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PREFACE

By the Grace and Blessings of Allah the Almighty, we would like to present, with great pleasure, the Volume 05 number 01 of *Food ScienTech Journal* (FSJ). This journal is part of the Universitas Sultan Ageng Tirtaya series of journal.

This journal was envisioned and founded to represent the growing needs of food technology as an emerging and increasingly vital field, now widely recognized as an integral part of agriculture and human living. Its mission is to become a voice of the food technology and science community, addressing researchers and practitioners in areas ranging from chemistry to management, from microbiology to industry, presenting verifiable methods, findings, and solutions.

The journal is intended as a forum for practitioners and researchers to share their research, idea, and solutions in the area of food science and technology. We would like to request for the reader to participate on writing the articles in this journal.

Thank you for your kind attention and support, hopefully this journal will provide lots of benefits for you and society.

Serang, July 2023

Editorial Team

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Physicochemical Properties of Tomato Paste Fortified Functional Cheddar Cheese

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ABSTRACT

The aim of this research is to fortify cheddar cheese's nutritional value by adding tomato paste. This study transformed ultra-heat treatment (UHT) milk into cheese through cheddaring. Tomato paste was added at 5 g/L, 10 g/L, and 15 g/L during the first curd formation, together with calcium chloride ($CaCl_2$). The type of rennet (animal and microbial) was varied at 0.25 ml/L of milk. Ripening was done in one month at 4°C. According to this study, animal rennet formed curd better than microbial rennet. The addition of tomato paste slightly decreases the curd formation, with approximately 0.37 % reduction per 5 gram of tomato paste. Increasing tomato paste to 15 g/L would increase lycopene to 0.993 – 0.996 mg/100 g. The cheese produced was categorized as extra-hard and low-fat based on the percentage of Moisture Non-Fat Basis (MNFS) and Fat on Dry Matter (FDM). The addition of tomato paste reduced the pH value, resulting in increased firmness and hardness and decreased chewiness and springiness.

Keywords: cheddar, functional cheese, animal rennet, microbial rennet, tomato paste

INTRODUCTION

Innovation in food products has been occurring rapidly. Many kinds of research are conducted to find a method to improve customers' health without sacrificing flavors and environment and offer unique customer experiences. Functional food is defined as food that provides nutrients and beneficially reduces the risk of diseases (Butnariu and Sarac, 2019).

Cheese is a food group from fermented milk produced in various flavors and textures. It is a popular product widely consumed around the globe, with the market increasing by 2.3% annually (Dairy Industries International, 2020). Cheesemaking is a complex process since it is biochemically dynamic and inherently unstable. It is comprised of milk conversion into curd and ripening. The cheese process and formulation produce different cheese products (Fox and McSweeney, 2017). Cheddar cheese is a type of cheese produced through the stirred-curd process (cheddaring) to improve the quality of the cheese due to faster acid production (Ong et al., 2017).

The initial of cheese making is to preserve the nutrients in milk. Recently, innovative functional cheese has been created for various purposes, such as extending shelf-life, dairy replacement, and improved flavors and nutritional ingredients (Farahat et al., 2021). Vegetable or fruit fortified into cheese is a popular method to ameliorate the cheese texture, tastes, and nutrients. Tomato has the potential to be incorporated into cheese due to its highly nutritious components, such as carotenoids, vitamin C, and flavonoids.

Lycopene is a major carotenoid that functions as a powerful antioxidant with a composition of approximately 8.8 – 4.2 mg/g in tomatoes. It is a natural preservative that provides human health benefits (Joshi et al., 2020). Tomato processed cheese spread was generated by fortifying tomato juice and tomato extract into processed Ras and cheddar cheese (Hassan et al., 2019; Mehanna et al., 2017). Based on the studies, the amount of tomato juice improved the lycopene content and free radical scavenging activity (%RSA). The sensory evaluation also verified the high acceptability of tomato processed cheese based on the firmness, spreading, stickiness, and crumbliness. Another study created tomato processed cheese by the addition of tomato powder (Solhi et al., 2020). The processed cheese with tomato powder had higher phenolic and lycopene content, and higher level of proteolysis. In the contrary, lipolysis index could be kept in low level. Tomato juice had also been incorporated into the making of mozzarella cheese (Abd El-Aziz and Refaey, 2017). Mozzarella cheese produced by tomato juice addition performed better in free radical scavenging activity (%RSA) and rheological tests. (Jeong et al., 2017) showed an improvement in lycopene content in Queso Blanco cheese by supplementing powdered microcapsule tomato extracts. In this method, lycopene could be protected during cheese making. The powdered microcapsules were produced using emulsion spray drying. Cheese' color and texture improvement have been reported due to the addition of tomato powdered microcapsules.

Despite many studies evaluating tomato functional cheese products, most of these studies used processed cheese. The tomato cheddar cheese has not yet been considered. Cheddar cheese is a hard cheese that gets through the cheddaring process and is usually applied in many savory food products, such as pizza, burgers, and soups. Fortified tomato into cheddar cheese was expected to promote texture, nutrients, and practicality innovation. Tomato paste was selected due to its higher lycopene content than tomato juice and powder (Górecka et al., 2020). The type of rennet used also varied between animal and microbial rennet to see the difference in cheese's composition and mechanical properties. This research aimed to produce fortified tomato paste cheddar cheese by varying tomato paste concentration (0, 5, 10, and 15 g/L of milk) and rennet type (animal and microbial rennet).

MATERIALS AND METHODS

Materials

Commercial ultra-high-temperature (UHT) milk (Ultrajaya, Indonesia) was employed as a milk base. The ripe and red tomatoes were obtained from a local store and selected for paste preparation. Mesophilic Aromatic Type B (Biena, Canada) was determined as cheese culture. Liquid animal and microbial rennet (Dupont Danisco, USA) and food-grade calcium chloride (CC Food Tetra, Finland) were used for curd coagulation. All chemicals (NaCl, K_2SO_4, and $CuSO_4$) and solvents (NaOH, HCl, H_2SO_4, H_3BO_3, Hexane, and Ethanol) were analytical grades obtained from Merck, Germany. Lycopene analytical standard (purity 90%, Sigma Aldrich, Germany) was applied to determine lycopene content.

The preparation of tomato paste

The clean tomato was blanched using steam for 5 minutes, and its seeds and skins were separated. The peeled tomato was crushed in a food processor until it formed tomato pulp, then cooked at 90 °C for 2 hours until the °Brix value was around 24–28. The °Brix was measured using a refractometer (ATAGO, Japan). The tomato paste was stored in a heatproof container and pasteurized for 15 minutes using boiling water.

The preparation of cheese

The cheese preparation followed the method by (Arlene *et al.*, 2015) with adjustment. Commercial UHT milk consisted of fat and protein content of around 8 g/L each. Milk was pasteurized at 62 °C for 30 minutes and then cooled to 30 °C. Milk was inoculated with a mesophilic culture of around 0.1 g/L of milk, then let sit for 30 minutes. Tomato paste (0, 5, 10, and 15 g/L) and $CaCl_2$ (0.25%-v/v) were added into the milk, followed by rennet (animal and microbial) addition around 0.025%-v/v to coagulate for 30 minutes. The coagulum was cut to around 3 x 3 x 3 cm^3 and left for 5 minutes. The curd was then slowly heated until it reached 38 °C then the whey was separated by cheesecloth, which was previously sterilized in boiling water. The curd yield was calculated by calculating the weight of curd obtained from the initial milk weight. The curd was stacked and formed block shapes, then cut and turn it over every 10 minutes. This cheddaring process was conducted four times. Approximately 2.5%-w/w NaCl was added to the curd. The curd was then placed in a container (10 x 15 cm^2) and pressed with 2 kg weight for 12 hours. The cheese was stored in a refrigerator at 4 °C for 30 days.

The chemical analysis

The moisture, fat, and protein content of cheese products were analyzed according to the Indonesia National Standard for processed cheese (SNI 2980:2018) (National Standardization Agency of Indonesia, 2018). The moisture, fat, and protein content used gravimetry, soxhlet, and Kjeldahl methods (Kjeltec 8100). The moisture non-fat basis (MNFS) and fat in dry matter (FDM) were calculated according to Equations 1 and 2, respectively. MNFS and FDM are parameters to categorize the type of cheese produced.

$$\text{MNFS (\%)} = \frac{m_{moisture}}{m_{cheese} - m_{fat}} \times 100 \quad (1)$$

$$\text{FDM (\%)} = \frac{m_{fat}}{m_{cheese} - m_{moisture}} \times 100 \quad (2)$$

The lycopene content was measured using the spectrophotometry method according to Ramadhany *et al.* (2021) method with adjustment. Approximately 0.1 g sample was extracted with 10 ml of solvent mixture (2:1:1 v/v/v of hexane, acetone, and ethanol) in the 50 ml Erlenmeyer flask. The solution was mixed for 10 minutes using a reciprocal shaker at 280 rpm. The mixture was filtered using a Buchner funnel to remove solid particles. The filtrate was combined with 1.5 mL of distilled water, followed by mixing for 5 minutes, and non-polar solution layers were formed. Around 4 mL of the top layer was pipetted. Absorbances were measured using a UV-Vis spectrophotometer (Genesys 20) at 503 nm.

The milk clotting activity (MCA)

The milk clotting activity was measured to determine the number of enzymes required to coagulate 1 mL of milk in 2400 s at 35 °C. Around 0.5 mL of liquid rennet was added to 1.5 mL of milk. The time until milk coagulated was measured. The measurement was conducted in triplicate. The MCA quantification was calculated following reports by (Silva *et al.*, 2014) and (Liburdi *et al.*, 2019) as shown in Equation 3.

$$\text{MCA (U)} = \frac{2400 \times \text{S}}{\text{T} \times \text{E}} \quad (3)$$

where S = milk volume (mL), T = coagulation time (s), and E = coagulant volume (mL).

