et al. (2005), which showed that by adding hydrocolloids to yogurt samples, the $L^*$ index has decreased. Staffolo et al. (2004) have reported a dropping in the brightness index with the addition of fiber to the samples, too.

## 4.Conclusions

In this research, the parameters of $L^*$ and yogurt viscosity were investigated. Also, the effects of two temperature levels (4 and 25 ºC), three pressing speeds of the probe (40, 60 and 80 mm/min), two types of yogurts (low-fat and high-fat) and the storage duration (21 days) on $L^*$ and yogurt viscosity were evaluated. Some of the main and mutual parameters had a significant effect on $L^*$ and yogurt viscosity at a probability level of 1%.

The results of the parameters effects on $L^*$ and yogurt viscosity ($\eta$) can be summarized as follows:

The increasing of probe speed was leads to the reduction of yogurt viscosity.

Yogurt viscosity generally was increased by the reduction of temperature from 25 to 4 ºC.

The storage duration did not have a significant influence on yogurt viscosity.

At both temperatures (4 and 25 ºC), the low-fat yogurt viscosity was less than the high-fat yogurt viscosity.

The high-fat yogurt had a smaller $L^*$ index compared to the low-fat yogurt.

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# RHEOLOGICAL, PHYSICAL AND SENSORY CHARACTERISTICS OF BREAD OBTAINED BY PARTIALLY REPLACING WHEAT FLOUR WITH HEN'S EGGSHELL POWDER

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**ABSTRACT**

Bread is one of the staple foods in many countries of the world. Currently, the consumption of bread made from wheat flour is of limited nutritional value. The aim of this investigation was to produce bread with high calcium content, performing different substitutions using eggshell powder in order to evaluate the effects it has on the product. The research began with the production of eggshell powder (HCH), and its proximal and microbiological composition. The eggshell powder was substituted in 3 different percentages (10%, 15% and 20%) in addition to a control group. The methodology included the conducting of a rheological study by evaluating parameters such as tenacity, extensibility, swelling index, dough's strength, tenacity/extensibility ratio and elasticity index. The rheological properties of the dough were evaluated with the Chopin alveograph. The physical characteristics (specific volume and height) were determined and the results showed no significant difference. On the other hand, the sensory evaluation was carried out using a hedonic scale of 5 points by 25 panelists; seven attributes were evaluated (color, smell, texture, taste, external appearance, internal appearance and overall appearance), in which there were significant differences.

## 1.Introduction

Nowadays, different food products for children are being developed. These are mainly fortified and nutritious products, within which we can find bakery products made by replacing wheat flour with flour of tubers, of cereals or of native grains, which increases their nutritional value (Obrego *et al*., 2013).

The World Health Organization (WHO) indicates that 80% of the world's population, that is, more than 2/3 (of 4 billion people) use traditional bread as a basic daily diet. The INEI - National Household Budget Survey (2009) – states that the average annual per capita consumption of bread is 24 kg/person, in comparison to the 2018 sectoral report of the Institute of Economic and Social Studies (IEES) which states that the average annual per capita consumption of bread in Peru is 35 kg/person, showing an increase of 11 kg/person in recent years.

A hen's eggshell is an excellent source of calcium. It is constituted in 96 % by calcium carbonate, and less by other minerals such as phosphorus and magnesium, which facilitate its absorption (Alais *et al.*, 1990).

In addition, fortification with calcium has been shown to be an economic way of obtaining additional calcium (Keller *et al.*, 2002). Moreover, the human body cannot produce adequate amounts of calcium without external support. Additionally, it is daily lost through hair, skin, nails, sweat, urine, and feces. This calcium loss must be replaced, or the body will take calcium from the bones to perform other functions (Kessenich, 2008).

On the other hand, in recent years environmental pollution rates have increased, causing a major problem for the planet and therefore for humanity. Large amounts of organic waste are discarded daily. The eggshell is classified as a waste material by the food industry, therefore it is not used despite having different properties, its calcium content being one of the most important of them.

For this reason, the present study aimed to investigate the rheological characteristics to evaluate the behavior of the dough, as well as the physical and sensory characteristics of the final product to determine its effect on it.

## 2. Materials and methods

### 2.1. Obtaining eggshell powder

Eggshells were collected from different sources such as restaurants, dealerships, bakeries, food trucks, etc. They were washed and disinfected with a concentration of 100 ppm for 5 min. Then they were dried at 120°C for 60 min. Afterwards, the shells were ground and passed through a mesh sieve for 10 min with an opening of 106 um.

### 2.2. Chemical and microbiological analysis

Proximal and microbiological analysis of the eggshell powder were determined by official methods such as moisture (NTP Method 209.085), ash (AOAC Method 2.173), fat (NTP Method 209.093), proteins (AOAC Method 2.057), fiber (NTP 209.074 method) and carbohydrates (AOAC Method 31.043) and salmonella (6579-1: 2017. Microbiology of food chain - Horizontal Method for the Detection, enumeration and serotyping of Salmonella. - Part 1: Detection of Salmonella spp).

### 2.3. Rheological analysis

The Chopin alveograph was used to describe the dough's resistance to the extention caused during fermentation in the different substitutions (0%, 10%, 15% and 20%). The parameters obtained were: tenacity (P), extensibility (L), inflation index (G), strength (W), tenacity/extensibility ratio (P/L) and elasticity index (ie), following the AACC 54-30.02 standard *(Peña et al. 2008).*

### 2.4. Bread production

The method used for the production of the product was the direct method. The loaves were prepared according to the standardized formulation with a slight modification of Mesas and Alegre (2002). The wheat flour was replaced by eggshell powder (EP) in 3 different percentages which were 10% (F1), 15% (F2) and 20% (F3), and compared with a blank sample with 0% (F0) EP substitution. The kneading lasted 5 min. and 50 gr were weighed for each bread. The bread was baked at 180°C for 15 minutes. The loaves were then removed, cooled and stored for further study.

### 2.5.Determination of the physical characteristics of bread

The bread was evaluated at room temperature after 30 minutes of being removed from the oven based by preliminary tests. The weight was determined by means of a precision analytical balance with a maximum capacity of 300 g and sensitivity of 0.1 g (Henkel – model BQ1001). For the determination of the specific volume, data were collected using the 10-05 of the AACC (2000) method proposed by Lainez et

al. (2008). Each parameter evaluated was measured with three repetitions.

### 2.6. Sensory analysis

Sensory evaluation was conducted with semi-trained judges consisting of 25 panelists, who evaluated the following sensory attributes: color, smell, texture, taste, external appearance, internal appearance and general appearance proposed by Larmond (1977).

### 2.7. Statistical Analysis

Data were statistically evaluated by a variance analysis (ANOVA) and the Tukey test with a reliability level of 95%, using the statistical software Minitab 18.

## 3.Results and discussions

### 3.1. Physical-chemical and microbiological analysis

The physical chemical characterization of the eggshell powder as a raw material used in the production of bread for cultivation is shown in Table 1.

**Table 1.** Proximal composition of eggshell powder

| Analyses | Results (%) |
|---|---|
| Moisture | 0.68 |
| Ash | 92.61 |
| Fat | 0.10 |
| Proteins | 5.34 |
| Fiber | 0.07 |
| Carbohydrates | 1.20 |

In the eggshell powder there was moisture (0.68%), ash (92.61%), fat (0.10%), proteins (5.34%), fiber (0.07%), and carbohydrates (1.20%). Similarly, Osonwa et al. (2017) states that eggshell powder has a humidity of 0.68 %, a value similar to that obtained in the present work. According to Velásquez and Obando (2017), humidity influences the conservation of quality since it is one of the factors of acceleration of chemical reactions, enzymes and growth of microorganisms.

On the other hand, the ash content in the eggshell powder was 92.61 %. This concentration is high due to the high content of minerals present, mostly calcium carbonate. Walton et al. (1973); Hassan (2015) and Ray *et al.* (2017) mention that the ash content in eggshell powder is 91.1 %, 90.2 %, 93.62 %. Burley and Vadhera (1989) and Shwetha *et al.* (2018) report that these differences may be related to nutrition and bird habitation.

**Table 2.** Microbiological analysis of eggshell powder

| Determination | Results | Units |
|---|---|---|
| Escherichia coli | <10 | UFC/g |
| Mohos | <10 | UFC/g |
| Salmonella spp. | Ausencia | En 25 g |

The table above shows that the count of Escherichia Coli and molds is less than 10 in UFC/g, this being a permissible parameter according to the Sanitary Standard that establishes the Microbiological Criteria of Health Quality and Safety Food and Beverages Human Consumption R.M. N°591-2008-MINSA. Detection of Salmonella spp. in the sample is negative showing to be a harmless product.

### 3.2. Moisture of substitutions by eggshell powder

Humidity is related to the water content necessary for the production of bread. As the humidity of the flour decreases, the absorption of water increases. This means that as wheat flour is replaced with eggshell powder, water absorption increases. This may be due to the fact that eggshell powder is a hydrophilic filling that

can absorb more water (Shuhadah and Supri, 2009).

**Table 3.** Moisture of flour according to the substitution

| WF (%) | EP (%) | Moisture (%) |
|---|---|---|
| 100 | 0 | 14.25 |
| 90 | 10 | 13.71 |
| 85 | 15 | 13.62 |
| 80 | 20 | 13.24 |

Legend: Where, WF= Wheat Flour; EP= Eggshell Powder

In the table above, it is noted that the humidity decreased as the substitution increased, presenting a behavior inversely proportional. These values are within the parameters as indicated in CODEX STAN 152 (1985). The moisture requirement for bread's wheat flour should not exceed 15.5 %. The moisture data in the table are necessary for the determination of the rheological parameters shown below.

### 3.3. Rheological characterization

**Table 4.** Mass parameters obtained from Chopin's alveograph

| Parameters | Partial substitution of WF by EP | | | |
|---|---|---|---|---|
| | F0 | F1 | F2 | F3 |
| P (mm $H_2O$) | 158 | 121 | 113 | 106 |
| L (mm) | 52 | 59 | 52 | 42 |
| G | 16 | 17.1 | 16 | 14.4 |
| W ($10^{-4}$ J) | 343 | 277 | 206 | 196 |
| P/L | 3.04 | 2.05 | 2.17 | 2.52 |
| Ie (%) | 59.4 | 56.3 | 39.5 | 54.2 |

Legend: Where, F0= White group, F1= Experimental group of 10% substitution, F2= Experimental group of 15% substitution, F3= Experimental group of 20% substitution, P= tenacity, L= extensibility, G= Inflation rate, W= Mass strength, P/L= tenacity/extensibility ratio, ie= elasticity index.

The substitution of flour influences the rheological characteristics of the dough and this is mainly due to the weakening of the gluten net (Ho et al., 2013) which is due to the substitution of wheat flour with eggshell powder. The results of the dough's parameters obtained from the Chopin alveograph based on the substitution of eggshell powder in different percentages are shown in Table 3.

#### *3.3.1. Tenacity (P)*

The tenacity of the dough refers to the dough's resistance to rupture and deformation. In addition, the value of P serves as a reference to the ability of the dough to retain gas (De la Vega, 2009; Vázquez, 2009; Wang *et al*., 2002).

Toughness depends on the glutenin content of wheat flour (De la Vega, 2009; Perego *et al*., 2002; Wang et al., 2002). Wheat flour, when partially replaced with eggshell powder, decreased the amount of these storage proteins, thus reducing the resistance to dough breaking (tenacity). This is because eggshell powder has a high content of ash, due to its composition of calcium carbonate. Ferreras (2009) indicates that ash is composed mostly of fiber, and consequently fiber is not composed of proteins, therefore, the higher ash content, the less amount of proteins, affecting the formation of gluten.

According to the requirements of the Uruguayan Institute of Technical Standards (UNIT), standard 951:94 (1994), the recommended value of tenacity for a baking flour should be in the range of 100 to 130 mmH2O. The tenacity value for F0 (100 % WF) shows that it is outside the recommended range, possibly because of the dietary additives of wheat flour used as raw material for this study. For example, the dough's tenacity increases through the use of glucose oxidase enzyme (Steffolani *et al*., 2011).

The tenacity of the different substitution percentages (10 %, 15 % and 20 %) is within the range set out in standard 951:94 (UNIT), showing that a good dough's resistance to rupture and deformation is obtained.

#### *3.3.2. Extensibility (L)*

Extensibility refers to the dough's ability to be stretched (Banu *et al.*, 2012) and depends on the gliadins present in wheat flour (De la Vega,

2009; Gómez, 2011; Perego *et al.,* 2002). According to the requirements of the Uruguayan Institute of Technical Standards (UNIT), standard 951:94 (1994), the recommended extensibility value for a bakery flour should be in the range of 100 to 130 mm.

The results obtained show that the values are below the recommended and this can be attributed to the wheat flour's additives used as raw material. For example, the enzyme glucose oxidase decreases the dough's extensibility (Preedy et al., 2011; Steffolani et al., 2011).

According to the study carried out by Kajishima et al. (2001), which produced French bread with calcium sulphate enriched flour, the extensibility of wheat flour without substitution was 38 mm, flour with 50 % IDR (Recommended Daily Intake) of Ca sulfate was 47 mm and flour with 100 % of Ca sulfate IDR was 38 mm. This shows that its results are also below the recommended value according to Standard (UNIT) 951: 94 (1994).

***3.3.3. Rate of swelling (G) Inflation rate***

Ferreras (2009) maintains that the rate of swelling is represented by the volume of air necessary to cause the rupture of the balloon dough. Morales (2014) indicates that G provides a value proportional to extensibility.

Hassan (2015) mentions that replacing wheat flour with sources of calcium reduces the amount of amylose and amylopectin, which are the main inflating components. The calcium particles found in EP are deposited within the structure of the starch gel causing changes in the structure. So higher levels of calcium substitution lead to smaller sizes of pores or air cells.

***3.3.4. Dough's strength (W)***

Dough's strength refers to the work necessary to deform the bread dough (Fálder, 2002). Polymeric proteins (glutenins) give strength to the wheat dough (Shewry *et al.,* 1992; Belton, 1999), meaning that there is a directly proportional relationship between the glutenin content and the dough's strength (Islands *et al.,* 2005). The gluten network is responsible for forming a viscoelastic dough, but by decreasing proteins the development of this network is hindered, and the dough's strength decreases (Mohamed *et al.,* 2010) according to the data obtained in Table 3.

Shewry and Halford (2002) and De la Vega (2009) mention that the dough's strength (W) must be between 200 and 300 10-4 J. Unlike Monleón and Collado (2008) who say that for fermented baked products, flour should be used between 180 and 200.

The substitution rates were found within the range recommended by Shewry and Halford (2002) for F1 (10 % EP) and F2 (15 % EP), while F3 (20 % EP) was found within the value recommended by Monleón and Collado (2008).

According to Quaglia (1991), if baking flour has W greater than 250, it is recommended to be used in mixtures with other flours. According to research work F0 (100 % EP) submitted 343 10-4 J, which confirms that eggshell powder can work with wheat flour in bread production.

The study carried out by Kajishima *et al.* (2001) obtained that the dough's strength was 250 (10-4 J) with 50 % IDR of calcium sulphate and 280 (10-4 J) with 100 % IDR of calcium sulphate, showing that its results are also within the recommended value according to Shewry and Halford (2002).

***3.3.5. P/L: tenacity/extensibility ratio***

The tenacity/extensibility ratio depends on the relationship between glutenins/glycine (De la Vega, 2009). It also indicates the balance of the dough (Ferreras, 2009).

The P/L scale is dimensionless and can range from 0,1 to 6 (Espitia *et al.,* 2003) or 0,5 to 1,5, with a balanced value of 1,1 for baking (Banu *et al.,* 2012; Martínez *et al.,* 2014; Vázquez, 2009).

The P/L values obtained were higher than recommended. This can be attributed to food additives used in wheat flour, such as the enzyme glucose oxidase that catalyzes the oxidation of glucose by generating o-D-gluconolacone and hydrogen peroxide. The latter oxidises the thiol groups of gluten and forms disulfide bonds that make up the dough (Steffolani *et al.,* 2011). With the values obtained in Table 3 the flour was classified as

strong and tenacious according to Espitia *et al.* (2003).

According to the study carried out by Kajishima et al. (2001) the values obtained from the tenacity/extensibility ratio were 3.45 (standard flour), 2.72 (flour with 50% of the Ca sulfate IDR) and 3.47 (flour with 100% of the Ca sulfate IDR) for French bread, showing that these values are also outside the recommended range in greater proportion in comparison to the results obtained experimentally.

***3.3.6. Ie: Elasticity index.***

The elasticity index is expressed as a percentage, which means that some of the curves, after reaching the maximum pressure, have a very abrupt fall and show a dough with little elasticity, On the contrary, a curve with a little steep fall will give us a more elastic dough, optimal for baking according to Ferreras (2009).

## 3.4. Physical Characteristics of bread

**Table 5.** Physical Characteristics of bread

| Substitution | Weight (g) | Volume ($cm^3$) | Specific Volume ($cm^3/g$) | Height (cm) |
|---|---|---|---|---|
| F0 | 41.70 | 307.39 | 7.36 | 4.11 |
| F1 | 39.66 | 289.74 | 7.31 | 4.05 |
| F2 | 38.86 | 275.91 | 7.10 | 3.83 |
| F3 | 38.62 | 270.03 | 7.01 | 3.81 |

***3.4.1. Bread's weight***

The results, with three repetitions, indicated that the average weights of the bread of the different formulations decreased as the substitution was greater; however, they did not show significant difference ($p > 0.05$). The average weight of F0 (100 % WF, 0 % EP) is 41.70 g and F3 (80 % WF, 20 % EP) is 38.62 g, these two values represent the maximum and minimum bread weight respectively, the difference between these formulations' ranges around 7.37 %. A similar result was obtained by Martinez et al. (2014) who used banana flour in two percentages 0 and 10 %. This type of dough does not allow gas retention because the dough shows resistance to expansion, reflecting the bread's low weight.

***3.4.2. Volume of the bread***

As the substitution level of wheat flour with eggshell powder increased, the bread decreased in volume; however, it did not show a significant difference ($p > 0.05$). This is due to the eggshell's lack of gluten. Previous research, such as that carried out by Pineda (1977) where tarwi flour was used in a panification trial, concluded that there was a descending variation in the volume of the replaced bread. The volume of the center of the bread is a very important parameter for consumers, because it is related to the perception of a light but not dense product, which means that the center of the bread's density and volume characteristics are associated with a specific bakery product (Hathorn *et al.*, 2008).

***3.4.3. Specific volume***

The results showed that the specific volume decreased according to the degree of substitution; however, they did not show any significant difference ($p > 0.05$). Seguchi *et al.* (2007), Lorenz and Coulter (1991) found that the specific volume decreases as the amount of wheat decreases, which was also observed in the present study.

Kajishima *et al.* (2001) analysed the effects of the addition of calcium sulphate on the physical characteristics of bread. It showed that, although the specific volume was not significantly modified, the addition of the highest dose (100 % of the Recommended Daily Intake) caused a slight decrease in its external characteristics. The specific volume of bread is one of its most important visual characteristics, strongly influencing the consumer's choice. It is therefore a fundamental parameter when assessing bread's quality (Hager and Arendt, 2013).

***3.4.4. Bread's height***

The bread that obtained the highest height was F0 (100 % WF, 0 % EP) with 4.11 cm while F3 (80 % WF, 20 % EP) obtained the lowest height with 3.81 cm. Statistical analysis revealed that there is no significant difference ($p > 0.05$). The height of the bread decreased as the percentage of substitution with eggshell powder

was increased, showing an inversely proportional ratio.

The height depends mainly on the gluten network and the production of CO2 during the dough's fermentation stage (Chinma *et al.*, 2014). Based on the results obtained from Table 4, it was observed that the gluten network was weakened as the percentage of replacement with eggshell powder was increased, thus affecting gas retention and the bread's height.

### 3.5. Sensory evaluation

The seven attributes evaluated (color, smell, texture, taste, external appearance, internal appearance and general appearance) showed significant difference (p 0,05) compared to the formulation. In other words, the substitution of eggshell powder influenced the sensory characteristics of the bread.

#### *3.5.1. Color*

Color, particularly color uniformity, has an important effect on the consumer's acceptability (Vasquez, 1982).

The possible cause of the significant difference could be due to the baking process, because it causes color changes. This is associated with a complex phenomenon commonly known as 'browning' or the Maillard reaction (Toaquiza, 2012).

#### *3.5.2. Odor*

The presence of a difference of smell in the bread could be due to the characteristic smell of the eggshell. The toasted smell of bread depends on the formation of active flavour compounds in the crust during the cooking process. The compound with the greatest impact on the smell of bread crust is the 2-Acetyl-1-pyrroline (Belitz and Grosch, 2009). It is an aroma and flavor compound that gives the usual smell to white bread.

Odor is a very important parameter in the acceptability of any product, but this characteristic is influenced by the raw materials used such as flour, flakes and bran. These inputs are not food that transmit strange or unpleasant odors to the product, as Coloma points out (2000).

#### *3.5.3. Texture*

Bradauskiene et al. (2017) found that to a higher content of eggshell powder, the texture of the bread is considerably reduced, since the granulometry was perceptible when chewing.

The possible cause may have been the characteristic texture of the eggshell powder, as Li-Chan and Kim (2008) indicate. They mention that the proteins in the eggshell matrix influence the crystalline growth process by controlling the size, the shape and orientation of the calcite crystals, thus affecting the texture and biomechanical properties of the eggshell.

In addition, Brun *et al.* (2013) point out that the most appropriate way to use the eggshell as a source of calcium is as powder added to pizza, bread or spaghetti, since it presents small changes in texture and no changes in flavor.

#### *3.5.4. Taste*

The result evaluated in taste showed a significant difference, possibly due to the texture provided by the eggshell powder that is perceived at the time of chewing the bread. Bradauskiene *et al.* (2017) made bread with added eggshell powder, finding that in all calcium concentrations the bread's taste score decreased in comparison to the control.

#### *3.5.5. External and internal aspects*

The external aspect was influenced by the color attribute, due to Maillard's reaction, presenting different appearances such as very light and dark colors in the bread.

#### *3.5.6. Internal aspects*

The internal aspect is reflected in the center of the bread, which is related to the amount of water added to the dough and the possible use of special flours in the process. But the most determining factors are the quantity and quality of the protein (Kihlberg, 2004).

In the process of making bread, when increasing the percentage of substitution, the center changed its sensory characteristics due to its low protein content.