The color analysis

The color of the cheese sample was determined by the Color Lab application and expressed in CIELAB parameters (L*, a*, b*). Variable L*, a*, and b* specify the

perceptual lightness, red-green chromatism, and blue-yellow chromatism, respectively.

The mechanical properties analysis

The mechanical properties were measured using Texture Analyzer (Brookfield CT 3). The texture analyzer was set to texture profile analysis (TPA) with initial condition 5.0 g trigger, 10 mm deformation, and 0.5 mm/s initial speed. The sample (3 x 3 x 3 cm^3) was measured for firmness, hardness, adhesiveness, cohesiveness, chewiness, gumminess, and springiness. Firmness is related to the force required to penetrate the sample with fingers, while hardness is the force required to perforate the sample with a knife. Adhesiveness indicates the work done to excel attractive forces between the surface of materials. Cohesiveness shows how a good product withstands deformation. Chewiness is related to the energy required to masticate the product, and gumminess is the energy required to disintegrate the product, ready for swallowing. Springiness specifies how a good product springs back after being deformed.

The statistical analysis

The ANOVA two-way test with replication defines variables that influence the product. The test was conducted in Microsoft Excel©. Partial Least Square Regression (PLSR) by Unscrambler™ was selected to evaluate further the correlation between rennet type and tomato paste to mechanical properties. Both statistical analyses were done at a confident interval of 95%.

RESULTS AND DISCUSSION

The pH, °Brix, and lycopene value of tomato paste

The pH, °Brix, and lycopene values of tomato paste can be seen in Table 1. The pH acquired in this study was around 4.45 ± 0.07. The acidity of tomato paste is related to its ripeness and acid compositions. Most commercial tomato pastes pH were in the range of 3.39 – 4.92. However, the tomato paste pH should be kept under 4.6 to intercept the activity of pathogenic microorganisms (Aykas *et al.*, 2020; FAO and WHO, 2013). Therefore, the result attained in this study was still low enough from the theoretical. The overall °Brix value of tomato paste was 25.24 ± 0.55. Similar values were also reported (Aykas *et al.*, 2020; Devseren *et al.*, 2021). According to (FAO and WHO, 2013), the tomato paste °Brix value should be at least 24 °Brix. Nevertheless, most commercial tomato paste usually ranges between 26 – 30 °Brix (Aykas *et al.*, 2020). Thus, the obtained value in this study was still close to the previous study.

The bioavailability of lycopene in tomato paste should be higher than the tomato juice due to the rupture of plant cell walls during the heating processes (Soares *et al.*, 2017). According to Table 1, the tomato paste consisted of 6.97 ± 0.34 mg lycopene/100 g. According to some studies (Joshi *et al.*, 2020; Soares *et al.*, 2017), lycopene in tomato paste was approximately 5.4 – 15 mg/100 g depending on the variety, ripeness, and processing condition.

The milk clotting activity (MCA) and curd yield

The enzyme plays a significant role in cheese coagulation and as a bioprotective agent. The milk clotting activity (MCA) is a variable to determine the capability of the enzyme to hydrolyze protein, specifically κ-casein. However, protein breakdown or proteolysis should not occur too much since it will degrade protein further into smaller size, causing whey loss and reducing curd formation (Ivens *et al.*, 2017).

According to Table 2, animal rennet only required 737 ± 136 enzyme units to coagulate 1 mL of milk at 35 °C, while

microbial rennet required 1224 ± 364 U/mL. It showed that animal rennet is a more effective milk clotting enzyme. Animal rennet consists of predominantly chymosin, followed by pepsin. Chymosin is effective in splitting κ-casein at Phe_{105} – Met_{106} and releasing macropeptide, while pepsin is less specific than chymosin (Jaros and Rohm, 2017). Microbial rennet refers to *Mucor miehei* lipase. Microbial enzymes are less specific than chymosin in proteolysis or protein breakdown, resulting in lower milk clotting activity. Microbial rennet is also more thermal stable than chymosin, requiring more enzymes to curd milk (Jaros and Rohm, 2017). In this study, each rennet was kept constant at 0.25 mL/L. Therefore, animal rennet with lower MCA resulted in better curd yield, as observed in Table 2. Previous studies also showed similar result (Manuelian *et al.*, 2020).

The pH is an essential parameter in the curding phase of cheese production. Its value can change due to culture activity or the addition of acid components. Low pH speeds up proteolysis, reduces electrostatic repulsion between casein micelles, and changes calcium distribution between the micelle and serum phases (Ong *et al.*, 2012a). Splitting κ-casein at low pH lessens the surface potential and steric repulsion between the casein micelle, permitting quicker protein aggregation (Holt *et al.*, 2013). Decreasing pH value also enhances Ca^{2+} activity that supports the salt bridges between the casein micelle and provides faster aggregation (Lazzaro *et al.*, 2017). However, the pH value should be controlled since lowering its value further can exacerbate the proteolytic process and cause lower yield (Ivens *et al.*, 2017).

The influence of tomato paste on curd yield was also assessed (Table 2). The control cheese had the highest curd yield, and adding tomato paste decreased the curd yield slightly. The addition of tomato pastes resulted in a slight pH reduction based on the pH profile during the curding process (as shown in Table 3). The initial milk pH was around 7.01 ± 0.03. However, after the addition of rennet (control), the value dropped between 6.60 and 6.43. The addition of tomato pastes even further reduced the pH to around 6.35. The standard renneting pH for cheddar cheese is around 6.5 (Ong *et al.*, 2012a). Thus, it was presumed that the pH decrement due to tomato pastes encouraged other proteolytic activity that caused curd loss due to protein degradation into smaller molecules. This result also follows the study by (Nugroho *et al.*, 2018) and (Wiedyantara *et al.*, 2017), where the addition of fruit extracts decreased the pH of the milk and curd yield.

The cheese composition

The cheese composition is summarized in Tables 4 and 5. The cheese is comprised of approximately 28.67 – 32.33 (30.52 ± 1.17) % moisture, 15 – 15.75 (15.42 ± 0.24) % fat, 19.10 – 20.24 (19.63 ± 0.30) % protein, and 0 – 1.069 (0.50 ± 0.39) mg lycopene/100 g. The average moisture content of the control cheese was about 32.17 ± 0.24 %, and its amount decreased by the tomato paste insertion. As shown in Table 3, the cheese product pH was lower by fortifying the tomato paste during the process and promoting syneresis. Similar results also occur in other studies (Farahat *et al.*, 2021; Jeong *et al.*, 2017). Under normal conditions, the protein appears as a (casein) micelle, a colloidal aggregate. Colloidal calcium phosphate (CCP) and counter ions make up this aggregate. This CCP maintains the integrity of the micelles and can hold a significant amount of water (3 g/g casein) (Huppertz *et al.*, 2017). However, these micelles lost their surface charge and steric repulsion during the proteolytic process. It causes CCP solubilization and an increase in protein interaction. Consequently, it decreases the water holding capacity and

stimulates syneresis (Meletharayil *et al.*, 2015). From Table 4, it can also be observed that microbial rennet resulted in higher moisture content than animal rennet. Due to the effectiveness of animal rennet's milk clotting activity (MCA), it advocated more syneresis than microbial rennet. In order to see the influence of rennet type and tomato paste on the moisture content, ANOVA two-way test was performed (Table 5). A p-value lower than 0.05 indicates the influential parameters. It is noticed that both rennet type and tomato paste had a p-value smaller than 0.05, proving a prominent influence of the moisture content. However, the interaction between rennet type and tomato was insignificant. These results confirmed the previous explanation.

Protein content in cheese was influenced by rennet type and tomato paste, as displayed in Table 4. Its value declined with the application of microbial rennet and tomato paste. As previously explained, animal rennet's chymosin is more selective in splitting κ-casein compared to microbial rennet. Thus, the microbial implementation resulted in lower protein content. The addition of tomato paste was related to pH reduction and protein loss in the whey during renneting. It was presented in Table 3 that the pH further decreased during the cheddaring and ripening process, in which pH dropped to around 6.03 – 5.47 with the addition of tomato paste. It was stipulated that more protein loss in the whey compared to control cheese. Similar events also occurred in other studies (Mehanna *et al.*, 2017; Ong *et al.*, 2012a). From ANOVA two-way test results (Table 5), rennet type, tomato paste, and interaction of both variables were corroborated to affect the cheese protein content ($p < 0.05$).

As indicated in Table 4, the rennet type and tomato paste did not significantly affect the cheese fat content. Since the process was constant for all samples, the mechanical process did not affect the cheese fat content. ANOVA two-way confirmed the evaluation, in which the p-value of rennet type, tomato paste, and interaction of both variables were larger than 0.05. It was stipulated that these variables did not affect fat content. However, comparing the fat content in this study to others (Ibáñez *et al.*, 2016; Ong *et al.*, 2012a; Zheng *et al.*, 2016), the cheese fat amount in this study was relatively small, with an average of 15.42 ± 0.24 %. Fat globules are generally trapped in the cheese pores and behave as a non-reactive filler. The size of fat globules is affected by forming a protein network during renneting (Ong *et al.*, 2012a). At low pH, the rate of protein network formation is faster and reduces the movement of fat globules. It creates restrictions for fat coalescence and derives smaller fat globules. Small globules increase the opportunity for fat loss, where the fat loss in cheese making is usually related to a mechanical process, such as agitation, cutting mechanisms, and whey removals (Logan *et al.*, 2015). This study's curd formation pH was lower than the typical renneting pH for cheddar cheese (pH = 6.5), so there was less entrapped fat in the curd during the whey removals.

According to SNI of Processed Cheddar Cheese (SNI 01-2980-1992), the cheddar composition should have composition of water (< 45%), protein (> 19.5 %), and fat (> 25%). The moisture and protein content of the obtained cheeses were according to the SNI standard. However, increasing the tomato paste to 10 g/L reduced the protein content less than the SNI standard. The fat content of fortified cheddar cheeses was lower than the SNI standard. Improvement is required to fulfill the SNI standard.