#### *3.5.7. General appearance*

Salem *et al.* (2012) studied the influence of the addition of eggshell powder at 10 % and 20 % as a source of calcium fortification on the sensory properties of butter cake. They reported

that no statistically significant differences were detected between the unfortified cake and the fortified cake with 10 % and 20 % eggshell powder for color and overall acceptability, while significant differences were found in texture, smell, taste and appearance.

Piscoya (2002) mentions in his research that bread fortified with calcium had a good acceptability.

**Table 6.** Average score of sensory characteristics by attribute of processed bread

| Sensory characteristics | Partial substitutin of WF by EP | | | |
|---|---|---|---|---|
| | F0 | F1 | F2 | F3 |
| Color | 4.6 | 3.4 | 3.36 | 2.4 |
| Smell | 4.16 | 3.4 | 3.4 | 3.04 |
| Texture | 4.16 | 3.28 | 3.52 | 2.52 |
| Taste | 4.36 | 3.44 | 3.84 | 3.12 |
| External aspect | 4.8 | 3.72 | 3.52 | 2.6 |
| Internal aspect | 4.48 | 3.52 | 3.32 | 3.16 |
| General appearance | 4.72 | 3.8 | 3.52 | 3 |
| **Average** | 4.47 | 3.51 | 3.50 | 2.83 |

In Table 6, the results showed the averages of each sensory characteristic of a total of 25 panelists out of a total of 5 points per attribute. The different formulations (F0, F1, F2 and F3) obtained an average score of 4.47, 3.51, 3.50 and 2.83 respectively.

According to the averages shown by F1, F2 and F3 (Fig. 1), the values are lower than the formulation F0 (100 % wheat flour). However, it is necessary to consider that bread with a 10 % substitution had similar characteristics of color, smell, texture, taste, external appearance, internal appearance and overall appearance in relation to F0 (the control test). Finally, it was shown that bread made from 10 % eggshell powder is accepted by the consumer.

In their research, Ali and Badawy (2017) carried out the sensory evaluation of bread strips, where they demonstrate and recommend using eggshell powder as a dietary calcium supplement to strengthen the bread in homes to a level of 10%. In Cuba, salted and sweet bread fortified with calcium was produced in such a way that each bread contained 477 and 538,80 mg, with an acceptability (over the target population) of 100 % and 96 % respectively (Riera *et al.,* 1999).

In Chile the bread was also fortified with calcium with a concentration of 120 mg of calcium per unit of bread. Later, the acceptability test obtained results of "liked" or "liked it a lot" in 80% of the population **(Toop *et al*., 1994)**.

## 4. Conclusions

According to the results, the formulation of bread with partial substitution of wheat flour (HT) with eggshell powder (HCH) at 10% (F1) has the highest calcium assimilation compared to bread with conventional formulation (F0). It is worth mentioning that this product rich in calcium and generated from an organic residue would contribute to solve calcium deficit in the body.

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# PRODUCTION AND CHARACTERIZATION OF 'SEKAKI' PAPAYA FRUIT PUREE AND POWDER

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**ABSTRACT**

This study was conducted to optimize enzyme liquefaction of papaya fruit using Pectinex Ultra SP-L, followed by spray-drying the liquefied puree into powder. Pectinex® Ultra SP-L was applied at different concentrations (0.5 - 2.5% v/w) and incubation time (0.5 - 2.0 hours). The puree after enzyme liquefaction was spray-dried with different temperatures of 160-200°C). Results showed that papaya puree treated with Pectinex® Ultra SP-L at 1.0% (v/w) with an incubation time of 2 hours gave the lowest viscosity (6510.10 ± 1616.37 cps), TSS value at 10.09 ± 0.68 °Brix, pH value at 4.42 ± 0.19 and color value of L*= 33.83 ± 1.61, a*=33.75 ± 1.18 and b*=44.37 ± 0.86. Spray-drying at 160°C inlet temperature yielded powder with good properties: moisture content at 5.45 ± 0.07%, water activity at 0.15 ± 0.004 Aw, hygroscopicity at 17.90 ± 1.34%, and color values L*=92.39 ± 0.01, a*=4.44 ± 0.001 and b*=12.27 ± 0.01. For proximate analysis, spray-dried papaya powder had the lowest ash content, fat content, protein content, and no fiber was detected in spray-dried papaya powder. The pH of the reconstituted powder was lower compared to the optimized puree, and the color was darker and yellow compared to the optimized puree.

## 1.Introduction

Food preservation is a method that has been used for a long time to prevent spoilage. Production of puree is generally performed by mixing the food and blended using a blender to the form of pulp (Sreenath et al., 1995). Enzymes, such as pectinase and cellulase, are then introduced to produce puree by enzyme liquefaction (Sreenath et al., 1995; Bhat, 2000). Enzyme liquefied puree has a lower viscosity, which is important for a spray-dryer feed to prevent clogging and an increase in feed flow rate.

Drying extends food stability and shelf-life by the prevention of microbial growth (McMinn and Magee, 1999). Besides, handling of food will be easier as the weight and volume of food is reduced as water is removed (Click and Ridberg, 2010). Spray-drying is used in the food industry, transforming a wide range of liquid food products into powder form (Jayasundera et al., 2011). The process of spray-drying involves spraying finely atomized solutions into a chamber where both hot and dry air rapidly evaporated the atomized solution leaving behind spray-dried particles Jayasundera et al., 2011; Nijdam and Langrish, 2006; Chew et al., 2019). Recent work were done on spray-drying of fruit such as 'cempedak' – a type of jackfruit, Kuini – a type of mango and 'Bintangor' orange (Pui et al., 2020a; Chng et al., 2020; Yu et al., 2021).

Papaya fruit is highly appreciated for its flavor, nutritional qualities, and digestive properties (Thomás et al., 2009). It contains

vitamins, minerals, enzymes, proteins, and phytochemicals such as alkaloids, glycosides, flavonoids, etc. (Krishna et al., 2008). Research had been done on papaya to produce papaya juice, papaya powder, and other products as well. However, producing spray-dried papaya powder with enzyme-treated puree is not fully explored yet. Therefore, this research aimed to produce papaya powder from the papaya pulp, which has been treated with an enzyme. The production of papaya powder was by using a spray-dryer. Physico-chemical properties of both enzyme-treated papaya puree and spray-dried papaya powder are determined.

## 2. Materials and methods

### 2.1. Materials

#### *2.1.1. Fruit sample and enzyme liquefaction*

'Sekaki' papaya, purchased from a local fruit store in Shah Alam, Malaysia, was cut and peeled and deseeded. The pulp was cut into smaller pieces (50 mm x 50 mm x 50 mm) and blended at high speed for 1 minute. The blended papaya pulp (200 g) was added with Pectinex® Ultra SP-L at 0 to 2.5% (v/w) and incubated at 50°C for 2 hours in a water bath (Memmert, Germany) with 100 rpm (Pui et al., 2020b). It was then placed into a water bath of 95°C for 5 minutes to inactivate the enzyme, followed by an ice-water bath for 5 minutes. After the optimum concentration for the each is obtained, the incubation time (0, 0.5, 1.0, 1.5, and 2.0 hours) was determined. For each enzyme liquefied papaya, the viscosity, TSS, pH, and the color is determined, while proximate analysis was conducted on the optimized enzyme-liquefied papaya puree.

### 2.2. *Spray-drying*

Enzyme-treated papaya puree (500 g) were sieved and added with maltodextrin DE 10 (Bronson and Jacobs, Australia) at a concentration of 20%, and water is added with 1:1 ratio (puree: water). The papaya puree was then fed and atomized in the mini spray-dryer (Büchi, Switzerland). The spray-drying process was conducted at different inlet temperatures (160, 170, 180, 190, and 200°C) (Chang et al., 2020a). The collected powder was determined in its physico-chemical properties: moisture content, water activity, color, and hygroscopicity.

### 2.3. *Reconstitution of powder*

Spray-dried papaya powder was rehydrated to the same total solid content, which is 10.07 before drying (Pui et al., 2021).

### 2.4. *Analysis*

#### *2.4.1. Water activity*

The water activity of the sample was measured by using a water activity meter (LabMaster - aw Water Activity Meter) at room temperature (Chang et al., 2020b).

#### *2.4.2. Color*

The color of the fresh papaya fruit, enzyme liquefied papaya with 2.0% Pectinex Ultra SP-L, papaya powders, and reconstituted powders were measured using a colorimeter (ColorFlez EZ, Hunter Associates Laboratory Inc, USA). The colorimeter was calibrated against a standard white tile (Loo and Pui, 2020). Colorimetric data were expressed in terms of L*, a*, and b*, representing luminosity or lightness, green-red component, and blue-yellow component, respectively.

#### *2.4.3. Total Soluble Solids (Brix)*

The total soluble solids content of the samples was measured using a Refractometer (0-32 °Brix) or (28-62 °Brix) (Pui et al., 2018).

#### *2.4.4. Viscosity*

Were measured using a Brookfield viscometer DV-II+ Pro, USA (Wong et al., 2015). 250 mL of samples were added with spindle no.2, and the reading measured at a speed of 150 rpm.

#### *2.4.5. pH*

The pH of the samples was measured using a digital pH meter (Mettler Toledo, USA) (Pui et al., 2018).

#### *2.4.6. Hygroscopicity*

The hygroscopicity of the papaya powder was determined by placing 2g of powder in n a glass desiccator at 25 ± 1°C containing saturated Na2SO4 solution (81% RH) instead of an airtight plastic container (Cai and Corke,

2000). After 1 week, the samples were weighed, and the hygroscopicity was expressed as g of moisture per 100 g dry solids (g/100 g).

***2.4.7. Proximate analysis***

The fresh fruit, papaya puree, and papaya powder were analyzed in crude protein, crude fat, crude fiber, ash content, and moisture content (AOAC, 2000).

**2.5. *Statistical Analysis***

Data (n=3) was expressed in terms of ± standard deviations, with the statistical analysis conducted with SPSS 22, one-way ANOVA. The Tukey's Honestly Significant Difference (HSD) was performed to determine significant differences with $p \leq 0.05$.

## 3.Results and discussions

### 3.1. Enzyme liquefaction on homogenized papaya

Table 1 shows the effect of enzyme Pectinex® Ultra SP-L with different concentrations at the incubation of 50°C on the viscosity, total soluble solids (TSS), pH, and color (L*, a* and b*) of homogenized papaya. From Table 1, the viscosities of the Pectinex Ultra SP-L treated papaya purees were lower than the control at 0% (v/w). The addition of 0.5% (v/w) enzyme did not decrease the viscosity of the papaya puree.

**Table 1.** Effect of different concentrations of Pectinex® Ultra SP-L on the viscosity, total soluble solids (TSS), pH, and color of papaya puree at 50°C for 2 hours

| Analysis | Enzyme Concentration (%) | | | | | |
|---|---|---|---|---|---|---|
| | Control (0) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
| Viscosity (cps) | 8783.59 ± 559.67$^{a}$ | 8920.76 ± 2365.64$^{a}$ | 6510.10 ± 1616.37$^{b}$ | 6064.17 ± 788.14$^{b}$ | 4651.02 ± 917.17$^{b}$ | 5849.43 ± 1916.49$^{b}$ |
| TSS (°Brix) | 9.57 ± 0.50$^{a}$ | 9.67 ± 0.48$^{a}$ | 10.09 ± 0.68$^{a}$ | 9.52 ± 0.41$^{a}$ | 10.06 ± 0.49$^{a}$ | 10.13 ± 0.34$^{a}$ |
| pH | 4.57 ± 0.24$^{a}$ | 4.41 ± 0.19$^{a}$ | 4.42 ± 0.19$^{a}$ | 4.38 ± 0.15$^{a}$ | 4.33 ± 0.10$^{a}$ | 4.40 ± 0.15$^{a}$ |
| Color ($L^{*}$) | 34.32 ± 0.45$^{a}$ | 33.56 ± 0.23$^{b}$ | 33.83 ± 1.61$^{ab}$ | 32.13 ± 1.58$^{c}$ | 32.90 ± 0.30$^{bc}$ | 32.77 ± 0.62$^{bc}$ |
| Color ($a^{*}$) | 33.76 ± 0.98$^{a}$ | 33.60 ± 0.84$^{a}$ | 33.75 ± 1.18$^{a}$ | 32.95 ± 1.22$^{a}$ | 33.33 ± 1.24$^{a}$ | 33.24 ± 1.75$^{a}$ |
| Color ($b^{*}$) | 42.73 ± 0.43$^{ab}$ | 43.23 ± 1.52$^{ab}$ | 44.37 ± 0.86$^{a}$ | 42.11 ± 1.52$^{b}$ | 43.05 ± 1.92$^{ab}$ | 43.05 ± 1.92$^{ab}$ |

Data on viscosity, TSS, pH, and color of papaya puree are means ± standard deviations where n = 3. For each row, superscripts of the same letter are not significantly different at $p \leq 0.05$, as measured by the Tukey HSD test.
Abbreviations: HSD = honestly significant difference, TSS = total soluble solids, L* = degree of lightness and darkness, a* = degree of redness or greenness, and b* = degree of yellowness or blueness.

However, the viscosity of the papaya puree decreases after the addition of 1.0% (v/w) enzyme, followed by no further increase in viscosity when enzyme with 2.5% (v/w) is added into papaya purees. The enzyme pectinases can help in the extraction, clarification, increasing the yield and decreasing the viscosity of fruit juices by the degradation of pectin-containing substances (Demir et al., 2001). The pectinaceous substances possess a high water holding capacity, and this developed a cohesive network structure (Tapre and Jain, 2014). The degradation of pectin by the enzyme Pectinex® Ultra SP-L lead to the reduction in the water holding capacity and thus released the free water into the system, which is responsible for further reduction in viscosity (Sin et al., 2006).

In the aspect of total soluble solids (TSS), there was no significant difference ($p > 0.05$) between the enzyme concentrations at 0% to 2.5% (v/w) on the TSS of the enzyme-treated homogenized papaya, which indicates that increasing the concentration of the enzyme did

not affect the TSS of the papaya puree. This is similar to pH as well, with no significant effect ($p > 0.05$) with the addition of pectinase concentration.

From Table 1, L* value at 0% (v/w) was 34.32 ± 0.45 and it decreases to 33.56 ± 0.23 and 32.13 ± 1.58 for the L* value at 0.5% (v/w) and 1.5% (v/w) respectively. With an increase to 2.0% (v/w) and 2.5* v/w, the puree shows no significant difference in the L* values. L* values range from black (0) to white (100). As the concentration of enzyme increases, the color of the enzyme liquefied papaya becomes darker. This was possibly due to the degradation of thermo-labile pigments resulting in the formation of dark compounds that reduced the luminosity (Dutta et al., 2006).

The a* value represents the redness and greenness of the powder, where positive value shows the redness intensity while a negative value shows greenness intensity. As for the value of a*, results show that there was no significant difference ($p > 0.05$) between the color of a* with the enzyme concentration, which indicates that increasing in enzyme concentration would not affect the redness of the enzyme liquefied papaya puree. Based on the value obtained, the color of the enzyme liquefied papaya appears reddish due to lycopene found in papaya (Quek et al., 2007).

The b* value represents the blueness and yellowness of the powder, where positive value shows the intensity of yellowness, while a negative value shows the intensity of blueness (Chang et al., 2020a). The b* value increases to 44.37 ± 0.86 at 1.0% (v/w), to 43.05 ± 1.92 for both 2.0% (v/w) and 2.5% (v/w). Based on the value, the color of the enzyme liquefied papaya appears yellowish but hard to observe as the redness covers the yellowness color. The yellowness of the papaya was due to the β-carotene found in the papaya (Quek et al., 2007).

The effects of incubation time on homogenized papaya were shown in Table 2. With the increase of incubation time, it was found that the viscosity decreases. The lowest viscosity was found at 2.0 hour incubation time with a value of 4260.90 ± 483.79 cps. The total soluble solids increase slightly, while its pH decreases with 0.5 hours, followed by no significant effect ($p > 0.05$), with a further increase in incubation time. L* values were not affected by an increase in incubation time, while there has not been any significant difference ($p > 0.05$) in the a* value until 2.0 hours. The b* value of papaya puree varies from 40.83 to 43.39 after incubation. The optimization of the parameters for enzyme liquefaction was determined by the viscosity and color of the end product. Pectinex® Ultra SP-L enzyme at a concentration of 1.0% (v/w) and an incubation time of 2.0 hours produced puree with more desirable properties, as it exhibits the lowest viscosity, which was 4260.90 ± 483.79 and the color values of (L*, a*, and b*) which were 30.26 ± 4.13, 32.70 ± 1.15 and 40.83 ± 0.69. The product, after liquefaction, will be used as a spray-drying feed.

### 3.2. Production of papaya powder by spray-drying

Figure 1 shows the yield of the powder obtained from the spray-drying at 5 different inlet temperatures (160, 170, 180, 190 and 200°C). The inlet temperature of 160°C had the highest yield, which was 11.12 ± 0.02%. With a further increase in inlet temperature, the powder yield continues to decrease. At inlet temperatures of 200°C, the yield was decreased by 7.12 ± 0.02%.

Higher inlet temperature causes the powder to stick to the chamber wall and thus reducing the amount of powder produced. Chengini and Ghobadian (2007) also reported that powder produced at high temperatures resulted in a powder that is stickier, as the inlet temperature was above the glass transition temperatures for the spray-dried powders.

**Table 2.** Effect of different incubation time of Pectinex® Ultra SP-L on the viscosity, total soluble solids (TSS), pH and color of papaya puree at 50°C for 2 hours

| Analysis | Time (hour) | | | | |
|---|---|---|---|---|---|
| | Control (0) | 0.5 | 1.0 | 1.5 | 2.0 |
| Viscosity (cps) | 11590.65 ± 182.82$^{a}$ | 9299.37 ± 941.24$^{b}$ | 6806.17 ± 421.53$^{c}$ | 5498.10 ± 420.67$^{d}$ | 4260.90 ± 483.79$^{e}$ |
| TSS (°Brix) | 9.81 ± 0.10$^{a}$ | 10.47 ± 0.20$^{b}$ | 10.47 ± 0.20$^{b}$ | 10.31 ± 0.20$^{b}$ | 10.51 ± 0.20$^{b}$ |
| pH | 4.49 ± 0.13$^{a}$ | 4.25 ± 0.15$^{b}$ | 4.23 ± 0.21$^{ab}$ | 4.20 ± 0.16$^{ab}$ | 4.18 ± 0.10$^{b}$ |
| Color (L$^{*}$) | 30.90 ± 0.01$^{a}$ | 31.81 ± 0.29$^{a}$ | 32.12 ± 0.11$^{a}$ | 31.12 ± 0.61$^{a}$ | 30.26 ± 4.13$^{a}$ |
| Color (a$^{*}$) | 33.65 ± 0.04$^{a}$ | 34.08 ± 0.38$^{a}$ | 34.38 ± 0.31$^{a}$ | 33.83 ± 0.61$^{a}$ | 32.70 ± 1.15$^{b}$ |
| Color (b$^{*}$) | 38.59 ± 0.04$^{a}$ | 42.23 ± 0.15$^{b}$ | 43.39 ± 0.37$^{c}$ | 42.65 ± 0.62$^{c}$ | 40.83 ± 0.69$^{ab}$ |

Data on viscosity, TSS, pH, and color of papaya puree are means ± standard deviations where n = 3. For each row, superscripts of the same letter are not significantly different at $p \leq 0.05$, as measured by the Tukey HSD test.
Abbreviations: HSD = honestly significant difference, TSS = total soluble solids, L* = degree of lightness and darkness, a* = degree of redness or greenness, and b* = degree of yellowness or blueness.

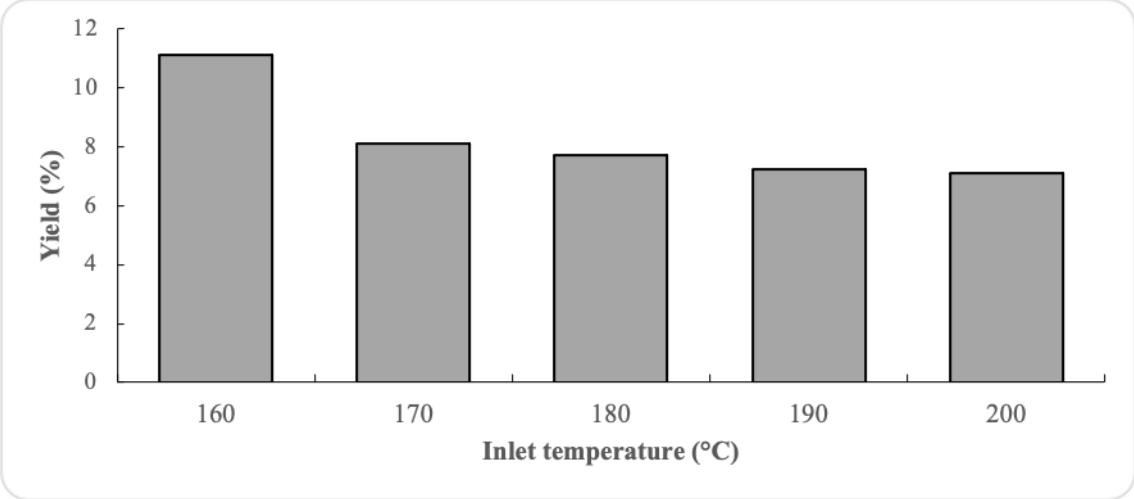


**Figure 1.** The yield of papaya powder spray-dried under different inlet temperature

Table 3 shows the comparison of analyses of hygroscopicity, moisture content, water activity, and color (L*, a* and b*) of spray-dried powders at different inlet temperatures. There was no significant difference ($p > 0.05$)s in the moisture content of the spray-dried papaya powder produced at 160-190°C. The moisture content of papaya powder decreases when produced at 200°C, with a value of 5.03 ± 0.97%. Water activity is different from moisture content as it measures the availability of free water in a food system that is responsible for any biochemical reactions (Wong et al., 2015). Higher water activity indicates more free water is available for biochemical reactions and hence, shorter shelf life (Quek et al., 2007). From Table 5, there was also no significant difference ($p > 0.05$) between the water activity of spray-dried powder, with a range of 0.14 – 0.16Aw. The powders can be considered microbiologically stable (Quek et al., 2007; Fávaro-Trindade et al., 2010). Besides, the hygroscopicity of the spray-dried papaya powder was also not significantly different ($p > 0.05$).

Based on the results in Table 3, the highest value of L* was at an inlet temperature of 160°C with a value of 92.39 ± 0.01, which means that the powder had become lighter in color. One explanation is that papaya contains

sugars, which could contribute to the browning of the powders at higher inlet temperatures (Quek et al., 2007). It is reported that the reduced lightness of the powder was due to the caramelization and Maillard reactions on the powder during the drying process (Jittanit et al., 2010). Following an increase in temperature from 170°C, with a value of 90.40 ± 0.01, which shows that the powder is darker. Beyond that temperature, there is no further increase in L*.

The papaya powder produced has values ranging from 4.44-4.62, indicating it is slightly reddish. On the other hand, yellowness, the b* of the papaya powder, show a decrease when spray-dried at 160°C to 180°C, followed by an insignificant decrease from 180°C to 200°C ($p > 0.05$). β-carotene in papaya undergo degradation by oxidation and heat (Goula and Adamopoulos, 2005). Dehydrated products have large surface-to-mass ratios that are easily susceptible to oxidative decomposition. β-carotene is easily oxidized because of the large number of conjugated double bonds in its structure (Phisut, 20012). These reactions of oxidation and thermal degradation cause color loss of carotenoids in foods (Quek et al., 2007).

Based on results obtained in Table 5, optimization of inlet temperature was determined based on 1 main parameter, which was the yield. The optimized inlet temperature for the spray-drying of papaya (160°C) produced powder with the moisture content and the water activity 160°C were 5.45 ± 0.07 and 0.15 ± 0.04, respectively. The values of hygroscopicity and color (L*, a* and b*) of spray-dried papaya powder at 160°C were 17.90 ± 1.34%, 92.39 ± 0.10, 4.44 ± 0.10 and 12.27 ± 0.01, respectively.

### 3.3. Proximate analysis

Table 4 shows the results of proximate analysis of fresh fruit, optimized enzyme liquefied homogenized papaya, and optimized spray-dried papaya powder. In comparison to the optimized puree, the moisture content of the fresh fruit of papaya was lower (89.82 ± 0.02%). The moisture content of puree was higher than the moisture content of fresh fruit due to the enzyme degradation of the pectin inside the fruit, which causes water holding capacity to breakdown and release free water into the system (Manjunatha et al., 2014).Moisture content in fruit powder was at 5.26 ± 0.02%. The low moisture content of spray-dried powder can prolong its shelf life as water content was too low for the growth of microorganism spoilage.

**Table 3.** Physico-chemical analysis of spray-dried papaya powder

| Analysis | Inlet temperature (°C) | | | | |
|---|---|---|---|---|---|
| | 160 | 170 | 180 | 190 | 200 |
| Moisture content (%) | $5.45 \pm 0.07^{a}$ | $5.35 \pm 0.57^{a}$ | $5.12 \pm 0.13^{a}$ | $5.27 \pm 0.74^{a}$ | $5.03 \pm 0.97^{b}$ |
| Water activity ($A_w$) | $0.15 \pm 0.04^{a}$ | $0.15 \pm 0.01^{a}$ | $0.14 \pm 0.01^{a}$ | $0.15 \pm 0.01^{a}$ | $0.16 \pm 0.01^{a}$ |
| Hygroscopicity (%) | $17.90 \pm 1.34^{a}$ | $17.29 \pm 0.04^{a}$ | $17.14 \pm 0.62^{a}$ | $17.06 \pm 0.22^{a}$ | $16.05 \pm 0.49^{a}$ |
| Color ($L^{*}$) | $92.39 \pm 0.10^{a}$ | $90.40 \pm 0.10^{b}$ | $90.70 \pm 0.60^{b}$ | $90.52 \pm 0.20^{b}$ | $90.91 \pm 0.40^{b}$ |
| Color ($a^{*}$) | $4.44 \pm 0.10^{b}$ | $4.62 \pm 0.04^{a}$ | $4.64 \pm 0.10^{a}$ | $4.48 \pm 0.07^{b}$ | $4.49 \pm 0.20^{b}$ |
| Color ($b^{*}$) | $12.27 \pm 0.01^{a}$ | $13.18 \pm 0.01^{b}$ | $13.40 \pm 0.05^{c}$ | $13.33 \pm 0.02^{bc}$ | $13.43 \pm 0.02^{c}$ |

Data on moisture content, water activity, hygroscopicity, and color of spray-dried papaya powder are means ± standard deviations where n = 3. For each row, superscripts of the same letter are not significantly different at $p \leq 0.05$, as measured by the Tukey HSD test. Abbreviations: HSD = honestly significant difference, L* = degree of lightness and darkness, a* = degree of redness or greenness, and b* = degree of yellowness or blueness.

**Table 4.** Proximate analysis and water activity of fresh papaya fruit, optimized papaya puree, and spray-dried papaya powder

| Analysis | Fresh fruit | Puree | Powder |
|---|---|---|---|
| Moisture Content (%) | $89.54 \pm 0.01^{b}$ | $89.82 \pm 0.02^{a}$ | $5.26 \pm 0.02^{c}$ |
| Ash Content (%) | $0.22 \pm 0.02^{b}$ | $0.41 \pm 0.01^{a}$ | $0.20 \pm 0.02^{c}$ |
| Crude Protein (%) | $1.34 \pm 0.01^{b}$ | $1.44 \pm 0.01^{a}$ | $0.31 \pm 0.02^{c}$ |
| Crude Fat (%) | $0.18 \pm 0.02^{a}$ | $0.13 \pm 0.02^{b}$ | $0.01 \pm 0.01^{c}$ |
| Crude Fiber (%) | $11.65 \pm 0.03^{b}$ | $24.81 \pm 0.02^{a}$ | $0.00 \pm 0.00^{c}$ |
| Water Activity ($A_w$) | $0.96 \pm 0.01^{b}$ | $0.97 \pm 0.01^{a}$ | $0.16 \pm 0.01^{c}$ |

Data on moisture content, ash content, protein content, fat content, and the crude fiber content of fresh papaya pulps, optimized enzyme-liquefied papaya puree, and optimized spray-dried papaya powder are meant ± standard deviations where n = 3. For each row, superscripts of the same letter are not significantly different at $p \leq 0.05$, as measured by the Tukey HSD test. Abbreviations: HSD = honestly significant difference.

Enzyme liquefied homogenized papaya had the highest amount of ash content due to the composition of minerals, which were released into the system upon the breakdown of tissues by the Pectinex® Ultra SP-L enzyme (Qin et al., 2005). However, the spray-dried powder produced the least amount of ash. The addition of maltodextrin increased the amount of non-papaya solids in the powders samples, which in turn lowered the ash content of maltodextrin added powders (Grabowski et al., 2006).

The protein content for fresh fruit was 1.34 ± 0.01%, which was lower compared to the protein content of enzyme liquefied homogenized papaya but significantly higher than spray-dried powder. The protein content was 1.44 ± 0.01% for the enzyme liquefied papaya puree and 0.31 ± 0.02% for the papaya powder. The low protein content in powder was due to the thermal degradation of the protein. Ignário and Lannes (2007) also reported similar results of reduced protein content in the spray-drying of egg yolk. Thus, the lower protein content of the powders could be attributed to the destruction of lysine through interaction with reducing groups of carbohydrates at high temperatures (Grabowski et al., 2006).

From Table 4, the fat content of fresh fruit was higher than the optimized puree, while fat in spray-dried powder was the lowest. The cause of the decrease of fat content in spray-dried papaya powder was due to lipid oxidation. Spray-drying greatly increases the surface area, which exposes more area in papaya powders for oxidation and degradation to take place, thus reducing the fat content (Gharsallaoui et al., 2007). Fiber content increases in enzyme liquefied papaya puree, and no fiber were found in spray-dried papaya powder (Table 4). The high fiber content in enzyme liquefied puree might be due to the breakdown of tissues by an enzyme that releases fiber into the system (Norjana and Noor Aziah 2011). The fiber was not detected in spray-dried powder as the feed was filtered before spray-drying. This is needed for preventing the clogging of the spray-dryer (Phisut, 2012).

As shown in Table 4, the highest water activity content was found in enzyme liquefied homogenized papaya with a value of 0.970 ± 0.01 and followed by the second-highest water activity content with a value of 0.960 ± 0.01, which was found in fresh papaya fruit. The lowest amount of water activity content was found in spray-dried papaya powder with a value of 0.158 ± 0.01. The water activity in both fresh papaya fruit and enzyme liquefied papaya puree were higher $Aw > 0.6$, which was considered to be suitable for microorganism growth. As for spray-dried powder, the water activity was $Aw \leq 0.6$ was considered to be microbiologically stable, which was suitable in increasing shelf life (Chang et al., 2020a).

### 3.4. Reconstitution of optimized powder

Total soluble solids (TSS), pH, and color (L*, a*, and b*) for optimized spray-dried powder were exhibited in Table 5. The value of

pH for the reconstituted powder was at 4.00 ± 0.01. When compared to the pH of the optimized papaya puree, the pH was lower. The pH was lower compared to the pH of the fresh papaya fruit might be due to the release of carboxyl groups from the pectin molecules during enzymatic liquefaction. The carboxyl groups, in turn, lower the pH of the due to its acidic properties (Gharsallaoui et al., 2007).

**Table 5.** Analysis of reconstituted optimized powder

| Analysis | Optimized Puree | Reconstituted Powder |
|---|---|---|
| TSS (°Brix) | $10.09 \pm 0.68^{a}$ | $10.07 \pm 0.00^{a}$ |
| pH | $4.42 \pm 0.19^{a}$ | $4.00 \pm 0.01^{b}$ |
| Color (L*) | $33.83 \pm 1.61^{a}$ | $22.67 \pm 0.23^{b}$ |
| Color (a*) | $33.75 \pm 1.18^{a}$ | $-1.54 \pm 0.15^{b}$ |
| Color (b*) | $44.37 \pm 0.86^{a}$ | $3.12 \pm 0.01^{b}$ |

Data on moisture content, ash content, protein content, fat content, and the crude fiber content of fresh papaya pulps, optimized enzyme-liquefied papaya puree, and optimized spray-dried papaya powder are meant ± standard deviations where n = 3. For each row, superscripts of the same letter are not significantly different at $p \leq 0.05$, as measured by the Tukey HSD test. Abbreviations: HSD = honestly significant difference.

The value of L*, which was the lightness, had a value of 22.67 ± 0.23. In comparison with the original L* of optimized papaya puree, the value was lower, which means the reconstituted powder had become darker. The decrease in lightness could be due to non-enzymatic browning occurring during the spray-drying (Fávaro-Trindade et al., 2010). As for a* value, the value obtained in reconstituted powder was -1.54 ± 0.15. The value of a* was lower when compared to the value of a* in optimized papaya puree. Obtaining a negative value in a* shows that the reconstituted spray-dried powder had lost the redness color. The degradation and oxidation of lycopene, which was responsible for the red color of the papaya due to the usage of high temperature in spray-drying, caused the redness to lose its color (Sousa et al., 2008). The value of b* for reconstituted optimized spray-dried papaya powder was lower when compared to the value of b* of optimized papaya puree. As a result of b* shows a positive value, the reconstituted spray-dried powder shows still contained yellowness. Thermal degradation and oxidation of β-carotene caused the yellowness to be lost (Quek et al., 2007).

## 4. Conclusions

Papaya has been one of the most popular fruits in many countries due to its slightly sweet, musky undertones with soft flesh. The optimization of enzyme liquefaction includes enzyme concentration and incubation time. The viscosity of papaya puree decreased with the increase of concentration and incubation time of both enzymes. 1.0% (v/w) Pectinex® Ultra SP-L with an incubation time of 2 hours was chosen as the optimum condition for enzyme liquefaction. In the determination of the optimum inlet temperature, powder sprayed at 160°C has the highest yield and favorable properties such as low moisture, water activity and hygroscopicity. The produced 'Sekaki' papaya powder has the potential to be used as functional ingredients for other food products.

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# THERMAL AND MICROSCOPIC PROPERTIES AND QUALITY CHARACTERISTICS OF LOW-FAT FRANKFURTERS AND EMULSIONS PRODUCED WITH CARBOXYMETHYL CELLULOSE, METHYL CELLULOSE AND PECTIN

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**ABSTRACT**

This study involves the effect of different concentrations of carboxymethyl cellulose (CMC), methyl cellulose (MC) and pectin (PEC) as fat replacers on thermal, microscopic and quality characteristics of low-fat frankfurters. Thermal analysis showed three peaks at 58.4, 66.6 and 81.9 for ground meat which were attributed to myosin, sarcoplasmic proteins and actin respectively. Addition of NaCl lowered the thermal denaturation temperature of myosin and actin. It was not possible to differentiate the second and third when phosphates and hydrocolloid were added to low-fat emulsion. The emulsion stability of the samples containing 0.5% MC, 0.5% and 1% PEC were significantly lower than control. The SEM result of the sample containing 1% PEC resembles most to that of the control. The sensory evaluations showed that addition of CMC decreased the acceptability of low-fat frankfurters, on the other hand MC and PEC at a concentration of 0.5% were acceptable.

## 1.Introduction

Meat and meat products are a valuable source of vitamins and minerals such as A, C, $B_{12}$, folic acid and Fe. Moreover meat is rich in proteins with high biological activity, amino acids and fat (Cierach et al. 2009; Schmiele et al. 2015). Fat in meat products like frankfurters is responsible for the stability of the batter, reduces cooking losses, and improves the texture, juiciness and mouthfeel. Also fat is an important source of energy, essential fatty acids and carrier of fat soluble vitamins in meat products (St.Clair Henning et al. 2016; Choi et al. 2009). On the other hand association of saturated fat consumption with many chronic diseases such as diabetes, obesity, cardiovascular diseases has led researchers to produced healthier meat products by reducing the fat content (Han and Bertram, 2017). Since fat has favourable effects on the quality of meat products, reducing the fat content causes undesirable effects such as increased cooking losses, undesired texture and flavour. In order to overcome these effects researchers have tried incorporation of different additives like carrageenan, pectin (PEC), guar gum, xhantan gum (Candogan and Kolsarici, 2003; Yilmaz et al., 2017; Méndez-Zamora et al., 2014), microcrystalline cellulose, carboxymethyl cellulose (CMC) (Schuh et al. 2013; Gibis et al. 2017). Addition of these additives and dietary fibers from different sources to low-fat meat products improved the water binding and water retention of the product. Therefore the shrinkage, cooking losses, drip losses during storage of meat products were

reduced (Almeida et al., 2014, St.Clair Henning et al., 2016).

Incorporation of dietary fibers like PEC, CMC and methyl cellulose (MC) to meat products as fa replacers may also enhance the nutritional attributes of the products since consumption of dietary fibers reduces the risk of obesity, colon cancer and cardiovascular diseases (Han and Bertram, 2017). Different studies have been carried out considering the effect of PEC, CMC and MC on the quality of low-fat frankfurters, however studies on the interactions between these additives and meat proteins were not fully investigated.

Meat proteins especially myofibrillar proteins (myosin and actin) are responsible for the three-dimensional gels during the production and these gels forms the desired structure in meat products. It was stated by Morin et al. (2004) that CMC may interact with meat proteins with its negatively charged carboxyl groups. And it is believed that the functional properties of proteins like gel formation, solubility and emulsifying capacity mat change due to the interactions between proteins and hydrocolloids (Ayadi et al. 2009). As a result, these interactions has an important part in the formation of desired product and its stability during storage. Differential scanning calorimetry (DSC) is an effective analytical method for the determination of interactions between meat proteins and hydrocolloids since it is accepted that changes in the denaturation temperature of proteins is an indication of these interactions (DeFreitas et al., 1997). In this study the quality characteristics of low-fat frankfurters formulated with different levels of CMC, MC and PEC and the interactions between meat proteins and CMC, MC and PEC were investigated by using DSC.

## 2. Materials and methods

### 2.1. Materials

The ground meat used in the DSC analysis and for preparation of emulsions were obtained from local butchers. The additives potato starch, pectin (PEC), carboxymethylcellulose (CMC) and methylcellulose (MC) were kindly provided by GMT-food (Istanbul, Turkey). The hermetic pans used for DSC analysis were supplied from Likrom Analytical Solutions (Ankara, Turkey). All the ingredients and additives except for hydrocolloids, used for production of frankfurters were obtained from Pınar Integrated Meat and Feed Industries (Izmir, Turkey).

### 2.2. Preparation of Low-fat emulsions, frankfurters and high fat frankfurter

Low-fat emulsions were prepared according to the established formulations of low-fat frankfurters which were formed by addition of 1.5% sodium chloride (NaCl), 0.3% sodium-phosphate, 3.2% potato starch and 0.5 or 1% of either one of PEC, CMC or MC, to the mixture of meat, fat and water.

**Table 1.** Amount of hydrocolloids used for each sample and abbreviations of the samples

| Sample | Hydrocolloid | Hydrocolloid Percentage (%) | Abbreviations |
|---|---|---|---|
| 1 | - | - | Control |
| 2 | Carboxymethylcellulose | 0.5 | 0.5CMC |
| 3 | carboxymethylcellulose | 1 | 1CMC |
| 4 | Methylcellulose | 0.5 | 0.5MC |
| 5 | Methylcellulose | 1 | 1MC |
| 6 | Pectin | 0.5 | 0.5PEC |
| 7 | Pectin | 1 | 1PEC |

The high fat frankfurter (control group) and low-fat frankfurters were produced by Pınar Integrated Meat and Food Industries (Izmir, Turkey). The high fat frankfurter (control group) was produced with 35% beef meat (with 15% fat), 25% beef meat (with 30% fat), 15% beef fat and 25% ice. The fat content of the low-fat frankfurters were reduced to 5% and different

levels of PEC, CMC and MC were used so as to obtain the targeted texture. Seven kilograms of batter (meat, fat and ice) was prepared for each group, in each group the ingredients used per kilograms of batter were as follows, 15g of sodium chloride, 3 g of sodium phosphate, 0.5 g of ascorbic acid, 0.5 g of ascorbate, 0.125 g of sodium nitrate, 2 g of black pepper, 2 g of red pepper, 0.5 g of coriander, 0.4 g of ginger, 8 g of sodium caseinate and 32 g of starch. Six different low-fat frankfurter formulations were produced and the different hydrocolloids and their quantities were given in Table 1.

For the production of frankfurters meat, fat, half of the ice and the ingredients other than potato starch and caseinate were mixed in a cutter (Kilia, vacuum cutter, Neumünster Germany) at low speed (1000 rpm). After incorporation of the hydrocolloid additives the mixture was blended at 3500 rpm for 1-2 min. the cutter speed was then raised to 5500 rpm. Until the mixture temperature reached 6°C. At this point potato starch, caseinate and the remaining of the ice were added and mixed at 5500 rpm until the temperature of the mixture reached 12°C. The mixture was stuffed into 18 Ø synthetic casings and hand-linked at 19 cm intervals. Stuffed mixtures were heat-processed and smoked in the smokehouse according to the following conditions, drying for 50 min at 60°C and 60 % relative humidity (RH), smoking for 50 min at 60°C 60 % RH and then steam cooking until the internal temperature reached 72°C. The frankfurters were then showered with cold water for 5 min. After cooling the frankfurters were peeled, vacuum packed and pasteurized for 30 min. at 78°C. Lastly, the frankfurters were transported to Hacettepe University, Department of Food Engineering under cold storage (4°C).

### 2.3 Determination of emulsion stability of the low-fat frankfurters

The emulsion stability of low-fat emulsions were determined according to the method of Zhou et al. (2010) and Hughes et al. (1997). 10 g of each sample were weighed into a 30 mL centrifuge tube and the samples were centrifuged at 3600 ×g, for 1 min (Sigma 3-30K, Germany) in order to remove the unbound water. Following the centrifugation the samples were placed in a water bath at 85°C for 35 min, the samples were then cooled to room temperature and then they were centrifuged once more at 3600 × g for 3 min. Analysis were performed in triplicate. After removal of the supernatant the samples were weight anew and the total expressible fluid (TEF) were measured by calculating the difference between the initial and the last weights. The percent TEF was determined according to the formula given below,

$$\% \text{ TEF} = (\text{TEF} / \text{Sample weight}) \times 100 \quad (1)$$

### 2.4. Determination of Thermal Properties of Potato Starch, Pectin, Hydroxymethylcellulose, Methylcellulose, ground meat, low-fat emulsion and frankfurters.

The thermal properties like denaturation and gelatinization temperature and glass transition temperature of ground meat, starch, hydrocolloids and low-fat emulsion and frankfurters were determined by using Q20 differential scanning calorimeter (TA instruments, Delaware, USA). DSC was calibrated with indium (melting point, 156.6°C and melting enthalpy, 28.5 J/g) before usage. DSC runs were performed under nitrogen atmosphere at a flow rate of 50 mL/min and all the analysis were performed in triplicate. Thermograms obtained from the DSC analysis were examined with TA universal analysis 2000. The solutions of potato starch (5%) and hydrocolloids (1%) were pr**e**pared in beakers and stored in refrigerator overnight before thermal analysis. Samples (composed of potato starch, hydrocolloids, ground meat, low-fat emulsions and frankfurters) were weighed (6.5 ± 0.5 mg) in an aluminium hermetic pan which were hermetically sealed. Samples were analysed between 20 and 100°C at a scanning rate of 5°C/min. The thermograms were evaluated to identify the denaturation and gelatinization peak temperatures, to this end an empty pan was used as a reference.

In order to determine the glass transition temperature of frankfurters the samples were

weighed (6.5 ± 0.5 mg) into an aluminium pan and hermetically sealed. The samples and the reference were equilibrated at 20°C, after equilibration the pans were cooled to -80°C and kept at that temperature for 15 min. then the pans were brought up to annealing temperature and were kept at that temperature for 60 min. At the end of this time the pans were cooled to -80°C and held again for 15 min. the samples were then scanned to 20°C at a scanning rate of 5°C/min against an empty reference. Annealing temperature was determined in line with preliminary studies. For the identification of glass transition temperature first derivative of the DSC thermograms were used. Glass transition was analysed for onset, mid- and conclusion- points and midpoint temperature was reported as glass transition temperature.

### 2.5. Determination of quality characteristics of the frankfurters

Moisture, fat, protein and pH values of the low-fat frankfurters and the control group were determined following the methods in Oztan and Vural (1996); and each analysis was performed in triplicate, at the beginning of the storage.

### 2.6. Water Holding Capacity (WHC) determination of samples

The WHC of the samples was determined according to the methods of Zayas and Lin (1988, 1989); which was modified by Oztan and Vural (1993), each analysis was performed in triplicate, at the beginning of the storage.