The lycopene composition

The lycopene and color for each cheese sample are displayed in Table 4. Control cheese consisted of no lycopene, but adding

5 g/L of tomato paste increased the lycopene content to 0.324 – 0.327 g/100 g. Further, increasing the tomato paste to 15 g/L improved the lycopene content to 0.993 – 0.996 mg/100 g. The obtained results were better than the study by (Solhi *et al.*, 2020), in which adding 20 g of tomato powder per kg of processed cheese increased the lycopene to 0.224 mg/100 g. Lycopene value in tomato paste is 3 – 16 times higher than in the dried forms (Górecka *et al.*, 2020). According to ANOVA two-way test (Table 5), the lycopene content was solely influenced by tomato paste ($p < 0.05$).

The CIELAB color parameters are displayed in Table 4. The color of the control cheese was white, and with the addition of tomato paste, the color shifted to orange (Figure 1). The color transformation is related to the cheese's concentration of tomato paste or lycopene.

The commercial tomato paste has the lycopene content in the range between 7 – 15.6 mg/100 g (Soares *et al.*, 2019). In this study, the lycopene content in the functional cheese was much lower than commercial products of tomato paste. Lycopene's oxidation during the cheese making might be the cause of lycopene reduction. Interaction between lycopene with other cheese composition (such as moisture) will also accelerate lycopene degradation though isomerization (Ramadhany *et al.*, 2021). Therefore, despite enhancement of nutritional value of fortified cheese, the cheese making processing and storage need to be evaluated to prevent further lycopene degradation.

The cheese classification

The moisture non-fat basis (MNFS) categorized the cheese from extra hard to soft. The fat in dry matter (FDM) classified the cheese from high fat to skim cheese. From Table 6, the MNFS was around 33.93 – 38.26 %, and the FDM was between 21.43 and 23.16 %. According to (Goosen, 2014), cheese with MNFS lower than 41% and FDM between 10 – 25% is listed as extra-hard and low-fat cheese. Low-fat cheddar cheese is suggested to have MNFS and FDM around 53.79 – 57.58 % and 14.24 – 29.57 %, respectively (Amelia *et al.*, 2013; Zheng *et al.*, 2016). The MNFS obtained in this study was much lower than the suggestion due to the low-fat content. Cheese with low MNFS has been suggested to require a longer time to mature (Ong *et al.*, 2017).

The FDM of acquired functional cheeses were still lower than 25% of SNI standard (SNI 01-2980-1992). Cheese with low FDM is categorized as skim cheese according to SNI.

The cheddar cheese's mechanical properties

The mechanical properties evaluated are firmness, hardness, adhesiveness, cohesiveness, chewiness, gumminess, and springiness, as shown in Tables 7 and 8. Firmness represents the force required to press and penetrate the sample by fingers. From Table 7, it can be noted that cheese from animal rennet was firmer than microbial rennet. It was suggested that the protein matrix formed by the animal rennet gives rise to the rigid form of the cheese. Fat content also played a role in the cheese texture. Around 15 – 30% of fat formed firm and less smooth cheese (Ong *et al.*, 2017). In this study, fat content was approximately 15.42 ± 0.24 %, resulting in a firm and solid cheese. Similar results were also performed in other studies (Zheng *et al.*, 2016). Tomato paste fortification also increased the firmness of the cheese to 34.07, and 40.48 N. Casein breakdown at low pH due to tomato paste greatly enhanced the solubilization of CCP and contributed to the change of texture. When the peptide bond is broken, two new ionic groups are formed, and each of these competes for the available water in the

system, resulting in a firmer, less easily deformed cheese (Brigiano *et al.*, 2022). According to ANOVA two-way test (Table 9), rennet type and tomato paste significantly influenced the firmness of cheese ($p < 0.05$). Hardness indicates the force required to penetrate the cheese with a knife. Similar to firmness, cheese hardness with animal rennet was higher than microbial rennet due to the effectivity of animal rennet in casein splitting and new protein network formation. Tomato paste also increased the cheese hardness for a similar reason as the increase of cheese firmness. Based on the ANOVA test, rennet type and tomato paste were the influential variables in determining the cheese hardness ($p < 0.05$).

Adhesiveness is the work necessary to remove the cheese's attractive force to the mouth surface. Protein and moisture content are the dominant factors in determining cheese adhesiveness. An increase of water–protein matrix interaction implies an elevation of cheese adhesiveness (Bulut-Solak and Akin, 2019; Zheng *et al.*, 2016). In this study, cheese from the microbial rennet has better water retention than animal rennet. It suggested a higher interaction between water and protein, resulting in a higher adhesiveness value. From Table 7, it can be implied that the control cheese had adhesiveness around 0.56 ± 0.01 mJ and 0.67 ± 0.01 mJ. Tomato paste increment reduced the cheese adhesiveness. As previously explained, low pH promotes syneresis and lower cheese moisture content. Due to weak water and protein content, the adhesiveness declines with the tomato paste insertion. ANOVA two-way test (Table 9) confirmed the significant influence of rennet type and tomato paste on adhesiveness value ($p < 0.05$).

Cohesiveness is the degree of strength of the internal bonds of the product or the degree of chewed mass held together. According to (Meletharayil *et al.*, 2015), altered protein influences cohesiveness value. The decrease in moisture and calcium content also correlates to cheese's cohesiveness. According to Table 8, the cheese cohesiveness increased with the addition of animal rennet and tomato paste. It is proposed that chymosin selectively reduces casein molecules' electrostatic interactions. Along with this occurrence, the inclination for micelles hydrophobic interaction increases, and pH drops to the isoelectric point. Around this point, the solid-like behavior surges and forms stronger and less permeable gels (Sadeghi *et al.*, 2014). During the ripening process, a thin fibrous casein matrix transforms into thicker and stronger structures. This strong matrix creates sturdy internal bonds and improves cohesiveness. Decreasing pH value due to tomato paste increased cohesiveness to around 0.65 and 0.74. It is indicated that syneresis encourages the tight protein matrix, so it improves the cohesiveness value. It was confirmed through the ANOVA test that rennet type, tomato paste, and interaction of both variables play an essential role in the cohesiveness value ($p < 0.05$).

Chewiness is the energy required to masticate the product to the ready-to-swallow state, while gumminess is the force required to disintegrate the product to the state of ready-to-swallow. Chewiness and gumminess are correlated to hardness, cohesiveness, and elasticity. In this study, the chewiness value is similar to gumminess (as seen in Table 8). It can be observed that the addition of tomato pastes decreases cheese chewiness and gumminess. The decrease of pH could increase the interaction between proteins and strengthen the chewiness and gumminess value. However, further pH reduction increases proteolytic activity and syneresis, resulting in loss of protein network and free oil, making the structure less elastic (Ong *et al.*, 2012b). According to ANOVA two-way test (Table 9), rennet type, tomato

paste, and interaction of both variables contribute to the value of chewiness and gumminess ($p < 0.05$).

Springiness is the rate of deformed product return to its undeformed condition. Its value is related to chewiness and gumminess. Like chewiness and gumminess, springiness is reduced when tomato pastes increase or lower pH. This decline might be due to the loss of elasticity. Similar to gumminess and chewiness' ANOVA test, rennet type, tomato paste, and interaction of both variables are significant in determining the springiness value ($p < 0.05$).

The statistical analysis (PLSR)

Partial Least Square Regression (PLSR) was applied to determine the effect of rennet type, tomato paste, and cheese composition on the mechanical qualities of cheese. The correlation of cheese composition to mechanical properties is displayed in Figure 2. Variables highly influence factors 1 and 2 located in the inner and outer circles. Factor 1 consists of 61% data input (X) and 66% data output (Y), while factor 2 consists of 14% data input (X) and 17% data output (Y). Cheese composition (moisture, fat, and protein) is the data input, and all mechanical properties are the data output. The response (Y) variables are utilized to interpret the relationships between X and Y variables. When predictors (X) are projected in the same direction as a response from the center, it suggests the predictors are positively related to the response. Predictors have a negative relationship if they are projected in the other direction. Predictors projected near the center are poorly displayed in the model and are therefore difficult to comprehend.

Figure 2 showed that fat, protein, and moisture were firmly represented by factor 1. Protein, moisture, and fat position opposite of firmness, hardness, cohesiveness, and gumminess. Meanwhile, adhesiveness, chewiness, and springiness were positioned in the same direction as protein, moisture, and fat. Hence, fat was less significant than protein and moisture due to its place in the inner circle. Opposite placement indicated that protein, moisture, and fat were negatively correlated to those mechanical properties. In comparison, the same direction implied a positive correlation to mechanical properties.

The correlation between rennet type and tomato paste is exhibited in Figure 3. Factor 1 represented 50% of the data input (X) and 90% of the data output (Y), whereas factor 2 represented 50% of the data input (X) and 2% of the data output (Y). Rennet type and tomato paste were the input data or predictors. The outputs or responses were firmness, hardness, adhesiveness, cohesion, chewiness, gumminess, and springiness. It can be seen in Figure 3 that tomato paste and all mechanical properties were strongly represented by factor 1. The increase of tomato paste positively enhanced cheese's firmness, hardness, cohesiveness, and gumminess. On the contrary, adding tomato paste negatively correlated to adhesiveness, chewiness, and springiness. Rennet type was a less significant variable than tomato paste in the mechanical properties value.

CONCLUSION

Tomato pastes fortified functional cheese was successfully obtained by varying rennet types and tomato paste. Animal rennet was more effective in milk clotting and curd formation than microbial rennet. Tomato paste fortification during cheddar cheese improved the lycopene content in the cheese. Nevertheless, tomato paste addition also encouraged the faster rate of cleaving of the casein peptide bond due to the low pH. The cheddar cheese obtained in this study was still listed as extra hard and low-fat cheese. The cheese's protein, moisture, and fat interaction created a unique cheese texture.