### 2.7. Colour Measurements

The colour measurements of the samples were performed at the inner cuts of the samples using a benchtop Spectrophotometer CM-3600 (Minolta, Osaka, Japan) using a Hunter colour scale. The lightness (L*), redness (a*) and yellowness (b*) of the samples were evaluated. Each analysis was performed in triplicate at the beginning of the storage.

### 2.8. Instrumental Texture Profile analysis

The texture profile analysis of the samples was performed with a texture analyser (Amatek Lloyd Instrument Ltd., United Kingdom) using Warner Bratzier shear blade. The test speed was 200mm/min, trigger was 0.05 N, the compression rate was 50% and the samples length was 15 mm. Changes in hardness ($Ncm^{-1}$), springiness (cm), gumminess ($Ncm^{-1}$) and chewiness between the samples were evaluated at the beginning of the storage and each analysis was performed quadruplicate for each sample.

### 2.9. Determination of cooking loss in the frankfurters

The cooking loss of the frankfurters was measured by weighing the linked frankfurters before and after cooking in the smokehouse using the following equation,

% Cooking loss = (Weight before cooking – Weight after cooking) / Weight before cooking ×100 (2)

### 2.10. Scanning Electron Microscope analysis

The microscopic properties of the samples were determined by means of SEM (Zeis EVO50, Germany). The frankfurters, ground meat and fat were directly placed on the samples holder for analysis. Hydrocolloid gels were coated with gold and were placed on an aluminum holder, analyses were performed at 15 kW at three different locations on each sample.

### 2.11. Sensory Evaluation

The frankfurters were evaluated in one session with two replicates. Ten untrained panellists, who were members of the faculty aged between 25 and 30 years old, evaluated both the control group and the low-fat frankfurters for their appearance, colour, texture and flavour on a 9-point hedonic scale; 9 representing strongly like and 1 representing strongly dislike on the hedonic scale. The samples were prepared by keeping the frankfurters in boiling water for 2 min and they were randomly served to the panellists. In order to evaluate the total acceptability of the samples the appearance, colour, texture and flavour scores were multiplied by specific weighing factors which are 1, 3, 3 and 3 respectively. To reach the final score, the sum of the multiplied criteria was divided by the sum of weighing factors which is 10.

### 2.12. Statistical analysis

The statistical analysis of the results was conducted using IBM statistics 21 and the statistical significance of the differences between means were determined by Duncan multiple range test.

## 3.Results and discussions

### 3.1. Thermal Properties of Potato Starch, carboxymethyl cellulose, methyl cellulose, pectin, ground meat and meat emulsions.

**Table 2**. Thermal denaturation temperatures and enthalpies of meat batters.
Values represents means (n=3)

| Sample | $T_{p1}$ (°C) | $\Delta H_1$ (j/g) | $T_{p2}$(°C) | $\Delta H_2$ (J/g) | $T_{p3}$(°C) | $\Delta H_3$ (J/g) | $\Delta H_T$ (J/g) |
|---|---|---|---|---|---|---|---|
| M | $57.50^{e}$ | $0.0145^{a}$ | $64.74^{a}$ | $0.3296^{a}$ | $78.72^{a}$ | $0.1628^{a}$ | $0.5069^{a}$ |
| M+S | $56.58^{bcd}$ | $0.0410^{b}$ | $68.21^{bc}$ | $0.3272^{a}$ | $74.00^{b}$ | $0.0176^{b}$ | $0.3858^{a}$ |
| Control | $56.86^{cde}$ | $0.0899^{c}$ | $67.58^{b}$ | $0.4572^{a}$ | | | $0.5472^{ab}$ |
| 0.5CMC | $57.26^{de}$ | $0.0967^{c}$ | $68.49^{cd}$ | $1.0274^{c}$ | | | $1.1240^{e}$ |
| 1CMC | $56.48^{bc}$ | $0.0747^{c}$ | $69.45^{e}$ | $0.7532^{b}$ | | | $0.8279^{cd}$ |
| 0.5MC | $54.55^{a}$ | $0.0229^{ab}$ | $69.10^{de}$ | $0.6691^{b}$ | | | $0.6987^{bc}$ |
| 1MC | $54.40^{a}$ | $0.0416^{b}$ | $69.05^{de}$ | $0.7017^{b}$ | | | $0.7433^{cd}$ |
| 0.5PEC | $56.33^{bc}$ | $0.0843^{c}$ | $68.44^{cd}$ | $0.7930^{b}$ | | | $0.8773^{d}$ |
| 1PEC | $56.05^{b}$ | $0.0251^{ab}$ | $68.83^{cde}$ | $0.4782^{a}$ | | | $0.5034^{a}$ |

M, Meat, M+S, Meat containing 1.5 g/100 g of NaCl, Control, High fat meat emulsion. CMC, Carboxymethyl cellulose, MC, Methyl Cellulose, PEC, Pectin, $T_{p1}$, Denaturation peak temperature for myosin, $\Delta H_1$, Denaturation enthalpy of myosin. $T_{p2}$, Denaturation peak temperature for sarcoplasmic proteins. $\Delta H_2$, Denaturation enthalpy of sarcoplasmic proteins. $T_{p3}$, Denaturation peak temperature for actin, $\Delta H_3$, Denaturation enthalpy of actin.
$^{a-e}$, Means with the same superscript at the same column do not differ significantly ($p>0.05$).

The thermal properties of the samples were examined by using TA Universal analysis 2000. According to the thermograms obtained from DSC the gelatinization temperature of potato starch was 64.41°C. This outcome was slightly lower than those reported in the literature, this could be due to the differences in sample preparation techniques and differences in the DSC analysis parameter like scanning rate (Li and Yeh 2003, Yassaroh et al. 2019). There were no transition peaks observed for CMC, MC and PEC between the temperature ranges of this study, which was 20-100°C. Akhtar et al. (2018) reported that CMC shows a glass transition peak ($T_g$) at 78.21°C and El-Sayed et al. (2011) reported a glass transition for CMC at 75°C. It could be argued that the reason for the absence of glass transition in our DSC results stemmed from the differences in the sample preparation. Several studies have been conducted on thermal properties of pectin and it was stated that the thermal behaviour of the pectin depends on its chemical composition as well as the source it was obtained from. In a study conducted by Iijima et al. (2000), phase transitions of pectins were analysed with DSC in a temperature range of -150 to 180°C and an endothermic peak was observed at 150°C for highly methoxylated pectin. Since the temperatures in our analyses did not exceed 100°C, thermal degradation temperatures for pectin were not observed.

The changes in the thermal denaturation temperatures of ground meat with and without other ingredients are presented in Table 2. The thermograms obtained from DSC for ground meat showed three peaks which were ascribed to the denaturation of myosin, sarcoplasmic proteins and actin and Tp values, for these proteins were 58.50, 64.74 and 78.72°C respectively (Table 2). Similar results were reported in the literature. Chen et al. (2007) found that transition temperatures for myosin, sarcoplasmic proteins and actin were 58.4°C, 66.6°C and 81.9°C respectively. Vasquez Mejia et al. (2018) reported that denaturation peak temperature for myosin, sarcoplasmic proteins

and actin were 54.84, 65.18 and 77.18°C. Slight differences in the denaturation temperature could be due to the variation in muscle type of the meat samples and analysis parameters studied. The enthalpy (ΔH J/g) gives information about the energy needed for denaturation of proteins. According to the results obtained the highest energy was needed for the denaturation of sarcoplasmic proteins which was 0.3296 J/g (Table 2) and the lowest enthalpy value was of myosin for ground meat.

Addition of NaCl to ground meat significantly lowered the denaturation peak temperatures ($T_p$) of myosin and actin. On the other hand, the Tp value of sarcoplasmic proteins were increased from 64.74 to 68.21°C. Graiver et al. (2006) reported that only two peaks can be observed in DSC when brining applied to meat samples with concentrations over 20g/L and that the peak temperatures for sarcoplasmic proteins and actin could not be differentiated. Furthermore $T_p$ values of actin and myosin were decreased. Similar results were obtained by Pighin et al. (2008) and by Aktaş et al. (2005) proving that addition of NaCl destabilizes myofibrillar proteins which results in a decrease in the Tp values of those proteins. According to the DSC results of low-fat emulsions only two denaturation peak temperatures were observed (Table 2). The first peak was attributed to myosin and the second one was attributed to sarcoplasmic proteins and actin. While the Tp value of sarcoplasmic proteins were increased the $T_p$ of actin was decreased. As a result the peaks could not be differentiated from each other and were observed as one peak in the thermograms. These results were in agreement with the results of Marchetti et al. (2013) and Graiver et al. (2006). The $T_p$ value of control samples which contains phosphate aside from NaCl was not significantly different from the sample containing only NaCl. It was reported by Pighin et al. (2008) and by Findlay and Barbut (1992) that when phosphates were used with more than 1% of NaCl their effects were minimized. The results revealed that the $T_p$ values of myosin for the samples containing 0.5% and 1% MC and 1% PEC was significantly lower than that of the control group. The $T_p$ values of the second peak for all the samples were significantly higher when compared to the $T_{p2}$ values of control group. Increasing the level of added CMC from 0.5% to 1% increased the $T_{p2}$ values significantly, whereas increase in the $T_{p2}$ values of samples containing MC and PEC were not significant. When the level of CMC increased from 0.5% to 1% the $T_p$ value for myosin were significantly decreased, yet increasing the level of MC and PEC had no significant effect of $T_p$ value of myosin. Morin et al. (2004) stated that CMC is an anionic water-soluble polymer and likely to interact with meat proteins via cross-linking its negatively charged carboxyl groups with the positively charged groups of amino acids in the myofibrillar proteins. In light of this, it can be argued that the changes in the denaturation temperatures of the low-fat frankfurters produced with CMC could be due to the interactions between CMC and meat proteins.

$\Delta H_T$ value gives information about the total amount of energy needed for the denaturation of all the proteins. Addition of NaCl to ground meat decreased the $\Delta H_T$ value, which was expected since myosin and actin are salt soluble proteins and destabilizes with NaCl. Even though, the total energy needed for the denaturation of proteins for control group was increased, it did not differ significantly from that of the ground meat and ground meat with NaCl. However, the increase in the $\Delta H_T$ value for low-fat emulsions was significantly different than that of the control except for the samples containing 0.5% MC and 1% PEC.

### 3.2. Quality Characteristics, Water Holding Capacity and pH results of Low-fat Frankfurters.

The moisture, fat, protein, WHC and pH result of low-fat frankfurters were given in Table 3. These results show that the moisture content of the samples varied between 70.59 and 62.02%. The highest moisture content was found for the sample produced with 1% MC and the lowest was for the sample produced with 0.5% PEC. The moisture content of the samples containing 1% MC and PEC were significantly higher than that of the control group ($p$<0.05).

On the other hand, the moisture content of the low-fat frankfurters produced with 0.5 and 1% CMC and 0.5% MC were found to be lower than that of the control but this difference lacked significance ($p$>0.05). The low-fat frankfurters had significantly lower fat content than that of the control. Among the low-fat frankfurters, the sample produced with 0.5% PEC had the highest fat content. The results show that increasing the level of hydrocolloids from 0.5% to 1% causes a decrease in the fat content of the samples. The protein content of the control group was 15.42% which was significantly higher than that of the low-fat frankfurters ($p$<0.05). The protein contents of the low-fat frankfurters varied between 12.54 – 13.75%. It was stated in the meat and meat products communique (Anonymous 2012) that for emulsifies meat products, the ratio of moisture content to protein content (M,P) and of fat content to total protein content (F,P) should be less than 6.5 and 3.2 respectively. The obtained results for M,P and F,P did not exceed this limit. It was also stated in the communique that the protein content of the emulsified meat products should not be less than 10% and the obtained results are higher than their threshold value.

**Table 3**. Moisture%, Fat%, Protein%, WHC and pH of the frankfurters. Values represent mean ± standard deviation (n=3).

| Sample | Moisture (M%) | Fat (F%) | Protein (P%) | WHC | M/P | F/P | pH |
|---|---|---|---|---|---|---|---|
| Control | $68.31 \pm 1.40^{b}$ | $24.53 \pm 1.70^{d}$ | $15.42 \pm 1.20^{b}$ | $0.42 \pm 0.14^{a}$ | 4.43 | 1.59 | $6.09 \pm 0.03^{b}$ |
| 0.5CMC | $67.43 \pm 0.73^{b}$ | $14.36 \pm 2.19^{b}$ | $13.75 \pm 0.29^{a}$ | $0.31 \pm 0.09^{a}$ | 4.90 | 1.04 | $6.17 \pm 0.01^{d}$ |
| 1CMC | $67.15 \pm 0.33^{b}$ | $14.08 \pm 0.32^{b}$ | $12.54 \pm 0.31^{a}$ | $0.47 \pm 0.24^{a}$ | 5.35 | 1.12 | $6.27 \pm 0.05^{e}$ |
| 0.5MC | $67.28 \pm 1.56^{b}$ | $11.96 \pm 1.04^{a}$ | $13.12 \pm 0.51^{a}$ | $0.27 \pm 0.10^{a}$ | 5.13 | 0.91 | $6.12 \pm 0.02^{bc}$ |
| 1MC | $70.59 \pm 0.81^{c}$ | $10.82 \pm 0.62^{a}$ | $12.73 \pm 0.30^{a}$ | $0.34 \pm 0.07^{a}$ | 5.54 | 0.85 | $6.05 \pm 0.02^{a}$ |
| 0.5PEC | $62.06 \pm 0.48^{a}$ | $18.67 \pm 0.14^{c}$ | $13.49 \pm 0.46^{a}$ | $0.39 \pm 0.11^{a}$ | 4.60 | 1.38 | $6.03 \pm 0.15^{a}$ |
| 1PEC | $70.33 \pm 0.16^{c}$ | $11.62 \pm 1.88^{a}$ | $13.25 \pm 0.40^{a}$ | $0.48 \pm 0.68^{a}$ | 5.31 | 0.88 | $6.14 \pm 0.01^{cd}$ |

WHC, Water Holding Capacity. M,P , Moisture%,Protein% F,P , Fat%,Protein%, Control, High-fat Frankfurter. κCGN, kappa carrageenan, λCGN, Lambda carrageenan, GG, Guar gum, XTH, Xanthan Gum, CHI, Chitosan.
a-e, Means with the same superscript at the same column do not differ significantly ($p$ <0.05).

Gupta and Sharma (2018) concluded in their studies that addition of different dietary fibers for production of hen meat slices, the moisture and protein contents and M,P ratio were decreased when compared to the control, which is in agreement with our study. Albeit; Gibis, Schuh and Weiss (2015) showed that addition of carboxymethyl methyl cellulose and microcrystalline cellulose for production of low-fat fried beef patties increased the moisture content, yet the fat levels were significantly lower. The differences in quality characteristics among the studies could stem from the differences in the applied processes during production. The moisture content of the control group in our study was as expected, the divergences in the moisture content of the low-fat frankfurters may result from the differences in the water binding capacities of the hydrocolloids used.

Table 3 presents the water holding capacity (WHC) of the samples. Although, the addition of hydrocolloids effected the WHC of the samples differently, these differences did not produce significant outcomes when compared with the control group. The results show that increasing the level of hydrocolloid used in the production of frankfurters increased the water holding capacity of the samples. Han and Bertram (2017) stated that addition of CMC and PEC increased the water binding of the fat reduced model systems when compared to the control. Méndez-Zamora et al. (2014) reported that the low-fat frankfurters produced with inulin and pectin showed lower WHC values than that of the control, and when the amount of pectin used was increased the WHC values were

increased as well. These findings were in agreement with our findings.

The pH values of the samples varied between 6.03 – 6.27. The lowest value was for the sample containing 0.5% PEC and the highest value was for the sample containing 1% CMC. The pH values of samples produced with 0.5 and 1% CMC, 0.5% MC and 1%PEC differed significantly from that of the control. On the other hand, the pH values of samples containing 1 % MC and 0.5% PEC were significantly lower when compared to the control. Méndez-Zamora et al (2014) reported that addition of pectin together with inulin lowered the pH of low-fat frankfurters. On the other hand, when pectin was used with carrageenan in the production of low-fat frankfurters the pH levels of samples were not significantly affected

### 3.3. Emulsion Stability and Cooking Loss

The lowest cook loss result was found to be that of the control group and the highest cook loss was for the sample produced with 0.5% MC (Table 4). The results show that addition of different additives for the production of low-fat frankfurters did not improve the cooking loss of the samples. When the quantity of hydrocolloids used were increased the cooking loss of the low-fat frankfurters were decreased because of the increased water holding capacity of hydrocolloids. Lin et al. (1988) stated that lowering the ratio of F,P would result in increased water loss and the process yield depends on the mobilization of fat and water by the proteins. However, for low-fat frankfurters the WHC and gelling properties of hydrocolloids are the main factors affecting the emulsion stability. Morin et al. (2004) also proposed that the ability of a meat system to keep water within the matrix depends on the protein network strength and the capacity of hydrocolloids the entrap water within that system. The divergence in the results in our study might have caused by the differences in the gelling properties occurred in the emulsions and the different origins of the hydrocolloids. Gibis et al. (2015) showed that when CMC was used at a level of 2 to 3% a significant decrease in the weight loss occurred when compared the control group. In our study, the amount of the hydrocolloids was not as high as in their study which could also explain the high cooking loss values. The cooking loss values of samples produced with 1% pectin were lower than other samples containing CMC and MC (Table 4), and also the moisture values were higher than other samples (Table 3). Han and Bertam (2017) pointed out that pectin fibers may cover and surround the myofibrillar proteins and lipid droplets like Chitosan, and this structure may prevent the water and fat expulsion during cooking.

Total expressible fluid results give information about the emulsion stability of the systems, showing that the higher the TEF value the greater the water and fat release from the emulsion. It was observed from the results that the emulsion stability of the low-fat emulsions were lower than that of the control. The lowest one being the sample produced with 1% PEC. Increasing the level on hydrocolloids MC and CMC increased the emulsion stability of the samples. The emulsion stability of samples containing PEC and 0.5% MC were significantly lower than that of the control. Samples containing pectin has the lowest emulsion stability among other samples. Candoğan and Kolsarici (2003) reported that pectin was not effective on improving the emulsion stability. Lurueña-Martinez et al. (2004) pointed out that differences between cooking loss and emulsion stability (TEF) could be caused by different cooking procedures applied. Although the final temperatures reached similar values for both processes', it took longer to reach the targeted temperature during the determination of cooking loss in the smokehouse. On the contrary, during the determination of emulsion stability a small amount of sample was used and brought to the desired temperature quickly which probably improved the formation and strength of the gel.

**Table 4**. Cook loss and Total Expressible Fluid results.
Values represents mean ± standard deviation (n=3).

| Sample | Cook Loss (%) | TEF (%) | F,P |
|---|---|---|---|
| Control | 6.38 | $1.00 \pm 0.01^{a}$ | 1.59 |
| 0.5CMC | 8.49 | $1.93 \pm 0.28^{a}$ | 1.04 |
| 1CMC | 8.12 | $1.83 \pm 0.73^{a}$ | 1.12 |
| 0.5MC | 9.42 | $3.34 \pm 0.26^{b}$ | 0.91 |
| 1MC | 7.31 | $1.51 \pm 0.06^{a}$ | 0.85 |
| 0.5PEC | 8.46 | $12.96 \pm 0.63^{c}$ | 1.38 |
| 1PEC | 6.62 | $13.60 \pm 0.52^{c}$ | 0.88 |

a-c, Means with the same superscript at the same column do not differ significantly ($p > 0.05$). TEF, Total Expressible Fluids, Control, High-fat frankfurter/emulsion,CMC, Carboxymetyl cellulose, MC, Methyl Cellulose, PEC, Pectin. F,P, Fat%,protein%

### 3.4. Texture Profile Analysis

The results reveal that the hardness values of the low-fat frankfurters were significantly lower ($p<0.05$) than that of the control (Table 5) except for the sample containing 0.5% PEC which was not significantly different ($p>0.05$).

**Table 5**. Texture Profile analysis.
Values represents mean ± standard deviation (n=3).

| Sample | Hardness | Springiness | Gumminess | Chewiness |
|---|---|---|---|---|
| Control | $13.23 \pm 0.34^{d}$ | $9.06 \pm 0.04^{a}$ | $5.92 \pm 0.31^{d}$ | $53.62 \pm 2.64^{e}$ |
| 0.5CMC | $10.39 \pm 0.51^{c}$ | $7.72 \pm 0.50^{a}$ | $4.38 \pm 0.18^{c}$ | $33.68 \pm 1.99^{cd}$ |
| 1CMC | $7.20 \pm 0.35^{a}$ | $8.18 \pm 0.85^{a}$ | $3.27 \pm 0.14^{b}$ | $27.12 \pm 3.64^{bc}$ |
| 0.5MC | $10.49 \pm 0.26^{c}$ | $9.07 \pm 0.01^{a}$ | $4.21 \pm 0.01^{c}$ | $38.28 \pm 0.17^{d}$ |
| 1MC | $8.58 \pm 0.37^{b}$ | $8.70 \pm 0.38^{a}$ | $2.84 \pm 0.22^{b}$ | $24.64 \pm 1.79^{b}$ |
| 0.5PEC | $12.62 \pm 0.57^{d}$ | $8.77 \pm 0.25^{a}$ | $4.31 \pm 0.52^{c}$ | $37.84 \pm 4.69^{d}$ |
| 1PEC | $6.10 \pm 0.07^{a}$ | $8.71 \pm 0.34^{a}$ | $1.72 \pm 0.05^{a}$ | $15.01 \pm 0.79^{a}$ |

a-c, Means with the same superscript at the same column do not differ significantly ($p > 0.05$). Control, High-fat frankfurter, CMC, Carboxymetyl cellulose, MC, Methyl Cellulose, PEC, Pectin.

Increasing the level of hydrocolloid used lowered the hardness values. The addition of hydrocolloids did not have a significant effect in the springiness of the low-fat frankfurters. The gumminess and the chewiness values of the low-fat frankfurters were significantly lower than that of the control ($p<0.05$). Like hardness values, increasing the quantity of hydrocolloids used decreased the gumminess and chewiness values. TPA results of our study were in agreement with the findings of Han & Bertram (2017), Méndez-Zamora et al. (2014) who showed that addition of hydrocolloids lowered the hardness, gumminess and chewiness values. Schuh et al. (2013) suggested that the decrease in the firmness of the low-fat frankfurters may be due to the destabilization of batter with the addition of CMC and PEC. As a result of this no coherent protein network was formed upon heating. The same effect could also be the reason for the lower hardness value of MC added samples.

### 3.5. Scanning Electron Microscopy Results

SEM results of the control group and the low-fat frankfurters were presented in Figure 1. It was observed from the results that a three-dimensional structure was formed with large or small holes in the control group (Figure 1a). Similar structures were observed by Li and Yeh (2002) who stated that these holes were formed due to the disruption of starch molecules during heat treatment. Results of samples containing CMC and MC showed clusters which probably belongs to those hydrocolloids indication of no-gel like structure formation. Samples produced

with MC showed cavities in the results. When the concentration of hydrocolloids CMC and MC was increased from 0.5% to 1% the number of those clusters like structures were increased. The SEM results of sample produced with 0.5% PEC resembled to the SEM results of the control group.

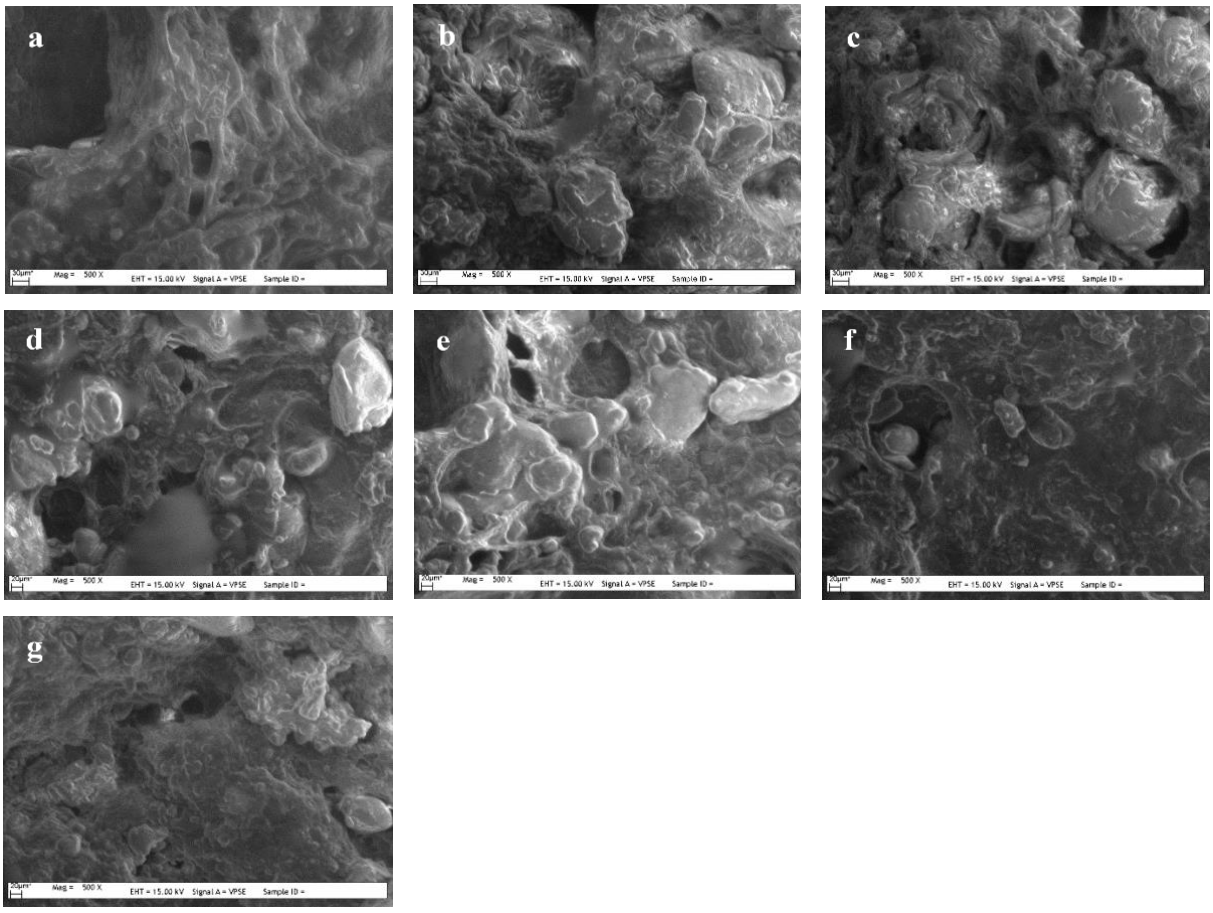


**Figure 1**. SEM images of the frankfurters.
a,Control, b, 0.5% CMC, c, 1% CMC, d, 0.5%MC, e, 1% MC, f, 0.5%PEC, g, 1% PEC containing samples

On the other hand, when the concentration of PEC was increased to 1% the structure formed was very different from all the low-fat frankfurters and the control. The hardness value of the sample 1PEC was the lowest and the samples were least acceptable among others, which indicate that the structure was not as requested.

Taking the hardness values into consideration together with SEM results, it was remarked that the sample with the hardness value closest to the control group (0.5% PEC) showed SEM results similar to that of the control. Moreover, total acceptability of these samples was also close to that of the control group. The protein networks in emulsified meat products are mainly formed by myofibrillar proteins (actin and myosin) which are soluble in the presence of NaCl (ionic strength). This network is formed during the heat treatment applied during production, which enhances the binding and consistency of the system (Li-Chan et al. 1984, Tornberg 2005). This system is affected by ionic strength, pH, formulation and temperature (Gibis et al. 2015). Gibis et al. (2017) stated that CMC at low concentration (0.5%) did not suffice to form a coherent protein network, and when the concentration was increased the fibers were able to retain the added water. On the other hand, Chattong et al. (2007) reported that no changes were observed when CMC was used at a level of 1%.

### 3.6. Colour Measurement Results

The lightness and redness values of the low-fat frankfurters were not significantly affected by the addition of hydrocolloids (Table 6, $p>0.05$). On the other hand, yellowness (b*) of the samples containing 0.5 and 1% CMC and 1% MC were significantly higher than that of the control ($p<0.05$). Increasing the level of hydrocolloids decreased the redness (a*) values of low-fat frankfurters, on the contrary increased the yellowness (b*) values, yet without a significance ($p>0.05$). Kim et al. (2016) found in their study that addition of pectin obtained by different methods decreased L* while increasing the b* value of low-fat meat emulsions. On the other hand, a* values were affected differently by the addition of pectin obtained by different methods.

Consumers demand the frankfurters to have pink-red colour which is a major factor influencing the purchase rates of the meat products. Although the colour measurement results showed no significant change in the redness of the samples, the sensory evaluation scores (Table 7) showed that the colour results of samples containing 0.5% CMC, 1% CMC, 1% MC and 1% PEC were significantly lower than that of the control which was not acceptable by the consumers.

**Table 6**.Color measurement results of frankfurters. Values represents mean ± standard deviation (n=3).

| Sample | Lightness (L*) | Redness (a*) | Yellowness (b*) |
|---|---|---|---|
| Control | $59.41 \pm 0.17^{ab}$ | $14.35 \pm 0.22^{a}$ | $15.44 \pm 0.53^{a}$ |
| 0.5CMC | $59.85 \pm 0.49^{b}$ | $14.63 \pm 0.32^{a}$ | $17.18 \pm 0.32^{cd}$ |
| 1CMC | $60.17 \pm 0.72^{b}$ | $14.05 \pm 0.52^{a}$ | $17.51 \pm 0.49^{d}$ |
| 0.5MC | $59.30 \pm 0.18^{ab}$ | $14.71 \pm 0.08^{a}$ | $16.02 \pm 0.53^{ab}$ |
| 1MC | $57.91 \pm 0.62^{ab}$ | $14.04 \pm 0.40^{a}$ | $16.53 \pm 0.90^{bc}$ |
| 0.5PEC | $59.76 \pm 0.27^{b}$ | $14.13 \pm 0.14^{a}$ | $15.35 \pm 0.28^{ab}$ |
| 1PEC | $59.53 \pm 0.69^{b}$ | $14.35 \pm 0.10^{a}$ | $16.00 \pm 0.49^{a}$ |

a-d, Means with the same superscript at the same column do not differ significantly ($p > 0.05$). Control, High-fat frankfurter, CMC, Carboxymetyl cellulose, MC, Methyl Cellulose, PEC, Pectin.

### 3.7. Sensory Evaluation Results

According to the sensory evaluation of the samples the outer appearance of the samples produced with 1% PEC was significantly lower than that of the control (Table 7, $p<0.05$). The TPA and SEM results of this sample also showed a poor formation of structure being the least acceptable sample by the consumers. Outer appearances of other samples were not significantly different from than that of the control ($p>0.05$). Contrarily, the colour and texture results were significantly lower when compared to the control except for the samples containing 0.5% MC and 0.5% PEC.

**Table 7**. Sensory Evaluation Results. Values represents mean ± standard deviation.

| Sample | Outer appearance | Color | Texture | Flavor | Total Acceptability |
|---|---|---|---|---|---|
| Control | $7.1 \pm 0.40^{bc}$ | $7.3 \pm 0.30^{b}$ | $7.0 \pm 0.36^{bc}$ | $6.6 \pm 0.54^{ab}$ | $6.9 \pm 0.30^{b}$ |
| 0.5CMC | $6.3 \pm 0.39^{ab}$ | $5.9 \pm 0.43^{a}$ | $5.8 \pm 0.44^{ab}$ | $6.1 \pm 0.37^{ab}$ | $5.9 \pm 0.34^{a}$ |
| 1CMC | $6.0 \pm 0.29^{a}$ | $5.4 \pm 0.22^{a}$ | $5.5 \pm 0.42^{a}$ | $6.2 \pm 0.29^{ab}$ | $5.7 \pm 0.14^{a}$ |
| 0.5MC | $7.7 \pm 0.30^{c}$ | $7.7 \pm 0.30^{b}$ | $6.3 \pm 0.66^{abc}$ | $6.9 \pm 0.50^{b}$ | $7.0 \pm 0.42^{b}$ |
| 1MC | $6.2 \pm 0.32^{ab}$ | $5.8 \pm 0.38^{a}$ | $5.6 \pm 0.42^{a}$ | $5.7 \pm 0.21^{ab}$ | $5.8 \pm 0.28^{a}$ |
| 0.5PEC | $7.3 \pm 0.15^{c}$ | $7.1 \pm 0.34^{b}$ | $7.2 \pm 0.41^{c}$ | $6.6 \pm 0.26^{ab}$ | $7.0 \pm 0.26^{b}$ |
| 1PEC | $5.6 \pm 0.22^{a}$ | $5.3 \pm 0.33^{a}$ | $5.1 \pm 0.17^{a}$ | $5.6 \pm 0.37^{a}$ | $5.4 \pm 0.22^{a}$ |

a-c, Means with the same superscript at the same column do not differ significantly ($p > 0.05$). Control, High-fat frankfurter, CMC, Carboxymetyl cellulose, MC, Methyl Cellulose, PEC, Pectin

The flavour scores of the low-fat frankfurters were not significantly affected from addition of different hydrocolloids. Total acceptability of the samples 0.5CMC, 1CMC, 1MC and 1PEC were significantly lower than the controls. In contrast, containing 0.5% PEC and 0.5% MC were the most acceptable ones which also had the highest hardness values. Furthermore, SEM results showed that the sample containing 0.5% PEC resembles the most to the SEM results of the control. The colour scores of the samples 0.5PEC and 0.5MC were also significantly different than other low-fat frankfurters. It was observed from the panellists preferences that low-fat frankfurters produced with PEC and MC at a level of 0.5% was more acceptable than the other low-fat frankfurters providing a close score to the total acceptability of the control.

## 4. Conclusions

Addition of different hydrocolloids at varying levels to low-fat frankfurters effected the quality properties differently. This effect was arguably caused mainly by the different sources of fibers and different behaviours of additives under certain pH, ionic strength and temperature. Increasing the level of hydrocolloids used in this study enhanced neither the acceptance of the final product in general nor the quality characteristics, yet it enhanced the moisture and cook loss. Low-fat frankfurters produced with methyl cellulose and pectin at a concentration of 0.5% were the most acceptable among the frankfurters. However, the cook loss of these samples was higher in comparison to others. Considering these effects, the level of the hydrocolloid should be determined carefully. The DSC results showed three distinct peaks for meat which were myosin, sarcoplasmic proteins and actin. While the addition of NaCl to the meat increased the denaturation temperature of sarcoplasmic proteins; destabilized the myofibrillar proteins and as a result their denaturation temperatures were decreased. Addition of phosphates and starch along with NaCl caused the peaks of sarcoplasmic proteins and actin to merge. Addition of different hydrocolloids increased the denaturation peak temperature of the second peak, on the other hand the denaturation peak temperature of myosin was either increased or decreased depending on the additive used. The changes in the denaturation temperatures of the meat proteins occurred due to the addition of hydrocolloids are accepted as a sign of interactions between proteins and the hydrocolloid. DSC is a promising method for the determination of interactions between hydrocolloids and proteins. For a detailed analysis the meat proteins could be extracted and their interactions with different hydrocolloids from different sources could be examined. It should also be considered to study different ionic strength, pH values and concentrations since hydrocolloids may behave differently under different conditions.

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# MECHANICAL SCARIFICATION OF QUINOA SEEDS (*CHENOPODIUM QUINOA* WILLD.) AND OBTAINING OIL FROM THE SEED COAT BY SUPERCRITICAL $CO_2$ EXTRACTION

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**ABSTRACT**

The paper presents development of a high-performance process for extracting oil from quinoa seeds (*Chenopodium quinoa* Willd.) by supercritical $CO_2$ extraction. The pre-treatment of seeds involving mechanical scarification was applied. The abrasive material gradation and time of abrasion were optimized. Optimum scarification conditions were obtained using the abrasive gradation P40 at scarification time of 50-100 min. Under these conditions, a seed coat was obtained in an amount of 10% of the seed weight and it contained 20 g oil/100 g seed. The oil was separated from the seed coats by supercritical fluid extraction. Approximately 61% of oil recovery was obtained under extraction conditions: pressure 25 MPa, temperature 40° C and extraction time 120 min. From seeds containing 5.6 g oil/100 g seed, after scarification and extraction with supercritical carbon dioxide, approx. 1.2 g oil/100 g seed was obtained from the seed coat.

## 1.Introduction

Oils from seeds, fruits and other raw materials are obtained by various methods. The conventional method of oil extracting from seeds with high content of oil, such as rapeseed and sunflower, is expression without heating or expression with heated press (Bogaert *et al.*, 2018; Savoireet *et al.*, 2012). However, in many cases, complex methods are used including pressing, heating, solvent extraction, ultrasounds and microwaves pretreatments or enzymatic extraction (Koubaa *et al.*, 2016, Kumar *et al.*, 2017). Oil content in quinoa is at a low level ranging from 2 to 9.5 g oil/100 g seed, with an average of 5.0–7.2 g oil/100 g seed, therefore, in order to achieve high extraction efficiency, complex extraction methods are required.

Quinoa (*Chenopodium quinoa* Willd.) is a food plant of the family *Amaranthaceae*, subfamily *Chenopodiaceae* and genus *Chenopodium*. The edible seeds of quinoa are small, round and flat, and they may measure from about 1.5 mm in diameter to 2.5 mm (Koziol, 1993; Maradini-Filho, 2017; Novak et al., 2016; Vega-Gálvez *et al.*, 2010).

Quinoa is a pseudocereal of Andean origin and is used principally in the same manner as wheat and rice (Maradini-Filho, 2017). Quinoa has been considered as an alternative oilseed crop, due to the quality and quantity of its lipid fraction being rich in essential fatty acids such as linoleic and α-linolenic and contains high concentrations of natural antioxidants such as α- and γ-tocopherol (Bhargava, 2006; Koziol, 1993; Maradini-Filho, 2017; Vega-Gálvez *et al.*, 2010). Quinoa is also an excellent example of "functional food" which may help to reduce the risk of various diseases. Its functional properties

may be related to the presence of fibres, minerals, vitamins, fatty acids, antioxidants and phytonutrients, which favourably contribute to human nutrition (Hager *et al.*, 2014; Maradini-Filho, 2017; Miranda *et al.*, 2010; Vega-Gálvez *et al.*, 2010).

Thus, quinoa is an alternative ingredient in the gluten-free diet and can be used by persons who suffer from celiac disease (Cordeiro *et al.*, 2012). Furthermore, due to the relatively high protein content with very favourable amino acid composition, it is a potential raw material for obtaining protein preparations (isolates, concentrates), which in turn can be used to enrich food (Bastidas *et al.*, 2016; Hager *et al.*, 2014). Quinoa can be eaten as a wheat replacement, as a hot breakfast cereal or can be boiled in water to make infant cereal food. The seeds can even be popped like popcorn. Seeds can be ground and used as flour (bakery products, noodles, flakes etc.).

Important methods of seed scarification include heat, freeze-thaw, mechanical and acid scarification (Bastidas *et al.*, 2016; Kimura and Islam, 2012). Heat scarification is the method that uses high temperatures to break seed coat. Freeze-thaw scarification is a method that breaks the seed coat by exposing seeds to temperature alternations between low and high. Acid scarification is chemical method to melt seed coat and soften hard seed (Kimura and Islam, 2012). However, due to ecological reasons, mechanical processes are used. The mechanical processing concerns abrasion of the outer layer of the seed coat and can be carried out using a thresher or scarifier with sandpaper. In the case of quinoa, this process is not easy to perform due to the small size of seeds. The scarification of quinoa seed is often applied before sowing. Seed scarification reduces the thickness and weakens the strength of seed coat, which improves germination (Alderete-Chávez *et al.*, 2010; Kimura and Islam, 2012; Martin *et al.*, 2013; Rostami and Shasavar, 2009). The residue after the scarification of seeds is used as an additive to animal feed.

Due to concentration of oil in the outer layer of seeds (pericarp) (Prego *et al.*, 1998), studies were carried out on the possibility of extracting oil from the coats by using supercritical fluid extraction (SFE). SFE is widely used as an alternative to traditional techniques like mechanical pressing, organic solvent extraction. The supercritical carbon dioxide (SC-$CO_2$) is the most common solvent because of its unique properties, namely, it is non-flammable, non-toxic, inexpensive, cost efficient and high selectivity to non-polar molecules such as oils (Rai *et al.*, 2016). Its solvent properties can be changed dramatically with small changes in pressure and temperature. SC-$CO_2$ also separates easily from the extract, once the pressure is released, leaving no traces on the extract (Benito-Román *et al.*, 2018). One possibility under consideration is to use supercritical fluids technology which provides extraction yields very similar to those obtained by conventional liquid solvents extraction processes in which solvent-free extracts are obtained in relatively mild conditions which avoid thermal degradation, thus making it the ideal solvent for natural products (Follegatti-Romero *et al.*, 2009; Gracia *et al.*, 2011). In previous papers, application of SFE for extraction of oil and oil with increased amount of tocopherols was presented (Przygoda and Wejnerowska, 2015; Wejnerowska and Ciaciuch, 2018). Under optimum conditions, high oil recovery was obtained (~ 89%) and content of tocopherols in oil was increased from ~ 73 to 336 mg tocopherol/100 g oil. Regardless of the method of extraction (extraction with solvent or SFE) and type of oil extracted, no significant differences were found in profile of fatty acids (Benito-Román *et al.*, 2018; Follegatti-Romero *et al.*, 2009; Wejnerowska and Ciaciuch, 2018). Additionally, quinoa oil extracted using $CO_2$ presented higher antioxidant capacity and tocopherol content than quinoa oil extracted with solvent (hexane), regardless the quinoa variety used (Benito-Román *et al.*, 2018).

The paper presents result of the studies on the method of oil extraction from seed coat after seed pre-treatment by mechanical scarification. In order to recover oil, the seed coats obtained

as a result of mechanical scarification was subjected to extraction with supercritical carbon dioxide.

## 2. Materials and methods

### 2.1. Materials

Quinoa seeds (*Chenopodium Quinoa* Willd.) were bought from a local shop (country of origin Bolivia). Total humidity of seeds was determined by moisture analyzer MA30 (Sartorius, Germany) and was equal to 8.2%. Size of seeds before grinding was 1.6-2.0 mm (91%). Size of seeds and ground seeds was determined by performing sieve analysis Fritsch analysette 3 (RoTH, Germany). For sieve analysis, sieves with mesh sizes from 0.5 to 2.24 mm were used.

### 2.2. Reagents and standards

Carbon dioxide (99.5%) was obtained from Linde Gas (Poland). HPLC grade *n*-hexane and ethanol were purchased from Merck (Darmstadt, Germany).

The fine-grain sand for Soxhlet and SFE was sieved on sieves and fractions of 0.2–0.3 mm were collected. Then, the sand was purified by successive elution with warm distilled water, methanol and hexane. The sand was dried after each stage of elution.

### 2.3. Soxhlet extraction

Samples of 10 g o

f ground quinoa seeds were weighted with accuracy of 0.0001 g and then were mixed with 10 g of sand to determine the oil content by Soxhlet extraction using *n*-hexane at 60 °C for 16 h. After extraction, *n*-hexane was evaporated under vacuum at 40 °C and subsequently the solvent was totally removed by nitrogen steam. After evaporation of solvent, the oil content was determined gravimetrically. The mass of extracted oil was assumed to be 100% of the extractable matter (Przygoda and Wejnerowska, 2015; Wejnerowska and Ciaciuch, 2018).

### 2.4. Scarification of seeds

The working chamber of the scarifier (designed in our laboratory) was made of stainless steel and inside covered with replaceable, fine-grained abrasive material. Moreover, a flexible clamp supporting the abrasion of seeds was mounted. After starting the device, the chamber with a diameter of 40 cm rotated at a speed of 20 rpm. 100.0 g of seeds were poured into the chamber and after the cover was applied, the abrasion process was started. The principle of the scarifier operation consists in rubbing the seeds by rotating abrasive material at the moment of passing under the pressing down element. Scarification was carried out at constant drum revolutions, changing the time (10, 50 and 100 min) and using three types of abrasive gradations P40 (425 μm), P60 (269 μm) and P80 (201 μm). Scarification was performed three times for each time and abrasive gradation. Seeds that were not subjected to scarification were accepted as a control sample. After scarification, the seeds along with the seed coats were removed from the chamber by means of a manual vacuum cleaner and sieve analysis of the obtained material was performed. Based on the results of sieve analysis of the tested samples, the equivalent diameter (d) and content of fractions in the set were calculated. The particle size distribution of the tested material was approximated by Rosin, Rammler, Sperling and Bennet (RRSB) distribution function:

Where:

$$\sum R = e^{\left(-\frac{d}{d^*}\right)^n} \quad (1)$$

ΣR – total residue on the sieve,

d – diameter of sieve fraction of seed weight, defined as a geometric mean of mesh sizes of two adjacent sieves [mm],
d* – mean of linear dimensions of all seeds in a set [mm],
n – uniformity coefficient of graining.