Due to its low-fat and high protein breakdown, the cheddar cheese had high firmness and hardness; and low chewiness, gumminess, and springiness.

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Table 1. The tomato paste composition

Parameters	Value
°Brix	25.24 ± 0.55
pH	4.45 ± 0.07
Lycopene (mg/100 g)	6.97 ± 0.34

Table 2. Milk Clotting Activity (MCA) and Curd Yield

No	Tomato Paste (g/L)	Rennet Type	MCA (U/mL)	Curd yield (%)
S1	Control	Animal	737 ± 136	17.50 ± 0.02
S2	5			17.24 ± 0.04
S3	10			16.84 ± 0.02
S4	15			16.35 ± 0.01
S5	Control	Microbial	1224 ± 364	16.63 ± 0.04
S6	5			16.41 ± 0.04
S7	10			16.07 ± 0.02
S8	15			15.53 ± 0.09

Table 3. The pH value in curding, cheddaring, and ripening process

No	Rennet Type	Tomato Paste (g/L)	pH		
			Curding	Cheddaring	Ripening
S1	Animal	Control	6.60 ± 0.02	6.42 ± 0.09	6.06 ± 0.04
S2		5	6.67 ± 0.02	6.35 ± 0.17	6.03 ± 0.03
S3		10	6.50 ± 0.06	6.33 ± 0.18	5.90 ± 0.03
S4		15	6.42 ± 0.11	6.27 ± 0.11	5.56 ± 0.01
S5	Microbial	Control	6.43 ± 0.03	6.05 ± 0.04	5.95 ± 0.03
S6		5	6.38 ± 0.00	6.02 ± 0.07	5.83 ± 0.07
S7		10	6.37 ± 0.00	6.00 ± 0.01	5.73 ± 0.06
S8		15	6.34 ± 0.01	5.91 ± 0.11	5.47 ± 0.06

Table 4. The cheese composition and color

No	Moisture (%-w)	Fat (%-w)	Protein (%-w)	Lycopene (mg/100 g)	L*	a*	b*
S1	31.83 ± 0.24	15.50 ± 0.35	20.19 ± 0.06	0.000 ± 0.000	78.25 ± 2.71	0.43 ± 0.21	19.51 ± 4.26
S2	30.50 ± 0.71	15.62 ± 0.18	19.84 ± 0.06	0.081 ± 0.004	76.38 ± 2.99	4.27 ± 0.38	41.60 ± 3.22
S3	30.33 ± 0.47	15.13 ± 0.18	19.62 ± 0.12	0.168 ± 0.022	77.07 ± 1.41	8.49 ± 0.70	61.75 ± 1.27
S4	28.67 ± 0.00	15.50 ± 0.00	19.49 ± 0.06	0.249 ± 0.015	63.66 ± 1.00	17.88 ± 2.37	64.75 ± 1.66
S5	32.17 ± 0.24	15.63 ± 0.18	19.62 ± 0.12	0.000 ± 0.000	75.34 ± 2.52	1.38 ± 2.12	28.60 ± 1.74
S6	30.83 ± 0.24	15.50 ± 0.00	19.67 ± 0.06	0.082 ± 0.003	65.61 ± 0.35	5.36 ± 0.51	41.73 ± 4.98
S7	30.67 ± 0.00	15.25 ± 0.35	19.41 ± 0.06	0.170 ± 0.006	64.97 ± 1.26	15.84 ± 0.79	58.54 ± 3.51
S8	29.17 ± 0.24	15.25 ± 0.00	19.19 ± 0.12	0.248 ± 0.027	57.37 ± 0.32	20.92 ± 1.02	51.73 ± 2.53

Table 5. ANOVA statistical analyses on moisture, fat, protein, and lycopene ($\alpha = 0.05$)

Variables	p-value			
	Moisture	Fat	Protein	Lycopene
Rennet Type (A)	$1.37x10^{-10}$	0.771	$1.78x10^{-4}$	0.949
Tomato Paste (B)	$9.69x10^{-10}$	0.095	$5.60x10^{-5}$	$2.20x10^{-11}$
Interaction (AB)	0.406	0.519	0.0481	0.999

Table 6. The MNFS and FDM of cheese

No	Rennet Type	Tomato Paste (g/L)	MNFS (%)	FDM (%)
S1	Animal	Control	37.67 ± 0.12	22.74 ± 0.44
S2		5	36.15 ± 0.91	22.48 ± 0.48
S3		10	35.74 ± 0.63	21.71 ± 0.40
S4		15	33.93 ± 0.00	21.73 ± 0.00
S5	Microbial	Control	38.12 ± 0.20	23.03 ± 0.18
S6		5	36.49 ± 0.28	22.41 ± 0.08
S7		10	36.19 ± 0.15	22.00 ± 0.51
S8		15	34.41 ± 0.28	21.53 ± 0.07

Table 7. The mechanical properties I

No	Rennet Type	Tomato Paste (g/L)	Firmness (N)	Hardness (N)	Adhesiveness (mJ)	Cohesiveness
S1	Animal	Control	33.48 ± 0.76	2.11 ± 0.01	0.56 ± 0.01	0.70 ± 0.02
S2		5	36.41 ± 0.83	2.18 ± 0.01	0.52 ± 0.02	0.71 ± 0.02
S3		10	38.28 ± 0.15	2.28 ± 0.01	0.45 ± 0.00	0.73 ± 0.02
S4		15	40.48 ± 0.41	2.52 ± 0.00	0.34 ± 0.04	0.74 ± 0.02
S5	Microbial	Control	31.51 ± 0.05	2.06 ± 0.00	0.67 ± 0.01	0.59 ± 0.01
S6		5	34.07 ± 0.42	2.15 ± 0.01	0.60 ± 0.04	0.65 ± 0.00
S7		10	36.76 ± 0.46	2.23 ± 0.00	0.50 ± 0.03	0.69 ± 0.00
S8		15	39.26 ± 0.13	2.48 ± 0.04	0.41 ± 0.00	0.73 ± 0.02

Table 8. The mechanical properties II

No	Rennet Type	Tomato Paste (g/L)	Chewiness (mJ)	Gumminess (N)	Springiness (mm)	Springiness (%)
S1	Animal	Control	42.62 ± 0.70	36.52 ± 0.50	1.17 ± 0.00	3.89 ± 0.01
S2		5	38.56 ± 1.91	33.61 ± 1.37	1.15 ± 0.01	3.81 ± 0.03
S3		10	30.60 ± 0.75	27.89 ± 0.54	1.10 ± 0.01	3.66 ± 0.02
S4		15	29.08 ± 0.08	26.80 ± 0.06	1.08 ± 0.00	3.62 ± 0.00
S5	Microbial	Control	54.51 ± 1.97	45.06 ± 1.41	1.21 ± 0.01	4.03 ± 0.02
S6		5	47.30 ± 0.83	39.88 ± 0.60	1.19 ± 0.00	3.95 ± 0.01
S7		10	38.47 ± 1.82	33.54 ± 1.31	1.15 ± 0.01	3.82 ± 0.03
S8		15	31.53 ± 1.30	28.56 ± 0.93	1.10 ± 0.01	3.68 ± 003

Table 9. ANOVA statistical analyses on mechanical properties (α = 0.05)

Variables	p-value						
	Firm.	Hard.	Adhes.	Cohes.	Chew.	Gumm.	Spring.
Rennet Type (A)	$8.3x10^{-5}$	$5.75x10^{-5}$	$1.2x10^{-4}$	$4.1x10^{-4}$	$2.80x10^{-6}$	$2.80x10^{-6}$	$4.27x10^{-7}$
Tomato Paste (B)	$1.4x10^{-7}$	$1.38x10^{-4}$	0.00	$6.6x10^{-4}$	$2.21x10^{-7}$	$2.21x10^{-7}$	$1.69x10^{-8}$
Interaction (AB)	0.428	0.887	0.249	0.0338	0.0069	0.0069	$9.70x10^{-4}$

Figure 1. The cheddar cheese with variation of rennet type (animal and microbial) and tomato paste

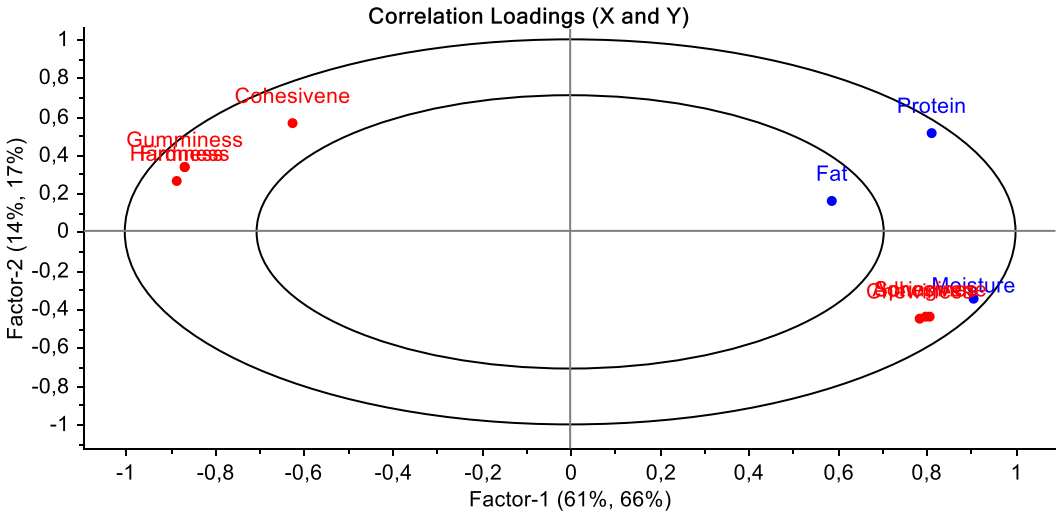

Figure 2. The Partial Least Square Regression (PLSR) of mechanical properties and cheese composition

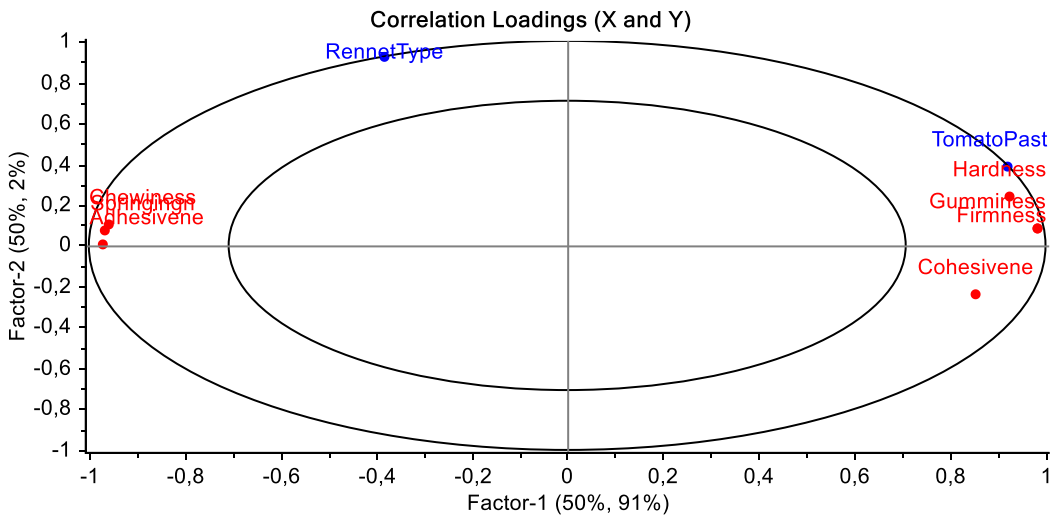

Figure 3. The Partial Least Square Regression (PLSR) of mechanical properties and variation

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Formulation and Validation of Turmeric and Black Pepper Flavored Probiotics

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ABSTRACT

The beneficial or harmless microorganisms naturally found in the digestive tract have major roles in modulating the physiological functions of the host. Probiotics are live microbial feed supplement that helps to improve the intestinal microbial balance and thus imparts beneficial health effects. Spices are established sources of natural agents for food flavor and color. In this study, probiotic curd formulations containing varying contents of turmeric and black pepper were prepared and evaluated for the viability of aerobic bacteria during the shelf life of 15 days and consumer acceptability. Based on the results of the viability studies and sensory evaluation, acceptable compositions of a probiotics curd formulation flavored with turmeric and black pepper were arrived at. The formulation with 1.0 and 0.5 % turmeric and black pepper respectively was found to maintain the required levels of probiotic bacteria during the storage for 15 days and was well accepted in the sensory evaluation.

Keywords: aerobic bacterial plate count, black pepper, probiotics, turmeric

INTRODUCTION

A large amount of beneficial or harmless micro-organisms are naturally found in our mouth, nostrils, stomach, intestine, lungs, urinary tract, vagina, skin, etc. This microflora has major roles in facilitating digestion and absorption of food components and medicines, production of vitamins, defending against pathogens, modulation of immunity, etc. They have also been found to produce many bioactive metabolites which can confer benefits to the host when consumed (Pandey et al., 2015). Conditions such as antibiotic use, travel, diet restrictions, etc. can result in the variation of nature and reduction in the content of microflora in the body, which may lead to negative physiological effects. Probiotics are generally used to restore normal microflora in the body and improve health. Probiotics are also recommended in the treatment of gastrointestinal problems, food allergies (Castellazzi et al., 2013; Tuohy et al., 2003), antibiotic-related health problems (Boyanova and Mitov, 2012), and microbial infections (Isolauri et al., 2002; Reid et al., 2003, Ried et al., 2001). Probiotic supplements are available not only in the form of foods and drinks but also in the form of tablets and capsules, sachets of encapsulated and flavored powders, etc. The common probiotic foods include dairy products like yogurt, curd, buttermilk, cheese, and kefir, fermented vegetable products like sauerkraut and kimchi, fermented soybean products like tempeh, natto, and miso, kombucha (fermented tea), fermented pickles, less cooked soup, etc. Microorganisms including

different bacterial strains belonging to *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, etc., and the yeast *Saccharomyces boulardii* (Czerucka et al., 2007; Pandey et al., 2015), are being used in probiotics formulations. Among these *Bifidobacterium* and *Lactobacillus* are the most common. *L. casei, L. paracasei*, and *L. rhamnosus* are some of the most widely researched and applied probiotic species of Lactobacilli (Saarela et al., 2000).

The nutraceutical and functional food sector, the fastest-growing segment of the food industry, is driven by the growing consumer demand for foods with health benefits and the continuous efforts of the industry to formulate foods with unique food ingredients and enhanced health benefits. Recent reports on the detrimental health effects of synthetic food ingredients lead to an increase in consumer demand for foods with coloring and flavoring agents and preservatives of natural origin. Spices and medicinal herbs being natural sources of functional flavoring agents are also being explored for their potential to flavor and color probiotics and impart medicinal effects to consumers (Illupapalayam et al., 2014). Black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) have been reported to possess a wide spectrum of health benefits including antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic, gastro-modulatory, and cardioprotective effects (Ahmad et al., 2012; Jiang, 2019; Lekshmi et al., 2014b, 2012). The presence of spices in the probiotics not only improves consumer acceptability by acting as a natural source of flavor and color but also provides some added health benefits. The presence of spices in the probiotics not only improves consumer acceptability by acting as a natural source of flavor and color but also provides some added health benefits. The antioxidant properties of herbs and spices are of particular interest as they can act as alternatives to synthetic antioxidants that are being used to improve the oxidative stability of probiotics.

Probiotic formulations should be able to provide healthy and viable microbes in a quantity sufficient to multiply and colonize the body to deliver the desired health effects. Hence it is important to ensure that the flavoring and coloring agents and other food additives will not hamper the conditions essential for the viability of microbes in the probiotics. Spices such as turmeric and black pepper contain antimicrobial components that can retard the growth and viability of microbes in probiotic formulations. Hence it is important to establish the composition of these spices sufficient to impart flavor to the probiotics without affecting the viability of microbes. In this study, probiotic curd formulations with varying compositions of black pepper, and turmeric were assessed for consumer acceptance and viability of microbes on storage to arrive at formulations with acceptable flavor and healthy microbial contents.

MATERIALS AND METHODS

Chemicals and Reagents

Plate count agar (PCA), potassium dihydrogen phosphate, (Himedia, Mumbai, India), sodium hydroxide, acetone, curcumin, piperine (Merck, Mumbai, India).

Spices

Dried black pepper berries and turmeric fingers were purchased from the local markets in Chennai, India. Black pepper berries and turmeric fingers were ground to fine powders in a domestic mixer grinder and passed through a mesh size of US 40 and stored at a cool and dry place. The powders were sterilized in an autoclave at 100^{o}C, 10 psi for 15 min. The samples were cooled to room temperature then opened and added to the formulations under aseptic conditions.

Flavor characteristics of turmeric and pepper

Volatile oil contents in the pepper and turmeric powders were determined by the AOAC 962.17 (1965). Piperin content in black pepper (AOAC 987.07-1990), and curcumin content in turmeric (ASTA 18.0-2004) were also determined.

Probiotic and raw curd

Probiotic curd of a well-known brand was collected from a local supermarket and coded as Pro-Curd for the study. The presence of Lactobacillus acidophilus and Bifidobacterium animalis with a minimum count of 106 CFU/mL was claimed on the label of Pro-Curd. Boiled milk (3 L) was inoculated with raw curd (50 mL) and allowed to ferment for 24h at room temperature and named Raw-Curd.

Preparation of Formulations

Pro-curd and Raw-curd samples were evaluated for the total aerobic bacterial count, and the samples with bacterial counts of more than 10^6 colony forming units (CFU)/mL were selected for the formulations. The formulations of Raw-Curd with turmeric were prepared by homogenizing (50 rpm, 5 Min.) 0.5, 1.0, or 1.5 g of turmeric powder with 100 mL Raw-curd and were respectively named 0.5% Raw-TUR, 1.0 % Raw-TUR and 1.5% Raw-TUR. Formulations of Raw-Curd with black pepper (0.5% Raw-BP, 1.0 % Raw-BP, and 1.5% Raw-BP) and Pro-Curd with turmeric (0.5% Pro-TUR, 1.0 % Pro-TUR, and 1.5% Pro-TUR) and Pro-Curd with black pepper (0.5% Pro-BP, 1.0 % Pro-BP and 1.5% Pro-BP) were also prepared in the same manner. Formulations of Raw-Curd or Pro-Curd with 0.5 g turmeric and 0.5 g black pepper, 1 g turmeric and 0.5 g black pepper, 0.5 g turmeric, and 1.0 g black pepper or 1.0 g turmeric and 1.0 g black pepper were also prepared. The formulations thus prepared are stored in airtight food-grade plastic containers at 4^{o}C for shelf-life studies. The containers once opened for the studies were discarded.

Total Aerobic Bacterial Plate Count

Total Aerobic bacterial count in the formulations of both Pro-curd and Raw-curd were evaluated at 0, 2, 4, 6, 8, 10, 12, and 15th days of storage as per the FDA BAM method (2001). 50g of the formulation was blended for 2 min. with 450 mL of Butterfield's phosphate buffered dilution water. The mixture was further diluted in phosphate buffer serially up to 10-10. The serial dilutions thus obtained were plated on PCA and incubated at 35^{o}C for 48h. The plates with viable colonies were enumerated in terms of CFU/mL.