Efficiency of the seed coat abrasion was investigated depending on time of scarification and abrasive gradation. The efficiency was expressed in amount of subscreen fraction obtained (diameter < 500 μm) (Table 1). The results were statistically analysed using one-way and multivariate analysis of variance (ANOVA) and Tukey's test to determine the significance of differences between variables.

In order to assess the effects of mechanical scarification, microscopic observation, analysis of oil content in the obtained seed coats and quantity of coat were used. A polarizing light microscope Eclipse E400 POL (Nikon, Japan) equipped with camera adapter for enlarged photos (20 x) was used for microscopic observations.

### 2.5. Supercritical fluid extraction procedures

A laboratory-scale SFE system Lizard 2001 SEKO-K s.r.o (Brno, Czech Republic) was used in this study. The seed coats (fraction < 0.5 mm) was loaded into the extractor cell of 1.2 mL capacity (0.5 cm internal diameter (I.D.) and 6.1 cm of effective height). 1 g of sample containing sand and seed coats at different ratios i.e. 1:1 - 1:3 (coat:sand w/w) were located into the cell and the content of cell was stirred for 5 min by use of rotary stirrer. The modifier (ethanol) was spiked directly into the sample in the extraction vessel before the extraction cell was attached to the SFE system. The extract was collected into 12 mL vials (previously weighted). The experiments were carried out in temperature 40 °C, at pressure 25 MPa and time 60, 90 and 120 min.

The adjustment of $CO_2$ flow is not possible in this SFE system. Measurements of $CO_2$ flow rate were performed at the end of capillary (restrictor) with diameter of 45 μm and length of 7 cm. The SC-$CO_2$ flows were dependent on extraction conditions and they were within the range from 15 to 22.0 L/h.

The extraction yield was determined by comparing the weight of oil obtained by SFE with the weight of oil obtained by Soxhlet extraction. All experimental results reported are average values from three repeated independent experimental runs.

## 3.Results and discussions

### 3.1. Scarification of quinoa seeds

The aim of studies was to carry out mechanical scarification in conditions allowing to remove as much of the seed coat as possible while maintaining the proper seed structure. Seeds after scarification can be used for food purposes and for sawing.

The scarification optimization was performed taking into account variable work parameters, i.e. time of scarification (10, 50 and 100 min) and the thickness of abrasive used (P40, P60 and P80). After each scarification process, a sieve analysis was performed, making allowance for the obtained amount of subscreen fraction. In the next stage of testing, oil was extracted from the subscreen fraction. Due to the small amount of obtained subscreen fraction after 10 min of scarification, regardless of the abrasive used, these results were not taken into consideration in statistical analysis of results.

**Table 1.** Coefficients of the RRSB equation

| | **Abrasive gradation** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **P80** | | | **P60** | | | **P40** | | |
| **Time (min)** | 0 | 10 | 50 | 100 | 10 | 50 | 100 | 10 | 50 | 100 |
| **d* (mm)** | 1.87 | 1.84 | 1.88 | 1.83 | 1.84 | 1.82 | 1.80 | 1.78 | 1.72 | 1.72 |
| **n** | 11.06 | 9.47 | 7.49 | 7.16 | 9.47 | 7.29 | 6.66 | 6.27 | 5.35 | 4.45 |
| **$R^2$** | 0.93 | 0.99 | 0.91 | 0.92 | 0.99 | 0.94 | 0.90 | 0.89 | 0.86 | 0.76 |

The seed coat layer obtained as a result of the scarification is a subscreen fraction of < 0.5 mm, the rest of the fraction are the different size seeds devoid of a hard shell (coat). The results of screening of control and scarified materials were subjected to linear regression analysis by determining the RRSB equation coefficients (Table 1). The calculated parameters indicate a good fitting of the RRSB model. The average grain diameter in the set (d*) decreases gradually with decreasing abrasive gradation and increasing time of scarification. The n parameter informing about the uniformity of the grain set is the smallest for the longest scarification time and the lowest abrasive gradation. It can be noticed that the equation well describes the granulometric distribution studied (high values of $R^2$).

Statistical analysis of the effect of the scarification time and the abrasive type on the amount of subscreen fraction obtained (Table 2) was carried out. Both the abrasive gradation and time of scarification have a statistically significant high impact on the amount of subscreen fraction obtained. Similarly, the analysis showed a highly statistically significant interaction between abrasive gradation and scarification time.

**Table 2.** Multifactorial analysis of variances with interactions (anova $\alpha = 0.01$) of the effect of abrasive gradation and time of scarification on the amount of subscreen fraction obtained

| **Factor** | **Statistical value** | |
|---|---|---|
| | ***F*** | ***p*** |
| **Abrasive gradation** | 2153.370 | < 0.0001 |
| **Time of scarification** | 331.464 | < 0.0001 |
| **Abrasive gradation × Time of scarification** | 152.992 | < 0.0001 |

The mean results of the amount of subscreen fraction obtained depending on the scarification parameters used were presented in Table 3. The statistical analysis showed no significant differences between the control sample (no scarification) and the samples subjected to scarification with abrasive gradation of P80. Similarly, no differences were found for abrasives gradation of 60 in time of 50 min. Abrasive gradation of P40 resulted in a larger amount of subscreen fraction compared to the other gradations in each applied scarification time. The highest share of subscreen fraction was obtained by scarifying quinoa seeds with abrasive gradation of 40 for 100 min (9.32%).

The amount of subscreen fraction increases on the average two- or four-fold with the longer scarification time for 50 and 100 min, respectively. The results presented in Table 3 show that due to the amount of subscreen fraction obtained, the most favourable is application of abrasive with gradation of P40 and scarification time of 100 min. According to our observations, it is the most favourable to remove 10% by weight of the outer layer of seeds (seed coat). The optimum conditions of scarification are indicated by a high oil content in the separated seed coat (Table 4) and the results of microscopic observations. This is clearly illustrated in Fig. 1 where the whole seeds (1a), seeds after scarification (1b), seeds after scarification and separation of seed coats (1c) and separated seed coats (1d) are presented. It can be distinctly seen in Fig. 1(c) that the seeds were not damaged in scarification process and the oil-rich layer of endosperm was separated from the seeds.

The selected fractions were subjected to analysis of oil content by Soxhlet method (Table 4). Satisfactory results were obtained which indicated that seed coats obtained by scarification (fraction < 0.5 mm) contained 20.0 g oil/100 g seed coats and in the other combined

fractions (0.5-2.4 mm) the oil content decreased from 5.6 (whole seeds) to 3.3 g oil/100 g seed.

**Table 3.** The amount of subscreen fraction obtained depending on the abrasive gradation and time of scarification

| Abrasive gradation | Time of scarification [min] | Subscreen fraction [%] |
|---|---|---|
| **control** | - | $0.60^{a} \pm 0.06$ |
| **P80** | 50 | $0.63^{a} \pm 0.05$ |
| **P80** | 100 | $0.84^{a} \pm 0.07$ |
| **P60** | 50 | $0.70^{a} \pm 0.09$ |
| **P60** | 100 | $1.61^{b} \pm 0.24$ |
| **P40** | 50 | $5.39^{c} \pm 0.27$ |
| **P40** | 100 | $9.32^{d} \pm 0.29$ |

a, b, c, d – values denoted with the same letter indicate belonging to a homogeneous group that does not differ statistically at $\alpha = 0.05$.

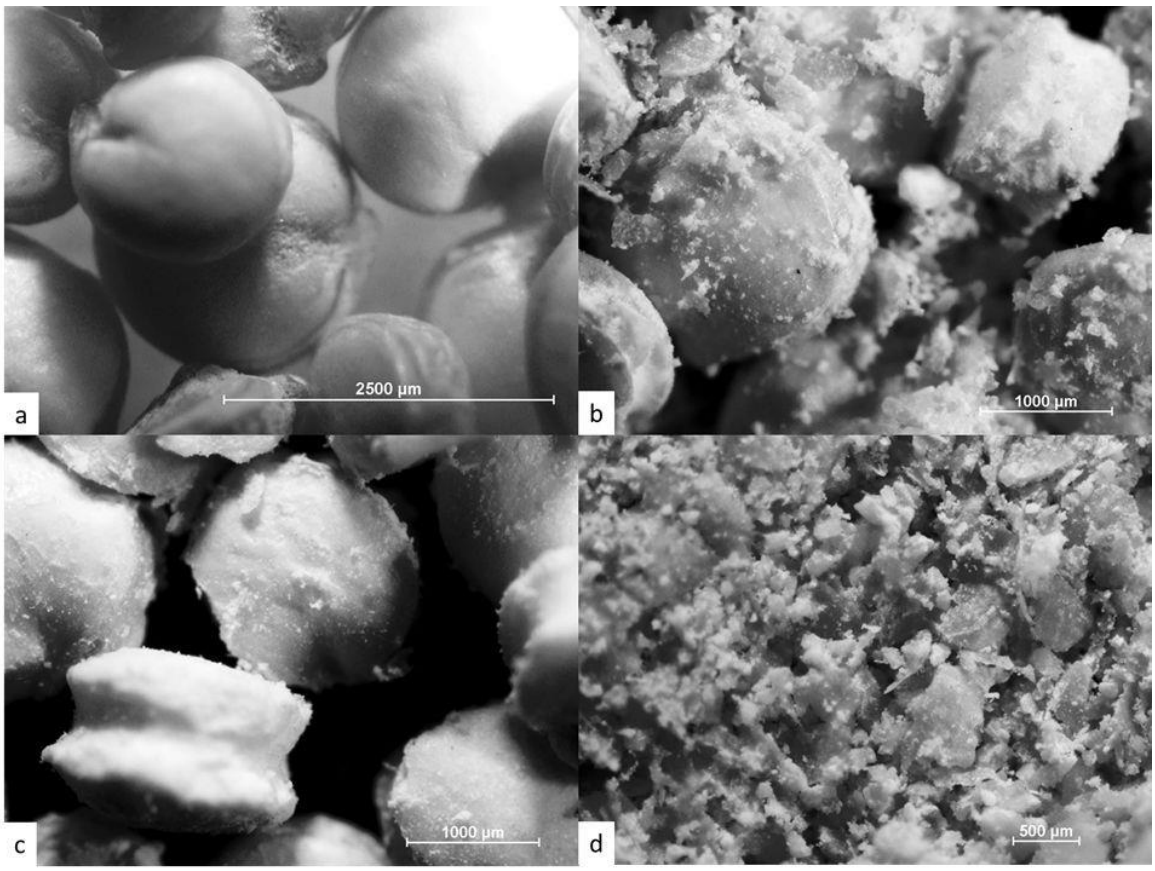


**Figure 1**. Quinoa seeds, a – whole seeds (control), b – seeds after abrasion (P40 grad., 100 min.), c – 0.5–2.25 mm, d – subscreen fraction (seed coat) < 0.5 mm

**Table 4.** Oil content (soxhlet method) in seeds and in the selected fractions after scarification of quinoa seeds for 100 min

| Operating conditions | Particle size (mm) | Oil content (Soxhlet) (%) |
|---|---|---|
| **Control (no scarification)** | > 2.24-0.5 | 5.6 ± 0.2 |
| **Seed coat scarified. P60** | > 2.24-0.5 | 5.5 ± 0.2 |
| **Seed coat scarified. P40** | > 2.24-0.5 | 3.3 ± 0.1 |
| **Seed coat scarified. P40** | < 0.5 | 20.0 ± 0.9 |

### 3.2. Supercritical fluid extraction

As a result of scarification of quinoa seeds, the material containing over three times (20.0 g oil/100 g seed coats) more oil was obtained compared to whole seeds (5.6 g oil/100 g seed).

The oil contained in the seed coats obtained by scarification was subjected to extraction with supercritical carbon dioxide. Optimization studies on oil separation from quinoa seeds have been presented in our previous paper (Wejnerowska and Ciaciuch, 2018). The most favourable parameters for extraction with supercritical $CO_2$ were determined during our previous studies. These parameters were also applied for extraction of oil from the seed covers, i.e. temperature of 40 °C and pressure of 25 MPa. In order to increase the extraction efficiency, ethanol was used as cosolvent in the amount of 10% w/w. The tests were carried out at a variable extraction time of 60, 90 and 120 min. Our previous studies showed that it was preferable to add an inert filling (sand) to a material such as ground seeds (Przygoda and Wejnerowska, 2015; Wejnerowska and Ciaciuch, 2018; Wejnerowska *et al.*, 2013). Extraction of oil from seed coats, obtained as a result of scarification, were carried out with addition of various amounts of filling i.e. 1:1-1:3 (coat:sand, w/w). The amount of oil obtained and the efficiency of SFE process are shown in Table 5.

**Table 5.** Amount of extract obtained from seed coats and degree of recovery depending on time of extraction and amount of inert filling added (25 MPa, 40 °C and 10% w/w ethanol as cosolvent)

| Seed coat:inert filling [w/w] | Amount of oil obtained [g/100 g of coat] | | | Yield [% w/w] | | |
|---|---|---|---|---|---|---|
| | 60 min | 90 min | 120 min | 60 min | 90 min | 120 min |
| **0** | 5.2 ± 0.2 | 6.4 ± 0.3 | 8.4 ± 0.4 | 24.5 ± 1.1 | 28.6 ± 1.2 | 40.1 ± 1.8 |
| **1:1** | 7.0 ± 0.3 | 8.2 ± 0.4 | 11.7 ± 0.5 | 35.1 ± 1.6 | 40.9 ± 1.8 | 58.5 ± 2.9 |
| **1:2** | 11.4 ± 0.5 | 11.8 ± 0.6 | 12.2 ± 0.7 | 57.2 ± 2.7 | 59.0 ± 3.0 | 60.9 ± 3.2 |
| **1:3** | 10.1± 0.5 | 10.1 ± 0.4 | 10.4 ± 0.5 | 50.5 ± 2.3 | 50.6 ± 2.4 | 52.2 ± 2.5 |

A positive effect of an inert filling addition was observed in the case of the seed coats. Addition of filling to the seed coat in a ratio of 1:2 results in an approx. 100% increase in extraction efficiency compared to extraction carried out without addition of filling. This is related to looseness of the batch structure and facilitated penetration of supercritical carbon dioxide between its particles. On the other hand, too much (1:3) of filling added results in a slight decrease in the yield.

A longer time of extraction caused that more oil from extracted material was obtained. Extending the extraction time from 60 min to 120 min has a significant effect on extraction efficiency if extraction is carried out without addition of filling or if it is in amount of 1:1. It can be concluded that adding the optimum amount (1:2) of filling to the seed coat results in maximum process efficiency in a shorter time. Extraction of oil from seed coat of quinoa with supercritical $CO_2$ allowed us to obtain about 12

g of oil from 100 g of seed coats. In the case of oil extraction from quinoa seeds, we received a maximum of 6.7 g of oil from 100 g of seeds (Wejnerowska and Ciaciuch, 2018). Taking into account that the coasts of supercritical extraction conducted on industrial scale are high, it is much more advantageous to extract from the material (seed coats) containing a larger amount of oil.

## 4. Conclusions

The paper presents a high-performance method of using seed coats of quinoa seeds to obtain an oil. Mechanical pretreatment with scarification prior to extraction was used. As a result of our tests, the optimum amount of seed coat which can be removed by any mechanical scarification method was determined and it was 9-10% of the seed weight. Seeds subjected to scarification can be used for seeding or after removal of hard shell and bitter saponins can be used for food purposes. A valuable product that is obtained as a result of scarification is a seed coat containing large amounts of oil (20 g oil/100 g seed coats). For this purpose, supercritical fluid extraction with supercritical carbon dioxide was applied. The recovery of oil from the seed coats was approx. 60% and the amount of oil obtained from 100 g of the coats was approx. 12 g.

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# ASSESSMENT OF PHYSICAL, FRICTIONAL AND AERODYNAMIC PROPERTIES OF CHAROLI (*Buchanania Lanzan Spreng*) NUT AS POTENTIALS FOR DEVELOPMENT OF PROCESSING MACHINES

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*Angle of repose.*

**ABSTRACT**

Charoli (*BuchananialanzanSpreng*) has a sustainable economic potential due to its nutritional and medicinal values. The moisture dependent physical, frictional, and aerodynamic properties play a key role while processing food and designing a processing machine. This study determined various physical, frictional, and aerodynamic properties of Charoli (*BuchananialanzanSpreng*) nut at increasing moisture content, and the importance of processing, machine designing were discussed. Different properties of Charoli nut were examined at 9.06, 10.92, 12.51, 15.29, and 17.86 % (d.b) moisture content. Various axial dimensions as length, width, and thickness revealed a linear increment by nut moisture content. The value of true and bulk density reduced as of 657.23 to 578.32 kg $m^{-3}$ and 917.94 to 851.21 kg $m^{-3}$, respectively, while sphericity and porosity increased from 86.42 to 88.64 %, and 28.40 to 32.06 %, respectively. The coefficient of static friction increases linearly and found highest for rubber. The value of static and dynamic angle of repose increased significantly as of 16.52° to 22.31° and 27.91° to 33.23° respectively. Also, the linear increment was noted in terminal velocity from 13.21 to 14.94 m/s by increasing moisture content. Data obtained by this study will be very much helpful for the development of novel equipment, which will be valuable for operations like separation, grading, cleaning, sorting, deshelling, packaging, and storage structures for Charoli (*BuchananialanzanSpreng*) nut.

## 1.Introduction

Charoli has a place with the family Anacardiaceae and mostly found in deciduous forests throughout India (Hiwale 2015). The plant expands on yellow sandy–topsoil and is regularly located inside the western, northern, and central India, notably inside the forest zone of Andhra Pradesh(AP), Bihar(BR), Chhattisgarh(CG), Gujarat(GJ), Jharkhand(JH), Maharashtra(MS), Madhya Pradesh(MP), and Uttar Pradesh(UP) (Pandey 1985). Along with India, this plant also observed globally, like in Burma and Nepal (Hemavathy and Prabhakar 1988).

The Charoli fruits grown–up in four to five months and are manually harvested in the summer season, generally in April / May(Shelare et al. 2020). Fig. 1 (a) shows the freshly harvested Charoli fruits and Fig. 1 (b) shows the Charoli nuts after expelling the skin. Fully matured Charoli fruits become blackish after storage. This blackish skin must be expelled prior to deshelling. For expelling, fruits are kept in water for a night and scrubbed within palms or by a jute sack. Again this is washed

with fresh water to get cleaned nuts. These nuts are then dried in solar light and put away for further processing, i.e., deshelling (Kumar et al. 2012).

It is a medicinally valuable tropical tree species and an essential cause of living for the bounded tribal community (Dhande et al. 2020). Practically all the parts of this plant are used for the therapy of numerous disorders (Jawalekar and Shelare, 2020). The Charoli kernel has a high amount of protein(19.0 – 28.19 %) which is exceptionally nutritious. The kernel yields sweet oil (33.50 %), of which 1.90 % is unsaponifiable. The 20.00 % of linolenic acid found in the saponifiable part. Charoli oil is healthy and fit for the consumption of humans (Banerjee and Jain 1988). Table 1 shows the proximate composition of Charoli, Sesame, Almond, Cashew, and Walnut kernals. Compared to other dried fruits, Charolikernals have significant fat, protein, ash, fiber, starch, and carbohydrate that is very significant for human health.

**Table 1.** Proximate composition of some dried fruit kernals

| Reference | Nut | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Fiber (%) | Starch / Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| (Sahu et al. 2018), (Hiwale 2015), (Banerjee and Jain 1988), (Dwivedi et al. 2012). | Charoli | 2.86 – 3.12 | 50.72 – 61.91 | 19.0 – 28.19 | 5.63 – 6.59 | 3.8 | 10.87 – 12.25 |
| (Onsaard 2012) (Badifu and Akpagher 1996) | Sesame | 4.50 –11.00 | 48.20– 56.30 | 19.10– 26.90 | 2.0–5.59 | 2.5–3.90 | 5.59–17.90 |
| (Ahrens et al. 2005), (Sze–Tao and Sathe 2000) | Almond | 2.84 – 5.86 | 43.3 – 56.05 | 16.42 – 23.30 | 2.69 – 4.56 | 1.98 | 23.6 – 27.0 |
| (Arogba 1999) (Alobo et al. 2009) | Cashew | 9.30 – 12.40 | 45.17 – 51.0 | 20.23 – 36.0 | 0.3 – 6.96 | 4.54 – 4.56 | 3.4 – 11.39 |
| (Dogan and Akgul 2005) (Sze–Tao and Sathe 2000) | Walnut | 3.00 – 3.62 | 66.75 – 67.15 | 16.23 – 17.47 | 1.81 – 2.26 | —— | 65.0 – 70.0 |

(a) (b)

**Figure 1.** (a) Freshly harvested Charoli Fruits, (b) Charoli nuts after expelling the skin.

The delicate piece of Charoli nut is the kernel that could be influenced by deshelling, its removal from a shell is the critical most practice (Kumar et al. 2012). Damaging the kernels in de-shelling process decreases the demand cost of nuts (Güner et al. 2003). Preserving the quality and decreasing the losses of this kind of items is an important subject and has pulled numerous researchers in the field. In the past, minimal research has been done on some physical properties of Charoli nut and the work was carried out on the samples from the specified local region (Deshmukh et al. 2017; Kumar et al. 2016; Nishad et al. 2019). Also, it

is essential to expand knowledge of the Charoli nut properties globally to produce an effective, efficient, and safe machine for kernel extraction followed by separation and grading because these processes on Charoli nuts are still carried out manually in India (Sahu et al. 2018). This investigation aimed to determine the physical, frictional, and aerodynamic properties of harvested nuts that will be valuable for researchers/scientists in designing and developing equipment for various postharvest treatments and processing.

## 2. Materials and methods

### 2.1.Sample preparation

Charoli nuts used in the study were procured from the nearby markets of various states/districts of India like Maharashtra, Madhya Pradesh, Chhattisgarh, Jharkhand, and Gujarat during April 2019 for randomization. The Charoli nuts were typically soaked overnight in plain water and scoured against the rough surface of the jute bag to remove the skin. Then the nuts were rewashed with clean water and dried in sunlight (Kumar et al. 2012). These dried, cleaned nuts were used for further experimentation.