Palatability studies

Two sets of fresh formulations were prepared as described above. The first set of formulations was made as sweet by adding 5g of sugar and the second set was added with 1g of table salt. Both sweet and salty formulations were subjected to sensory evaluation in terms of color, appearance, texture, flavor, and overall acceptance by a panel of 10 volunteers.

Statistical Analysis

Results were reported as mean ± standard deviation of three trials. The significant difference between the mean was determined by ANOVA followed by Tukey's pairwise comparison test at a level of $p<0.05$.

RESULTS AND DISCUSSION

Raw and commercially available probiotic curd flavoured with turmeric and black pepper at varying concentrations were evaluated for the viability of aerobic bacteria for 15 days and the results are summarized in Figure 1. The initial aerobic bacterial counts of the Raw-curd and Pro-curd used for the experiments were in the orders of 7 to 10 log

CFU/mL respectively. Raw-Curd mixed with 0.5% turmeric (0.5 % Raw-TUR) did not show significant variation in the aerobic bacterial count for 15 days. At 1% concentration (1.0 % Raw-TUR), a significant reduction in the aerobic count was observed after the 2nd day followed by a slight increase on the 6th day, and thereafter, no significant variation was observed for 15 days. Turmeric powder at 1.5% seemed to exert significant inhibition to the growth and development of bacteria in 1.5 % Raw-TUR, during the storage, at the 15th day the TPC was found to be reduced to 3.4 log CFU/mL. Black pepper at higher concentrations also reduced the aerobic bacterial count in raw curd formulations during storage. In 0.5% Raw-BP, the count reduced to the order of 4.3 log CFU/mL after 6 days and remained stable up to the 15th day. In the case of 1.0 % Raw-BP, the count significantly reduced from 7.6 to 4.3 log CFU/mL between the 6th and 12th day of storage followed by an increase to 6.3 log CFU on the 15th day. At 1.5% black pepper concentration, the aerobic count seemed to reduce continuously from 7.6 to 3.3 log CFU/mL within 15 days. The variations in the viability of bacteria in Pro-curd in presence of turmeric and black pepper were found to follow trends similar to those of Raw-curd. The aerobic bacterial counts in 0.5 % Pro-TUR, 1.0 % Pro-TUR, and 0.5% Pro-BP seemed to be maintained for 15 days of storage without much reduction from the initial value of 1010. In the case of 1.5 % Pro-TUR, 1.0% Pro-BP, and 1.5 % Pro-BP, the bacterial counts were found to be decreased gradually during storage to 10^4, 10^6, and 10^4 CFU/mL respectively. The results showed that the formulations with turmeric and black pepper at concentrations 1.0 and 0.5 % respectively were able to maintain the aerobic bacterial count without much reduction from the initial values both in Raw-curd and Pro-Curd formulations.

The fragrance of spices directly depends on the content of volatile oils. The black pepper and turmeric used for the formulations respectively contained 3.6 and 4.2 % (v/w) of volatiles. The content of piperine, the compound responsible for the characteristic flavor and pungency of black pepper was 6.5 % (w/w). The turmeric powder used in the formulations contained 4.2 % (w/w) curcuminoids. Curcuminoids which impart orange-red color to turmeric have been shown to possess many health benefits (Lekshmi et al., 2014a).

The formulations were subjected to sensory evaluation by a panel of 10 volunteers to get an indication of the palatability and acceptability of formulations. The results of the sensory evaluation of Raw-curd formulations are summarized in Table 1. Similar results were obtained for Pro-curd, hence the data is not included in the table. Salted formulations were preferred by the volunteers over sweet formulations. Salted formulations of raw curd containing 1% of both turmeric and black pepper were found to have more acceptance among volunteers followed by their 0.5% formulations. The assessment of the aerobic bacterial count showed that the formulations with 1 and 0.5% of turmeric and black pepper maintained the bacterial count without much variation. Considering the results of sensory evaluation and aerobic bacterial viability studies, a raw curd formulation containing both turmeric (1%) and black pepper (0.5%) with salt was prepared and evaluated for aerobic count and palatability. The formulation was found to reduce the aerobic count from 10^9 to 10^7 CFU/mL for up to 10 days and then increased up to 10^8 CFU/mL and maintained for 25 days (Data not shown). This formulation was also found to be well accepted by the volunteers.

On consumption, the healthy and viable microbes in the probiotic formulations are expected to multiply and colonize the body to

deliver the desired health effects. Hence it is important to ensure that the flavoring and coloring agents and other food additives would not hamper the viability of microbes in the probiotics. Most spices possess considerably higher antimicrobial properties (Keskin and Toroglu, 2011) which might stand as a retarding factor for the use of spices as flavoring agents in probiotics. However, recent studies have revealed that spices in lower proportions can be used to impart the required color and flavor to probiotics without affecting the growth and development of the biota (Illupapalayam et al., 2014). The present study also showed that turmeric and black pepper could be used as flavoring agents to the curd at lower concentrations of 1 and 0.5 % respectively.

Both turmeric and black pepper have been demonstrated to have wide spectra of health benefits (Ahmad et al., 2012; Amalraj et al., 2017; Lekshmi et al., 2014b). The probiotics formulations containing these spices could be evolved as a medium for delivering their bioactive components to the consumers. The antioxidant nature of these spices might be helpful to improve the oxidative stability of probiotic formulations. There is no fixed definition for the adequate amount of probiotic organisms in the formulations. A minimum dose of 10^9 CFU/mL of probiotic organisms is stipulated in the regulations of Canada and Italy. It would be better to add an overage dose of probiotics to the formulations containing turmeric and black pepper to compensate for the possible loss of viability due to the inherent antibacterial nature of these spices.

CONCLUSION

This study reports the development and assessment of probiotics curd flavored with turmeric and black pepper. The formulation containing 1.0 and 0.5 % turmeric and black pepper respectively was found to maintain the required levels of probiotic bacteria during the period study and was well accepted in the sensory evaluation. A better understanding of the interactions between spice components and probiotic organisms is the key element in developing spice-flavored probiotic food products. Efforts to utilize spices as natural flavoring and coloring agents for probiotics are expected to open a new area of foods with health benefits.

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Table 1. Sensory evaluation of spice-flavored formulations of raw curd

Formulation (w/v)		Colour (Max Score:10)	Appearance (Max Score:10)	Texture (Max Score:10)	Flavor (Max Score:10)	Overall acceptance (Max Score:10)	Score (Max Score:50)
Turmeric 0.5 %	Sugared	7.2 ± 1.1	7.6 ± 1.0	8.0 ± 1.1	4.0 ± 0.6	4.9 ± 1.0	31.7 ± 0.8[a]
	Salted	7.1± 1.0	7.6 ± 1.2	7.9 ± 0.8	7.2 ± 0.3	7.2 ± 0.8	37.0 ± 0.6[b]
Turmeric 1 %	Sugared	8.2 ± 0.2	8.2 ± 0.3	7.6 ± 1.2	5.2 ± 0.6	4.8 ± 0.7	34.0 ± 0.7[c]
	Salted	7.8 ± 0.7	8.0 ± 0.7	7.6± 0.4	8.0 ± 0.9	7.9 ± 1.0	39.3 ± 0.6[d]
Turmeric 1.5 %	Sugared	8.2 ± 1.0	8.2 ± 0.8	7.6 ± 0.7	4.1 ± 0.5	5.2 ± 0.9	33.3 ± 0.7[c]
	Salted	8.0 ± 0.9	8.0 ± 0.5	7.2 ± 0.7	7.5± 0.9	7.2 ± 0.8	37.9 ± 0.7[b,d]
Black pepper 0.5 %	Sugared	7.0 ± 0.9	7.0± 0.8	6.8± 0.6	4.1± 0.1	4.5± 0.1	29.4 ± 0.5[e]
	Salted	7.0 ± 0.7	6.9 ± 0.9	6.9± 0.7	7.9± 1.1	8.2± 0.9	36.9 ± 0.8[b]
Black pepper 1 %	Sugared	8.2± 0.9	7.6± 0.9	7.2± 0.7	3.9± 0.0	4.6± 0.2	31.5 ± 0.4[a]
	Salted	8.2± 1.1	7.6± 0.9	7.2± 0.7	7.6± 0.8	8.0 ± 0.7	38.6 ± 0.8[d]
Black pepper 1.5 %	Sugared	8.2± 0.9	7.2± 0.7	7.4± 0.4	3.9± 0.6	4.1± 0.6	30.8 ± 0.7[a,e]
	Salted	8.1± 0.9	7.0± 0.7	7.5± 0.5	6.2± 0.7	5.9± 0.6	34.7 ± 0.8[c]
Turmeric 1 % + Black pepper 0.5 %	Salted	7.9± 0.7	7.6± 0.5	7.8± 0.4	8.1± 0.7	7.9± 0.2	39.3 ± 0.7[d]

Mean ± n =3, Different superscripts indicate the values are significantly different ($p < 0.05$)

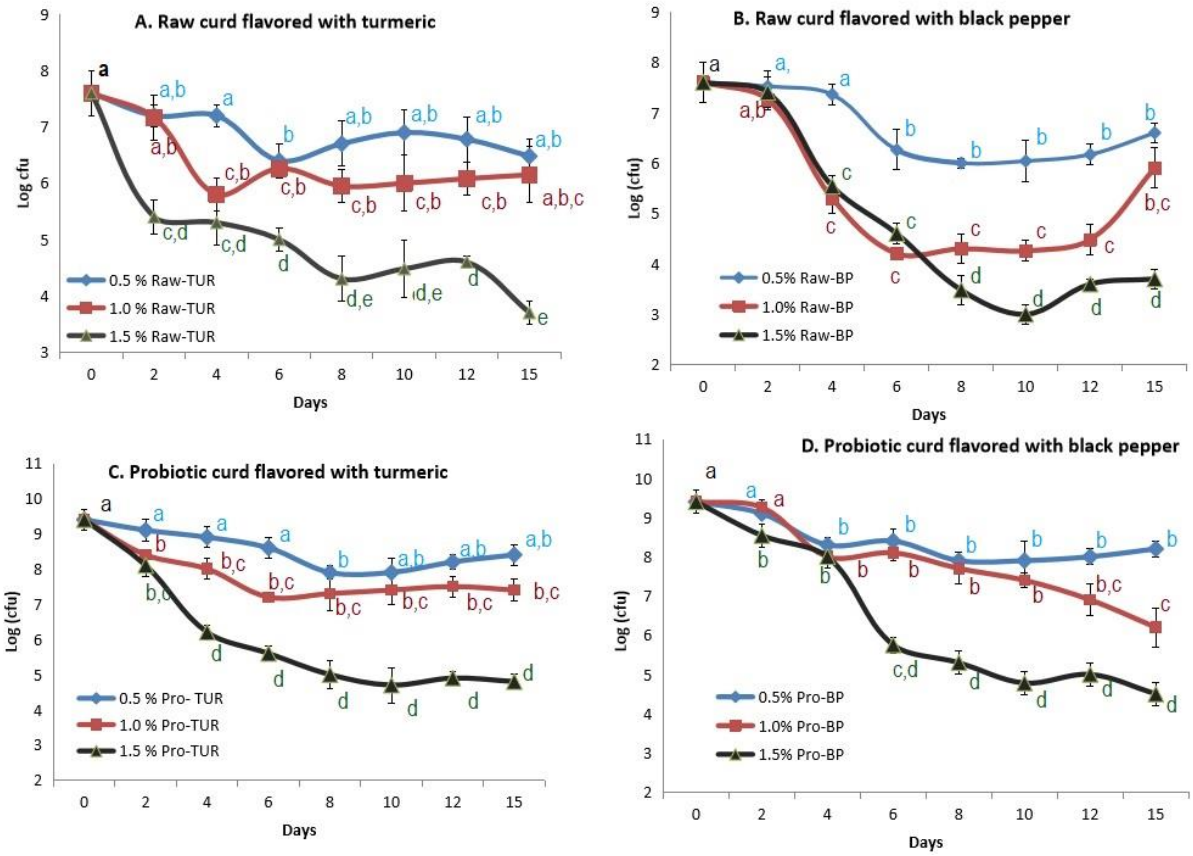

Figure 1. Variations in total aerobic bacterial count in curd flavored with turmeric and black pepper (Mean ± SD, n =3. Different notations indicate the values are significantly different ($p < 0.05$).

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Physicochemical Characteristics of Edible Film Sodium Caseinate with Sappan Wood Extract Addition

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ABSTRACT

Packaging will protect food from physical and chemical deterioration and maintains the economic value during storage. Generally, food packaging was made by plastic material which is cheap and easy to use. Plastic waste makes it difficult to degrade, so it is necessary to develop technology that aims to reduce the impact of plastic waste pollution by biodegradable packaging included edible film. Casein is one of the hydrocolloid ingredients which is utilized to made of edible film. Casein has flexibility, transparency, and tasteless film properties also it has a carrier antioxidant capacity. The addition of sappan wood extracts to the edible film that is interesting to observed as a carrier of antioxidant compounds. This research was conducted to obtain the concentration of sappan wood extract, which produces the best physicochemical characteristics of sodium caseinate edible film. The method used in this study was a one-factor Randomized Block Design (RBD) consisting of 5 levels of sappan wood extracts (0%, 2.5%, 5%, 7.5%, and 10% w/v_{total}) with 5 replications. Based on this research to obtained the best physicochemical characteristics of edible film sodium caseinate with the addition 10% sappan wood extracts, which has a thickness value of 0.20 mm, solubility 24.421%, color L* (79.0) a* (8.3) b* (32.8), water holding capacity of 6.863 (g/g), and antioxidant activity of 24.170%.

Keywords: antioxidant, edible film, sodium caseinate, sappan wood

INTRODUCTION

Packaging protects food from physical and chemical damage and maintains the economic value of stored food products. One of the packaging that are still being developed is edible packaging. Edible packaging is used to wrap (coating or wrapping) various foods to extend the shelf life of products that can be eaten together with food (Pavlath and Orts, 2009).

The edible film is a thin layer made of edible material and placed on top of food components which functions as a barrier to mass transfer such as moisture, oxygen, fat, and solutes (Julianti and Nurminah, 2006) and serves as a carrier for food components including vitamins, minerals, antioxidants, antimicrobials, preservatives, and ingredients to improve the taste and color of packaged products (Yulianti and Ginting, 2012). Edible films can be made from hydrocolloid, lipid, and mixed groups of these two materials (Prasetyaningrum et al., 2010). Hydrocolloids that can be used in the manufacture of edible films are proteins. Edible films made of protein are better at inhibiting water vapor, gas, or solutes and are also more biodegradable to reduce

environmental problems (Yoshida and Antunes, 2004). Casein is one of the proteins used to manufacture edible films (Sabil et al., 2021).

Casein is the leading milk protein that primarily contains the amino acid glutamine, which gives it a particular property that is difficult to decompose at high temperatures. Casein comprises 80% of the total milk protein and has polar functional groups, such as amino and hydroxyl groups (Ningsih et al., 2019). Based on these properties, casein is generally used as the main ingredient for forming edible film structures that can produce specific characteristics (Sabil et al., 2021). Casein has flexible, transparent, and tasteless film properties and has good nutritional value. Casein can also be a carrier for additives such as antimicrobial and antioxidant substances (Rai and Poonia, 2019).

Muin et al. (2017) stated that in addition to essential components, edible films also require additional constituent materials, such as plasticizers, antioxidants, antimicrobials, dyes, and flavors. One of the natural ingredients that can be added to manufacture of edible films is sappan wood. Sappan wood contains a lot of phenolic and flavonoid compounds that have antioxidant properties. These compounds are brazilin, 3'-O-methylbrazilin, saponins, chalcone, and sappancalchone, which can be used as primary and secondary antioxidants (Rina, 2013). Based on the potential, further research is needed to determine the best physicochemical properties of adding sappan wood extract to the sodium caseinate edible film. So that the edible film produced can protect food products from physical and chemical damage, increase the functional value of the product, and is eco-friendly, which can help reduce the problem of plastic waste that is difficult to decompose.

MATERIALS AND METHODS

Tools and Materials

The materials used for making edible film were sodium caseinate "Excellion DMV" obtained from Subur Kimia Jaya shop, and sappan wood obtained from Traditional market at Pontianak. Glycerol and aquadest were obtained from the Kalimantan Research store. The material for analysis is DPPH (1,1-diphenyl-2-picrylhydrazyl) (HIMedia). The research equipments were blender (Miyako), 80 mesh sieve, coarse filter paper, glass plate measuring 13 cm x 18 cm x 1.5 cm, analytical balance (Mettler Toledo), thermometer, hot plate (Cimarec Thermolyne), magnetic stirrer, cabinet dryer (Control egp (IL-80EN)), vortex mixer (VM-300), UV-VIS Spectrophotometer (Shimadzu UV mini-1240), digital colorimeter AMT506, oven (Phillip Harris Ltd), desiccator (Duran), Erlenmeyer (IWAKICTE33), measuring cup (IWAKICTE33), Beaker glass (IWAKICTE33), test tube, digital caliper and centrifuge (Hettich EBA III).

Methods

The research was carried out in three stages: sappan wood powder production, sappan wood extract production, and edible film production. The physicochemical tests included film thickness, solubility, color, water holding capacity, and antioxidant activity.

Sappan Wood Powder Production

The production of sappan wood powder is carried out by Dewi (2021) with modifications. The sappan wood is cleaned thoroughly, then cut into small pieces with a size of ± 3-5 cm to be dried for 5 hours at 60ºC using a cabinet dryer. The dried wood was then blended until smooth and sieved using an 80 mesh to obtain sappan wood powder.

Sappan Wood Extract Production

Sappan wood extract was made using the method of Karyantina et al. (2021) modified. 10 g sappan wood powder was added 100 ml of aquadest, and the solution was heated at 60℃ for 60 minutes using a hot plate. The resulting sappan wood suspension was filtered using filter paper to collect the filtrate. The filtrate obtained will be used for edible film productions.

Edible Film Production

The production of edible films refers to the research of Kadam et al. (2015), which has been modified. 5 g of sodium caseinate was dissolved in 100 ml of aquadest using a hot plate at 65℃ for 10 minutes. 1.5 ml of glycerol was added to the solution under the same conditions for 15 minutes of stirring. Sappan wood extract as much as 0%, 2.5%, 5%, 7.5%, and 10% (w/v_{total}) was added to the film-forming solution then stirred using a stirrer for 15 minutes to make it homogeneous.

The film-forming solution was cooled to 40℃ and filtered using filter paper. The filtering results are poured into a glass plate with a size of 13 cm x 18 x 1.5 cm^3, dried for 48 hours at a temperature of 35℃ using a cabinet dryer, then cooled at room temperature for 15 minutes.

Thickness

Thickness testing was carried out by Huri and Nisa (2014). Samples were measured using a digital caliper at five different places, different than the average measurement results due to film thickness. Thickness is expressed in mm, while the micrometer used has an accuracy of 0.01 mm.

Solubility

The solubility testing was carried out by Indrarti and Indriyati (2016). Solubility was measured by taking a 2 cm x 2 cm film sample and weighing it as the initial weight. Film samples were soaked in 50 ml of distilled water and stirred for 30 minutes with a stirrer. The soaked sample was filtered using filter paper, dried in an oven at 105°C for 2 hours, stored in a desiccator for 15 minutes, and then weighed.

Color

The color testing was carried out by Huri and Nisa (2014). Color measurement using a digital colorimeter with target readings of L, a*, b* determined at five different points.