### 2.2.Moisture content

Primarily experiments were conducted for moisture content determination of dried Charoli nut. The experiments were performed at the Metallurgical lab of the Mechanical Engineering Department, Priyadarshani College of Engineering, Nagpur, Maharashtra, India. The drying in the oven (hot air oven; Universal, New Delhi, India) method was adopted to find the moisture content of Charoli nuts. The nuts are kept in an oven at 105°C for 6 hrs as per the ASAE standard (ASAE 1982). The moisture content of dried Charoli nut was observed as 9.06 % (db) and was considered base moisture level to other examination. The moisture content was varied from 09.06 to 17.86 % (d.b) for an examination. The required moisture level of Charoli nuts was obtained by the addition of a specific amount (Equation 1) of distilled water and packing into the isolated polyethylene packets. For consistency of moisture, Charoli nut samples were stored at 5° C in the fridge around 15 days. Prior to each test of nuts, the required Charoli nut samples were brought out of the fridge and kept in room temperature (Bajpai et al. 2019). The distilled water amount to be added to achieve the required moisture content was computed using relation (1) (Galus and Lenart 2019; Aghbashlo 2013).

$$Q = \frac{W_i\,(M_f - M_i)}{(100 - M_f)} \qquad (1)$$

Where, $W_i$– initial sample mass (g); $M_i$– initial moisture content of the sample (% d.b.); $M_f$– final moisture content of the sample (% d.b.); and Q– amount of distilled water added (g).

### 2.3.Measurement of dimensional properties

Dimensional properties of samples were assessed on five levels of moisture content, namely 9.06, 10.92, 12.51, 15.29, and 17.86 % d.b. To determine the dimensions of the Charoli nut, 100 nuts of five moisture content levels were picked randomly from the sample (Igbozulike and Amamgbo 2019). The axial dimensions like length (L), width (W) and thickness (T) of a nuts, as shown in Fig. 2 were measured by loading between two parallel jaws and applying equal pressure using digital micrometer screw guage (Mitutoyo, Japan) having accuracy of ± 0.001 mm (Kacal and Koyuncu 2017; Aydin 2003).

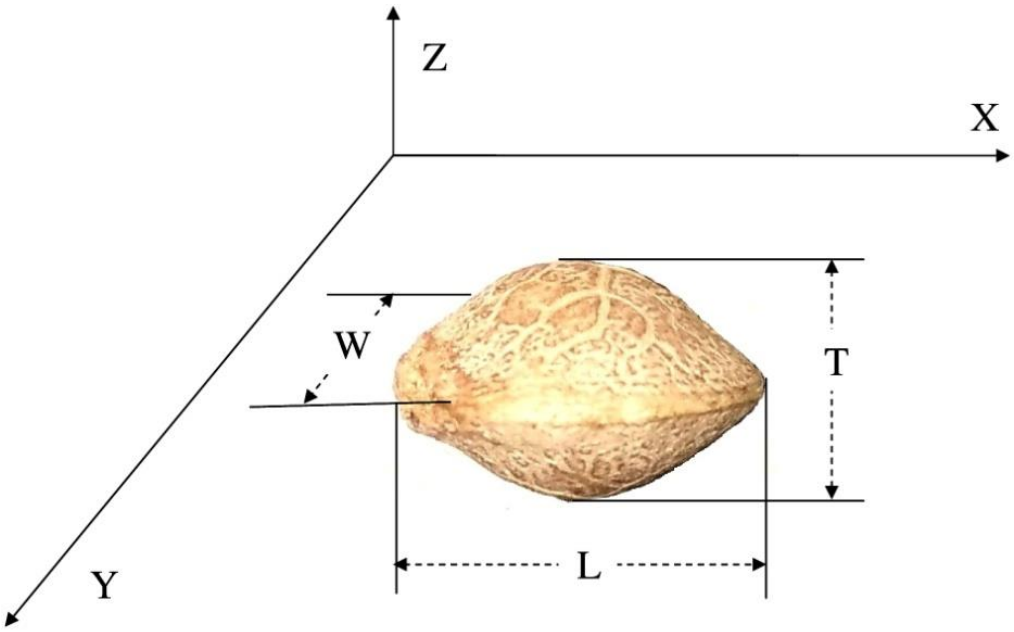



**Figure 2.** Axis and dimensions of Charoli Nut.

Geometric ($D_g$) and arithmetic ($D_a$) mean diameter were calculated through relations (2)–(3) (Moradi et al. 2019; Galedar et al. 2010; Celli 2015).

$$D_g = (L+W+T)^{1/3} \quad (2)$$

$$D_a = \frac{(L+W+T)}{3} \quad (3)$$

The sphericity (φ) and surface area (S) of the Charoli nut were found through the relationship as in equation (4)–(5) (Jahanbakhshi et al. 2019).

$$\varphi = \frac{D_g}{L} \quad (4)$$

$$S = \pi (D_g)^2 \quad (5)$$

## 2.4.Measurement of gravimetric properties

### *2.4.1.Thousand nut mass ($M_{1000}$):*

Thousand unit masses were obtained by an electronic balance (Model FA-2204B) of 0.001g accuracy. 100 nuts of the cleaned sample were randomly picked and weighed in the balance. Output was multiplied with 10 to get 1000 nuts mass. The measurements were replicated ten times for individual moisture content (Coskuner and Karababa 2007; Mohsenin 1980)

### *2.4.2.Bulk density*

The bulk density ($\rho_b$) was calculated by pouring nuts from 15 cm height into an empty 50 ml graduated cylinder (Mohsenin 1980). For achieving the throughout consistency, the cylinder was stabbed ten times to consolidate the nuts. A similar process was performed ten times individually for five moisture content level, and the bulk density was computed using the formula (6) (Sonawane et al. 2020)

$$\rho_b = \frac{W_s}{V_s} \quad (6)$$

Where, $W_s$– nut weight (kg); and $V_s$– Occupied Volume by nuts ($m^3$)

### *2.4.3.True density*

True density ($\rho_t$) can be calculated by toluene displacement method. Toluene ($C_7H_8$) has low absorptivity compared to water; hence it was used (Singh et al. 2019). Due to the property of low surface tension, it occupies the fills shallow dips of the nuts. A sample of nuts was immersed in toluene of a measuring cylinder with 0.1 ml accuracy. The graduated scale of the cylinder shows the amount of displaced toluene. A similar process was performed ten times (Thakur and Nanda 2018). The true density was computed using the formula (7)

$$\rho_t = \frac{M}{V} \quad (7)$$

Where, M– mass of each nut (kg); and V– volume displaced ($m^3$)

***2.4.4.Porosity***

Porosity (ε) indicates a number of pores into sample at given moisture content. It was computed by true and bulk density values and appears into the percentage. The porosity was determined by the use of the given formula (8). (Garnayak et al. 2008).

$$\varepsilon = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100 \quad (8)$$

Where, $\rho_t$– true density of nut (kg $m^{-3}$); and $\rho_b$– bulk density (kg $m^{-3}$).

## 2.5.Measurement of frictional properties

***2.5.1.Coefficients of static friction***

Coefficients of static friction (μ) of Charoli nut at five moisture contents were estimated for distinct frictional surfaces, specifically aluminium, plywood, and rubber (Obi et al. 2018). The samples were kept in an open-ended wooden container of 150 × 150 × 50 mm size, and the container was kept on the adjustable friction material tilting surface (Mansouri et al. 2017). The schematic of the arrangement is as shown in Fig. 3. A friction material surface was inclined slowly until a container began sliding on the surface. The tilting angle was recorded with the help of a graduated scale provided near surface. A coefficient of static friction (μ) was determined with equation (9).

$$\mu = \tan\theta \quad (9)$$

Where μ– coefficient of static friction and θ– tilt angle (degree).

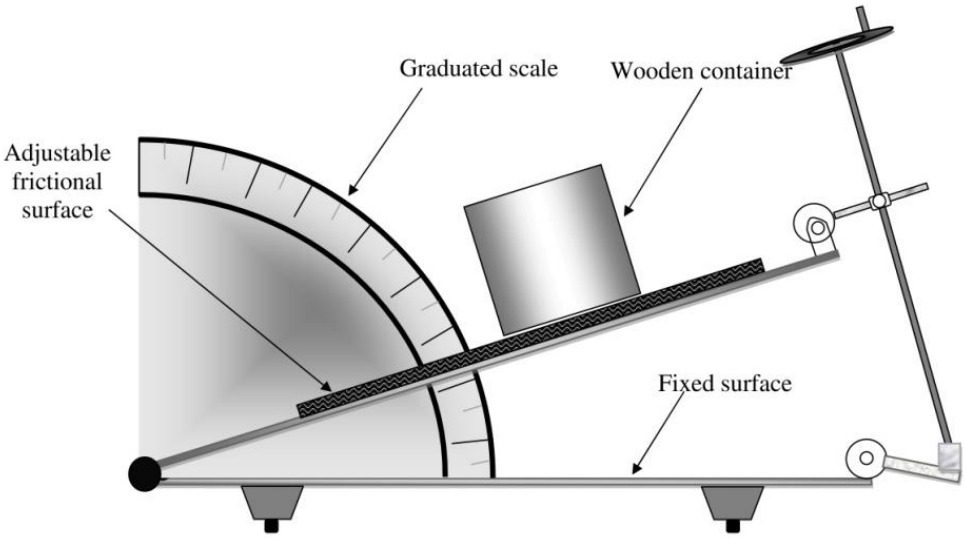



**Figure 3.** Schematic of the arrangement for measurement of coefficient of static friction of Charoli nut.

***2.5.2.Static and dynamic angle of repose***

A cylinder having 150 mm diameter and 250 mm height was used to find a static or filling angle of repose. The cylinder was filled by Charoli nut kept on the middle portion of the circular plate. Then the cylinder was then removed gradually until nut sample formed of the cone shape on the circular plane (Mirzabe et al. 2017). The cone height and diameter weremeasured to calculate an angle of repose ($\theta_f$) utilizing a formula (10).

$$\theta_f = \tan^{-1}\left(\frac{2H}{D}\right) \quad (10)$$

Where D and H are the cone diameter and height, respectively.

A fibreglass box of dimension 200×200 ×200 mm was utilized for finding a dynamic or emptying angle of repose. The complete box was filled by Charoli nut and front panel of the

box was immediately slid upwards. Because of this sudden slid the sample nuts were flowing outside and form a heap. Samples height between two points (b1, b2) in the sloping Charoli nut heap and horizontal dimensions at a different point (a1, a2) were noted. A dynamic or emptying angle of repose ($\theta_e$) was determined with a relationship (11). (Galedar et al. 2010)

$$\theta_e = \tan^{-1}\left[\frac{(b2-b1)}{(a2-a1)}\right] \quad (11)$$

Dynamic and static angle of repose plays a key role in designing hoppers for Charoli nuts where the bulk of the materials is in motion. (Khodabakhshian et al. 2010).

### 2.6.Measurement of aerodynamic properties

Terminal velocity of Charoli nut was measured by air column at 9.06, 10.92, 12.51, 15.29, and 17.86 % db moisture content. The schematic of the arrangement for measurement of terminal velocity is as shown in Fig. 4. During the test, Charoli nut sample was dropped inside stream of air from upper side of the air column. (Gezer et al. 2002). Then the flow of air was increased gradually until nut sample got suspended into stream of air. The digital anemometer (least count 0.1 m/s) was utilized for measurement of air velocity near nut suspension point (Ünal et al. 2006).

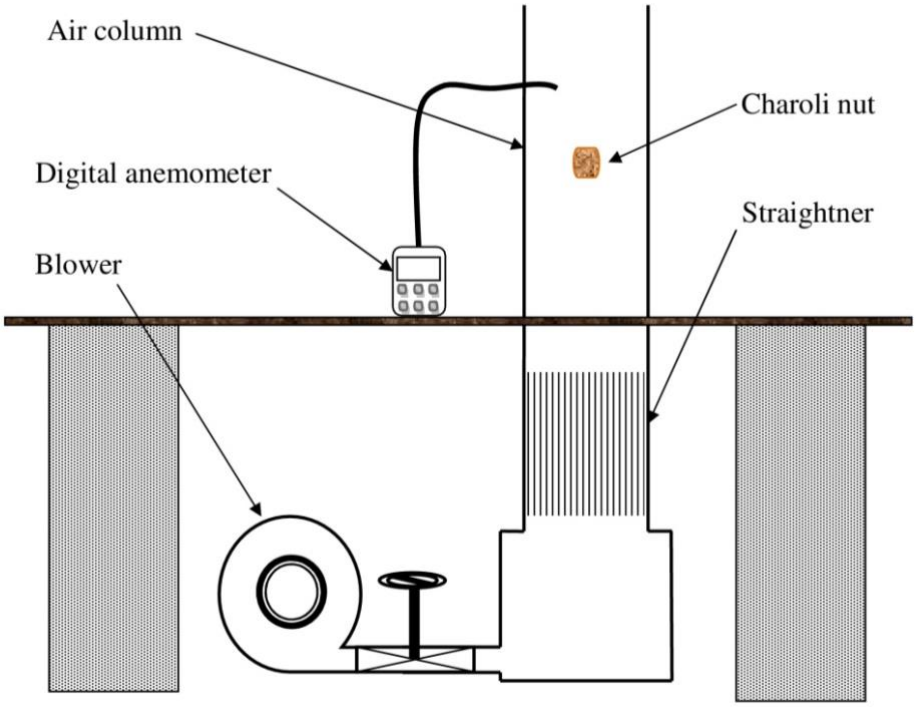



**Figure 4.** Schematic of the arrangement for measurement of terminal velocity of Charoli nut.

## 3. Results and discussions

### 3.1.Dimensional properties

Table 2 shows the various mean ± SD values of various dimensional properties of Charoli nut.Dimensional properties of nut help in the design process of sorting, deshelling, and separating equipments. Fig. 5 (a) show the bar chart for the variation in mean dimensions like length (L), width (W), thickness (T) and geometric mean diameter ($D_g$) of Charoli nut for individual moisture level. It can be seen from Fig. 5 (a), dimensions of L, W, T and $D_g$ increases by an increment in moisture from 9.06 to 17.86%. The dimensions of nut thickness significantly lower than the other two dimensions (length and width). Fig. 5 (b) shows a linear dimension for variation in mean dimensions at an individual moisture content of Charoli nut. The dimensions of nut length were mostly recorded more than the width, while in

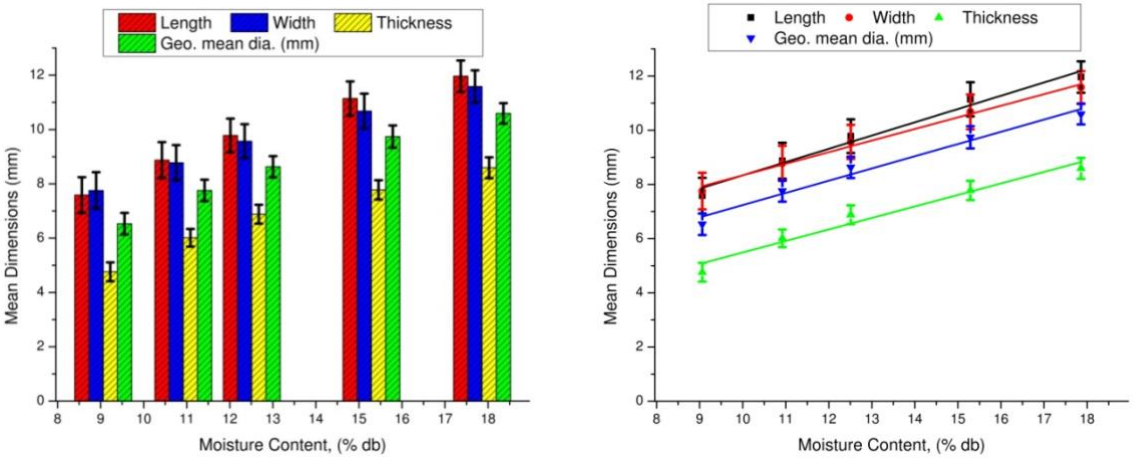
few cases, the nut widths were found more than the length.

**Table 2.** Moisture content dependent mean ± SD values of various dimensional properties of Charoli nut.

| Moisture Content | Length (mm) | Width (mm) | Thickness (mm) | Geo. mean dia. (mm) | Surface area ($mm^2$) | Sphericity (%) |
|---|---|---|---|---|---|---|
| 9.06 | 7.5844 ± 0.6601 | 7.7543 ± 0.6740 | 4.7608 ± 0.3462 | 6.5318 ± 0.3974 | 134.53 ± 16.07 | 86.427 ± 4.936 |
| 10.92 | 8.8725 ± 0.6615 | 8.7765 ± 0.6477 | 6.0136 ± 0.3247 | 7.7575 ± 0.3959 | 189.54 ± 19.08 | 87.662 ± 4.227 |
| 12.51 | 9.7811 ± 0.6202 | 9.5680 ± 0.6291 | 6.8819 ± 0.3482 | 8.6291 ± 0.3929 | 234.41 ± 21.11 | 88.379 ± 3.602 |
| 15.29 | 11.141 ± 0.629 | 10.678 ± 0.643 | 7.7786 ± 0.3562 | 9.7389 ± 0.4101 | 298.49 ± 24.98 | 88.428 ± 3.012 |
| 17.86 | 11.962 ± 0.579 | 11.583 ± 0.595 | 8.5923 ± 0.3853 | 10.593 ± 0.378 | 352.98 ± 25.12 | 88.643 ± 2.780 |

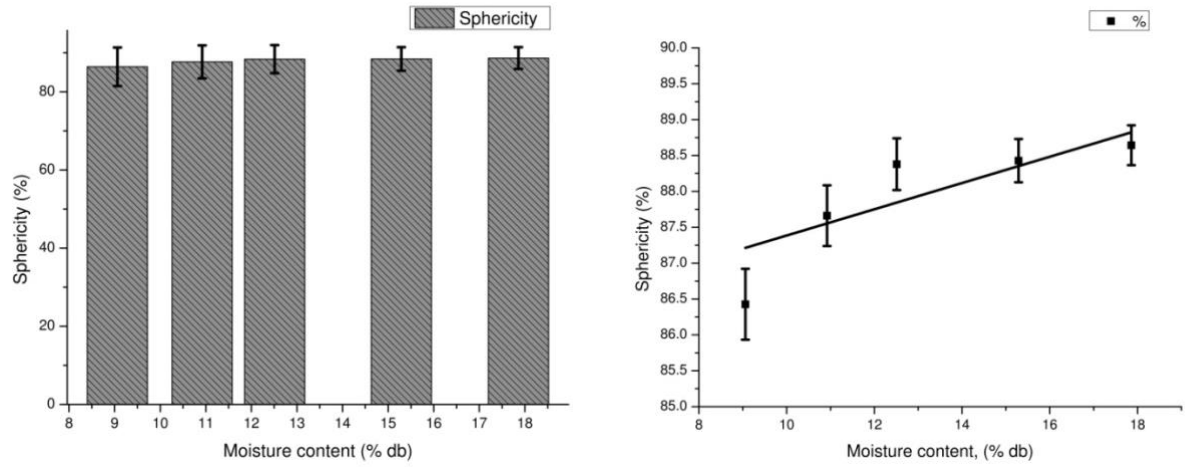
The L, W, T and $D_g$ of the Charoli nuts varied linearly from 7.58 to 11.96 mm, 7.75 to 11.58 mm, 4.76 to 8.59 mm, and 6.53 to 10.59 mm, respectively by varying moisture level between 9.06 to 17.86% on dry basis. The extension in length and width were seen as very closer than the thickness, which could be because of a cellular arrangement of Charoli nuts. The percentage increment in L, W, T and Dg dimensions are 57.72, 49.37, 80.47, and 62.17 %, respectively. Comparative patterns in the measurements were also found for Jamun (Syzgiumcuminii) seed with respect to dimensions of L, B, T, and Dg within the moisture level within 11.54 – 26% d.b. (Bajpai et al. 2019). The moisture content ($M_C$) dependent relationship of the L, B, T, and Dg dimensions are shown in Equations (12), (13), (14) and (15)

$$L = 0.4953\ M_C + 3.367\ (R^2 = 0.9809) \quad (12)$$
$$B = 0.4309\ M_C + 4.015\ (R^2 = 0.9914) \quad (13)$$
$$T = 0.4211\ M_C + 1.278\ (R^2 = 0.9678) \quad (14)$$
$$Dg = 0.4532\ M_C + 2.700\ (R^2 = 0.9798) \quad (15)$$




(a) (b)

**Figure 5**. (a) Bar chart and (b) linear dimension for variation in mean dimensions at individual moisture content of Charoli nut.

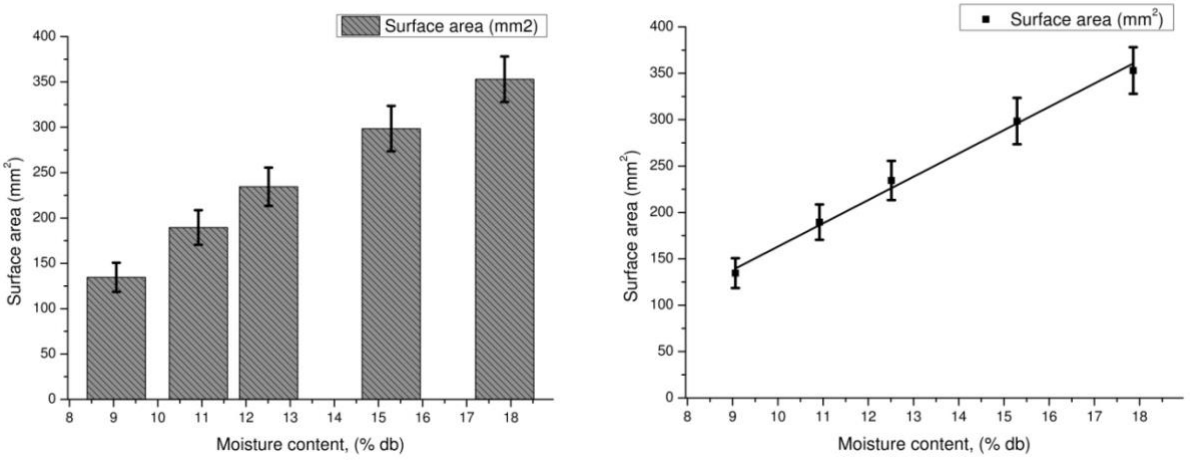



(a) (b)

**Figure 6.** (a) Bar chart and (b) linear dimension for variation in sphericity at individual moisture content of Charoli nut.

Mean values for the sphericity (φ) of Charoli nuts were 86.42, 87.66, 88.37, 88.42, and 88.64 %, respectively, with varying moisture content between 9.06 to 17.86% on a dry basis. Fig. 6 (a) shows the bar chart, and Fig. 6 (b) shows the linear dimension for the variation in sphericity of Charoli nut for individual moisture level.This indicates that average values of sphericity increased with increment in moisture content, which depicts nut's becoming more spherical. This result gives confirmation with results of Arjun et al. (2017) for makhana and Vilche et al. (2003) for hemp seeds. The higher value shows the tendency of rolling than sliding upon the surface. Therefore while designing the transfer, transport and sorting systems, make nut roll rather than slide. The moisture content effect on the sphericity of Charoli nuts is represented by regression equation (16).

$$\varphi = 0.2226\ M_C + 84.99 (R^2 = 0.7367) \quad (16)$$




(a) (b)

**Figure 7.** (a) Bar chart and (b) variation in surface area at individual moisture content of Charoli nut.

The surface area (SA) of the Charoli nuts varied between 134.52 and 352.97 $mm^2$. Values show a notable increment in surface area by means of increment of moisture content. The moisture content effect on the surface area of Charoli nuts is shown in Fig. 7 (a) and (b).

A similar linear increasing trend found in Taghi Gharibzahedi et al. (2011) for castor seeds; and Malik and Saini (2016) for sunflower seeds. The surface area plays a significant function in sizing systems design. Apart from this, surface areas are utilized to model material and heat transfer while freezing or drying nuts (Vivek et al. 2018; Pathak et al. 2019). Equation (17) shows the moisture content effect on the surface area of Charoli nuts

$$SA = 24.66\ M_C - 81.7\ (R^2 = 0.9948) \quad (17)$$

## 3.2.Gravimetric properties

### *3.2.1.Thousand nut mass ($M_{1000}$)*

Table 3 shows the mean ± SD values of various gravimetric properties of Charoli nut. In contrast, variation in a thousand Nut Mass of Charoli nut by the moisture content is illustrated in Fig. 8 (a) and (b). A result indicates the linear increment in mean value from 0.134 kg to 0.421 kg with moisture content.

**Table 3.** Moisture content dependent mean ± SD values of various gravimetric properties of Charoli nut.

| Moisture Content | Thousand gram Weight | Bulk Density | True Density | Porocity |
|---|---|---|---|---|
| 9.06 | 0.1342 ± 0.01579 | 657.23 ± 15.30 | 917.94 ± 43.9 | 28.25249± 3.84 |
| 10.92 | 0.196667 ± 0.0364 | 638.99 ± 10.01 | 898.43 ± 41.3 | 28.77164± 2.671 |
| 12.51 | 0.2765 ± 0.01893 | 616.58 ± 13.35 | 883.25 ± 45.7 | 30.04625± 3.46 |
| 15.29 | 0.3524 ± 0.02210 | 599.32 ± 19.87 | 874.65 ± 46.5 | 31.3178± 4.07 |
| 17.86 | 0.4215 ± 0.02371 | 578.32 ± 14.93 | 851.21 ± 46.9 | 31.9583± 2.050 |

The increase in thousand nuts mass illustrates the considerable moisture absorption by the Charoli nut for increase in moisture range. The nut mass is the important parameter while considering the flow rate of the equipment, therefore, deciding Charoli nut mass for acknowledgement of the impact of moisture content on increment of each nut weight.

Similar incremental tendency for thousand Nut mass were observed by Izli et al. (2009) and Sacilik et al. (2003) for rapeseeds and hemp seed respectively. The variation of the thousand nut mass (kg) is mathematically expressed as in equation (18)

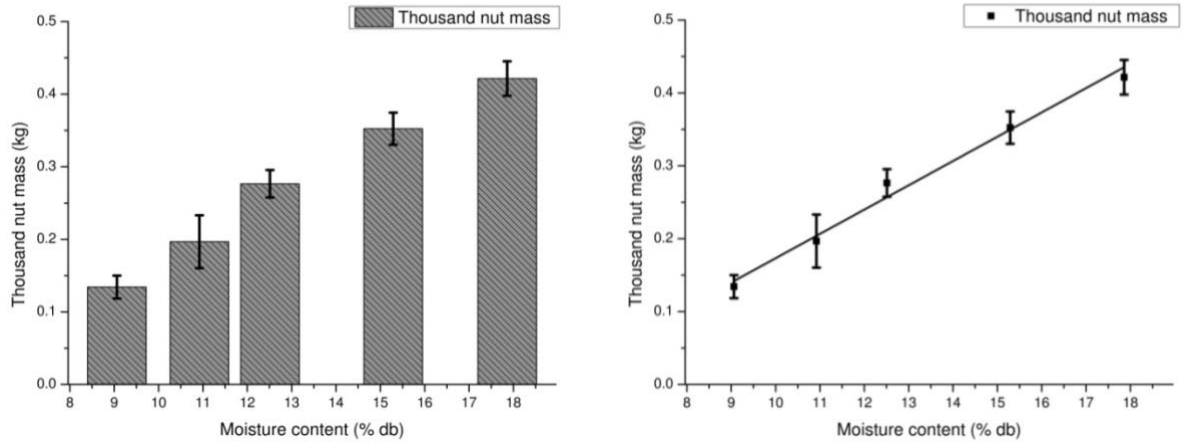
$$M_{1000} = 0.03285\ M_C - 0.1550\ (R^2 = 0.9874) \quad (18)$$

### *3.2.2.Bulk and true density*

Because of moisture content increment in nuts, bulk and true density values are significantly reduced from 657.23 to 578.32 kg $m^{-3}$and 917.94 to 851.21 kg $m^{-3}$. The bulk and true density relationship of Charoli nut is illustrated in **Fig.** 9 (a) and (b).

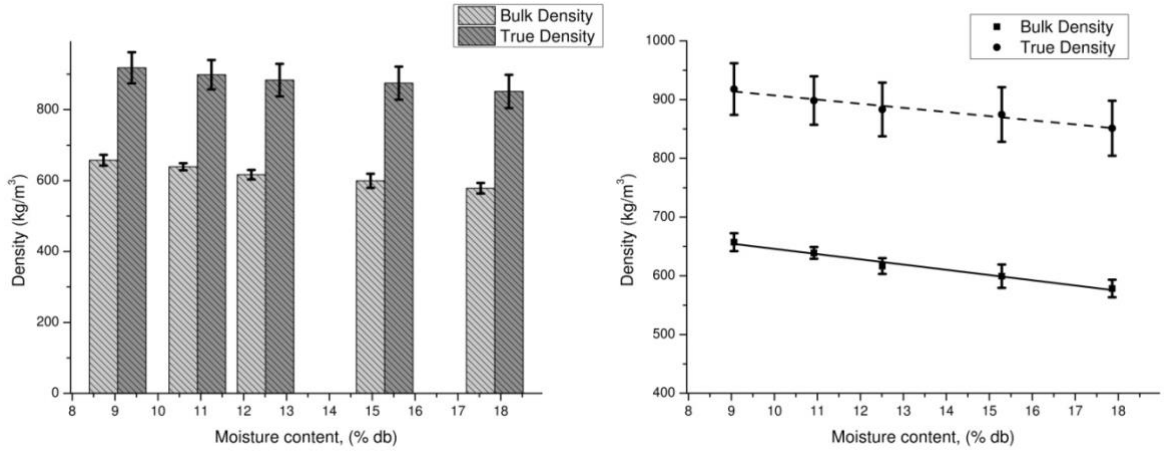
Bulk and true density consideration is very important while designing different devices for cleaning, separation, and conveyance systems of nut. The mass of the nut increased because of moisture gaining of Charoli nuts ultimately decreased the bulk density. A similar trend of contrary connection between moisture content and bulk density has been concluded by Ghosh et al. (2017) for Jamun seed and Pradhan et al. (2008) for Karanja kernel. Bulk density and moisture content relationship of Charoli nut has expressed as in equation (19)

$$\rho_b = -8.864\ M_C + 734.46 (R^2 = 0.9834) \quad (19)$$




(a) (b)

**Figure 8.** (a) Bar chart and (b) variation in thousand nuts mass at individual moisture content of Charoli nut.




(a) (b)

**Figure 9.** (a) Bar chart and (b) variation in bulk and true density at individual moisture content of Charoli nut.

The true density is a real mass of Charoli nuts with excluding a pores / empty spaces. Decrease in true density might be because of a higher true volume. The true density signifies the nuts are heavy in weight and will not float on water. Similar trend of contrary connection between moisture content and true density has been reported for pistachio nuts (Kashaninejad et al. 2005); sorghum (Mwithiga and Sifuna 2006); and spinach seeds (Kilickan et al. 2010). The variation in true density has expressed as in equation (20)

$$\rho_t = -7.056\, M_C + 977.7 (R^2 = 0.9666) \quad (20)$$

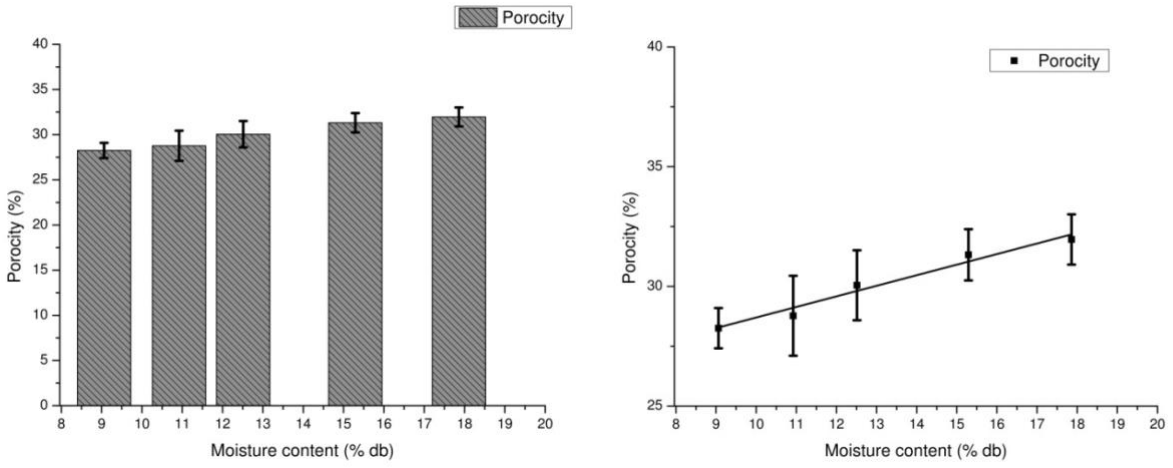
### *3.2.3. Porosity*

Porosity (ε) % value communicates the inter–granular to a total occupied space of the nut. Porosity dependency on a bulk and true density is diverse for individual nut with moisture content increment. A slight increment in porosity value of Charoli nut was observed with increment in moisture content. Porosity of Charoli nuts increased from 28.40% to 32.05% with moisture content increment from 9.06% to 17.86% (d.b.). A moisture content effect on porosity is shown in Fig. 10 (a) and (b).

The porosity of nuts provides the significance of the resistance to the air flow at aeration or drying. A similar linear increment

in porosity has been found for gram (Chowdhury et al. 2001); chickpea seed (Konak et al. 2002); and Brachystegia Eurycoma seed (Aviara et al. 2014). While linear decrement into porosity has been observed in the research by Sacilik et al. (2003) and Suthar and Das (1996) for hemp and karingda seed, respectively. Morphological characteristics play an important role in increase or decrease of porosity of nuts observed by the result. The linear correlation was observed in porosity and moisture content of nuts, and it can be shown by a regression equation (21):

$$\varepsilon = 0.4461\ M_C + 24.345 \quad (R^2 = 0.9654) \quad (21)$$




(a) (b)

**Figure 10.** (a) Bar chart and (b) variation in porosity at individual moisture content of Charoli nut.

### 3.3.Frictional properties

#### *3.3.1 Coefficients of static friction*

The result of the coefficient of static friction (μ) of Charoli nut having different moisture contents (9.06, 10.92, 12.51, 15.29, and 17.86 % db) on three frictional surfaces as aluminium, plywood, and rubber is presented into Fig. 11 (a) and (b). A value of μ increased

significantly with moisture content. The μ increases linearly from 0.367 to 0.495, 0.381 to 0.512, and 0.432 to 0.585 for aluminium, plywood, and rubber respectively within the moisture range between 9.06–17.86% (d.b.).

The value of (μ) varies linearly with increment in moisture content. A highest value of (μ) found at high moisture content in all of three surfaces. An increment in value of (μ) may be because of an increment in cohesive force between Charoli nut and contact surfaces. Nut becomes rough with increase in moisture content which ultimately reduces its sliding characteristics. Coefficient of friction results were similarly stated by Mirzabe et al. (2017) for cucumber seeds and Tabatabaeefar (2003) for wheat.

The correlation in coefficient of static friction with moisture content of aluminium, plywood, and rubber are presented in equations. (22), (23), and (24), respectively:

$$\mu_{al} = 0.01509\ M_C + 0.2331 \quad (R^2 = 0.9766) \quad (22)$$

$$\mu_{ply} = 0.01552\ M_C + 0.2428 \quad (R^2 = 0.9790) \quad (23)$$

$$\mu_{ru} = 0.017847\ M_C + 0.2699 \quad (R^2 = 0.9944) \quad (24)$$

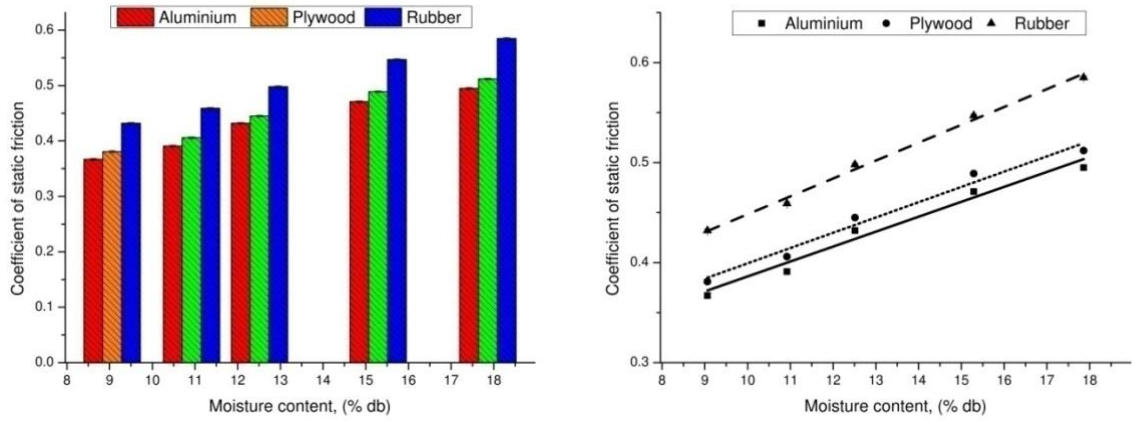



**Figure 11.** (a) Bar chart and (b) variation in coefficients of static friction at individual moisture content of Charoli nut.

***3.3.2.Static ($\theta_s$) and dynamic angle of repose ($\theta_d$)***

Variations of the static and dynamic angle of repose of Charoli nut within moisture range of 9.06–17.86% (db) are presented in Fig. 12 (a) and (b). A value of static and dynamic angle of repose increased significantly by 16.52° to 22.31° and 27.91° to 33.23° respectively with respect to moisture content ranging from 9.06–17.86% (db).

The angle of repose indicates the nuts flow–ability. It is also characteristic indicating the cohesion between nut and the surface layer of moisture of nut. With increase in moisture content, surface of nut becomes rough. Therefore, for easier flow–ability of nut, hopper angle must be more than 35°.

Similar linear increasing trends with increasing moisture content were reported by Oje and Ugbor, (1991); and Chowdhury et al. (2001) for oilbean and gram respectively.

The relationship between static and dynamic angle of repose of Charoli nut with respect to moisture content is represented in equation 25 and 26.

$\theta_s = 0.6503\ M_C + 10.777\ (R^2 = 0.9965$ (25)

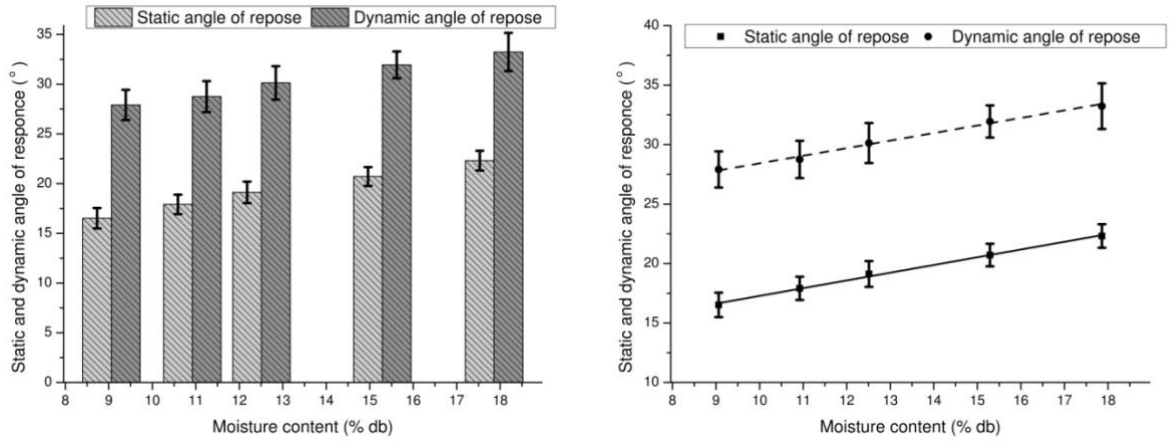
$\theta_d = 0.6274\ M_C + 22.156 (R^2 = 0.9928$ (26)

***3.3.3 Aerodynamic properties***

An experimental result for variations of the terminal velocity ($V_t$) of Charoli nut with respect to moisture content ranging from 9.06–17.86% (db) are shown in Fig. 13 (a) and (b). With increment in moisture content (9.06–17.86% (db)), increment into terminal velocity noted from 13.21 to 14.94 m/s.

Similar results were stated by Suthar and Das (1996); Nimkar and Chattopadyay (2001) and Sacilik et al. (2003) for karingda seed, green gram, and hemp seed respectively. An increment into terminal velocity with respect to moisture content is due to an increment in frontal area mass of a nut offered to a stream of air. Terminal velocity data may be useful for various post-harvest operations like separation from impurities, cleaning and transportation. The relationship between the terminal velocity of Charoli nut with respect to moisture content is represented in equation 27.

$V_t = 0.1784\ M_C + 11.634 (R^2 = 0.9465)$(27)




(a) (b)

**Figure 12.** (a) Bar chart and (b) variation in coefficients of angle of repose at individual moisture content of Charoli nut.

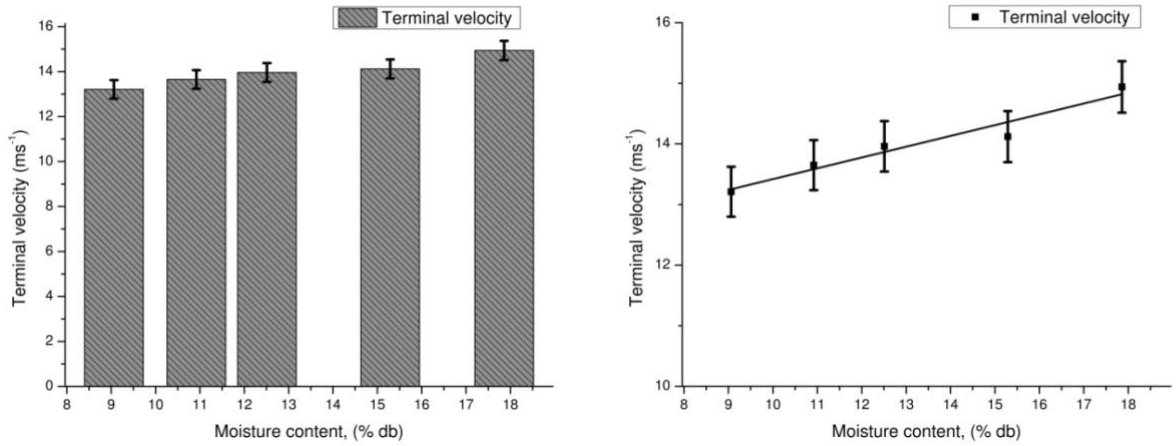



**Figure 13.** (a) Bar chart and (b) variation in terminal velocity at individual moisture content of Charoli nut.

## 4. Conclusions

Charoli has extremely high nutritional and medicinal values. Because of ignorance of its value and its processing difficulty, this ultimate product has been underestimated and unexplored. For making extra efficient and simple processing, a present experiment has been conducted to find various characteristics like physical, frictional and aerodynamic characteristics of nut. The results of this study indicated moisture content significantly influences and modifies the physical, frictional and aerodynamic properties. Following major conclusions are obtained by the study.

In all properties, only the true and bulk densities decreased with increment in moisture content whereas remaining properties were increased. Increments in the properties are attributed mostly to the cellular arrangements and water absorption by the nut.

The mean values for the sphericity of nuts were increased from 86.42 to 88.64 % with moisture content which shows the tendency of rolling than sliding upon the surface. This is helpful while designing handling and sorting systems for the nuts.

The surface area increased from 134.52 and 352.97 $mm^2$ which shows more attention needs to take while designing of sizing system. The nuts having higher moisture content needs more space for heat transfer while freezing or drying.

The gravimetric properties such as thousand nut mass and porosity increased linearly from 0.134 kg to 0.421 kg and 28.40% to 32.05%, respectively, whereas bulk and true density decreased. Decrease in a bulk density was noted because of increase of mass due to gaining of moisture. Whereas the true density signifies the nuts are heavy in weight and will not float on water.

The coefficient of static friction increased with moisture content and found highest in case of rubber as compared to aluminium and plywood. An increment in the value of (μ) is because of cohesive force increment.

The static and dynamic angle of repose increased significantly from 16.52$^{o}$ to 22.31$^{o}$, and 27.91$^{o}$ to 33.23$^{o}$. Therefore, for easier flow–ability of nut, hopper angle must be more than 35$^{o}$.

Terminal velocity of nut increased in linear manner from 13.21 to 14.94 m/s. This terminal velocity data may be useful in different post–harvest operations like separation from impurities, cleaning and transportation.

These results provide valuable information to various scientists, technologists, and engineers having keen interest for development of sustainable mechanization for postharvest processing of Charoli nut.

## 5.References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