Water Holding Capacity

The water holding capacity testing was carried out by Deden et al. (2014). Test tube in the oven for 1 hour and put in a desiccator then weighed. 0.25 g of sample was put into a test tube and added with 10 ml aquadest. Homogenize using a vortex mixer. Let stand for 1 hour, then centrifuged for 20 minutes.

Antioxidant Activity

The antioxidant activity was determined by Miranda et al. (2018). Each sample weighed 500 mg and dissolved in 5 ml methanol. The solution was allowed to stand for two hours and filtered through filter paper. 0.1 ml of solution filtrate was added to 3.9 ml of DPPH solution, then incubated at room temperature for 30 minutes.

Data Analysis

The results of testing the physicochemical properties of edible films were statistically analyzed using Analysis of Variance (ANOVA) to determine the effect of the treatment and further tested with Tukey's Honestly Significance Difference (HSD) at a 5% level. The best treatment is determined by index effectivity value (De Garmo et al., 1984).

RESULTS AND DISCUSSION

Thickness

Film thickness is included in the physical characteristics of edible films. According to the Japanese Industrial Standard (JIS), thickness is an essential characteristic in determining the suitability of films as packaging for food products. The thicker the edible film indicates that the retaining ability is also more significant, or it is more difficult for water vapor to pass so that the product's shelf life will be longer (Afriyah et al., 2015). The results of the thickness of the edible film sodium caseinate at various concentrations of adding sappan wood extract are shown at Table 1.

Table 1 shows that the thickness of the edible film ranges from 0.14 to 0.20 mm. The results of this study indicate that although it has no significant effect on the Analysis of Variance, an increase in the concentration of sappan wood extract tends to increase the thickness of the edible film. Kusumawati and Putri (2013), several factors affect the thickness of the edible film, including the area of the mold plate and the volume of the suspension, as well as the components that make up the edible film. The components of the edible film that are thought to affect the thickness of the sodium caseinate edible film are sappan wood.

Previous research from Emam-Djomeh et al. (2015) investigated adding pomegranate peel extract to edible film sodium caseinate. The addition of pomegranate peel extract causes the formation of a sponge-like structure in the film, which can increase the thickness of the edible film. Water molecules trapped in this structure's pores will increase the film's moisture content and swelling, which ultimately causes an increase in thickness. Mulyadi et al. (2016) said that the material's total solids affect the film's thickness. Hosseini et al. (2009) reported that adding cinnamon, clove, and thyme extracts to chitosan-based films caused an increase in film thickness. Li et al. (2014) found that polyphenolic compounds affected gelatin film thickness. Film thickness was higher when natural antioxidants were added. The increase in the thickness of the edible film is related to the nature of colloid compounds as thickeners and suspenders and the interaction between the components that make up the edible film (Galus and Lenart, 2013). Glycerol can absorb edible film moisture to a certain extent which causes an increase in the thickness of the edible film due to the swelling process (Ahmadi et al., 2012). Cahyo et al. (2017) stated that the more compositions of materials used in the manufacture of edible films, the thicker they will be due to the various constituent components. The higher the concentration of the constituent polymers at a specific limit can increase the thickness and stability of the edible film (Warkoyo et al., 2014).

Solubility

Solubility is one of the physical properties of edible films. Related to their solubility in water and the ability of edible films to retain water (Bourbon et al., 2011). Films with a high solubility value showed film hydrophilicity and lower water resistance (Fardhyanti and Julianur, 2015). The results of the solubility of the edible film sodium caseinate with the addition of sappan wood extract are shown at Table 2. The solubility of edible film sodium caseinate between 20.219 – 24.421%, with the highest value addition of 10% sappan wood extract.

According to Fardhyanti and Riski (2015), sappan wood contains brazilin compounds which contain many hydroxyl groups (OH^-). The hydroxyl group can bind to water molecules (H_2O), so the more hydroxyl groups are included in the edible film matrix, the higher the solubility or percent solubility (Santoso et al., 2016). Glycerol used in the ingredients for the edible

film is also thought to affect the solubility of the edible film. Glycerol is known to have hydrophilic properties, so it is easily soluble in water. Adding glycerol as a plasticizer can attract water from the environment to increase the solubility of edible films in water (Negara and Simpen, 2014). Edible films with higher solubility indicate that films are easier to consume (Krisna, 2011). The edible film, with the addition of sappan wood extract which has higher solubility, can be applied to products that can be eaten directly.

Color

The CIELAB color space is a color model similar to the perception of human vision by implementing three components: L*, a*, and b* (Harnis et al., 2019). The notation L* states showed that the brightness level with a value of 0-100 (black-white), and a* states the type of color with a negative value for green (0 - (-80)) and positive for red (0 - 80), and b* states the color type with negative values for blue (0 – (-70)) and positive for yellow (0 – 70) (Sinaga, 2019). The color results of edible film sodium caseinate with the addition of sappan wood extract shown at Table 3.

Table 3 shows that adding the concentration of sappan wood extract significantly affects the edible film sodium caseinate at the level of $\alpha = 5\%$. The value of L* decreases with adding the concentration of sappan wood extract. The highest L* value was found in the addition of 0% sappan wood extract which was 87.2, and the lowest value was in the addition of 10% sappan wood extract, which was 79.0. This happens because the more significant the concentration of sappan wood extract in the manufacture of edible films, the brightness of the edible film will decrease. According to Romruen et al. (2022), sappan wood has a red pigment compound called brazilin which can affect the brightness of the edible film itself. Colors of the edible film sodium caseinate at various concentrations of adding sappan wood extract are shown in Figure 1.

Huri and Nisa (2014) stated that the thickness of the edible film could also affect the brightness level. The higher the thickness value of the edible film, the lighter will diffuse so that the object of the edible film will appear cloudy, and the brightness will be lower. The uneven thickness also affects the color of the edible film, which causes the color of the edible film to be inhomogeneous. The same thing was reported by Emam-Djomeh et al. (2015), who investigated adding pomegranate peel extract to edible film sodium caseinate. Adding pomegranate peel extract increased the a*, b*, and ΔE indexes and decreased the L* and WI (Whiteness Index) values. Wang et al. (2012) stated that adding extracts affected the color, which was thought to come from the polyphenol component. According to Karlina et al. (2016), sappan wood contains phenolics, flavonoids, tannins, polyphenols, cardenolin, anthraquinone, sappan chalcone, caesalpin, resin, resorcin, brazilin, d-alpha phellandrene, ocimene, and essential oils. Increasing the concentration of sappan wood extract will increase the edible film's redness, yellowness, and darkness. Based on the color produced, this edible film is suitable for protecting products from protein sources, such as milk candy. Milk candy is generally only white, so applying colored edible film to milk candy can produce more attractive colors.

Water Holding Capacity

Water Holding Capacity (WHC) is defined as the ability to bind water in the material or added during the processing process and the ability of the material to hold water. WHC can also be related to the magnitude of the attraction of water (Deden et al., 2020). According to Ulfah et al. (2018), the value of WHC is closely related to the value of solubility. The higher the solubility

of edible films, the lower their ability to hold water. The WHC value of sodium caseinate edible film with the addition of sappan wood extract between 6.863 – 8.091 (g/g). The results of WHC edible film sodium caseinate with the addition of sappan wood extract shown at Table 4.

Several factors can cause the solubility of the film in water. According to Schmidt et al. (2013), the interaction between the components that make up the film matrix, such as hydrophilic and hydrophobic components, and the resulting film structure. This is presumably because sappan wood extract has a hydroxyl group that makes it hydrophilic (Fardhyanti and Riski, 2015). Lindriati et al. (2014) also stated that the main ingredients for making edible films affect the WHC value, in which protein gels have a lower water binding ability than starch gels. Protein gels have fewer hydroxyl groups than starch gels, so the ability to bind water is also lower. Edible films with low water binding capacity can be applied to food products that do not contain much water or are dry, such as candy.

Antioxidant Activity

The antioxidant activity of edible film sodium caseinate with the addition of sappan wood extract shown in Table 5. The antioxidant activity of sodium caseinate edible film range from 1.678 – 24.170%, with the highest value addition of 10% sappan wood extract. This indicates that the higher the concentration of sappan wood extract added, the higher the antioxidant activity.

Another study conducted by Oka et al. (2016) regarding the addition of cinnamon leaf extract to edible films can increase the phenol content by 4.24 (mg/100 g GAE) with the addition of 20% cinnamon leaves. These phenolic compounds are the main compounds that act as antioxidants in the product. So, with the increasing concentration of cinnamon leaf extract, the antioxidant activity produced also increases. The increase in antioxidant activity at various extract concentrations was thought to be due to increased phenolic and flavonoid compounds in the extract (Cepeda et al., 2018). Kadam et al. (2015) studied edible film made from sodium caseinate, which was incorporated with brown seaweed extract, producing antioxidant activity of 78.107% and total phenolic 5.381 mgPGU/100gdb. According to Kusumawati and Putri (2013), the antioxidant activity of edible films is influenced by the antioxidant compounds contained in the ingredients and the ability of these compounds to reduce free radicals.

Phenol compounds have a significant effect on the antioxidant activity of edible films. The higher the total phenol, the higher the antioxidant activity (Huri and Nisa, 2014; Nuansa et al., 2017). Antioxidant activity with the addition of 0% sappan wood extract showed a value of 1.678%, which means that the manufacture of sodium caseinate edible film without the addition of sappanwood extract already has antioxidant activity. This is possible due to edible films uses sodium caseinate and glycerol which antioxidant activity (Elias et al., 2008) and glycerol can be protected phenolic compounds (Nuansa et al., 2017).

Adding antioxidant compounds to the packaging aims to protect the product from oxidation. Edible films with high antioxidants can be applied to food products such as sausages and jelly candies. Both of these products are products that can undergo oxidation. According to research by Manuhara et al. (2009), sausage wrapped in edible film with added ginger extract had a lower free fatty acid value. This shows that adding natural antioxidants can reduce the breakdown of fat in the product. This antioxidant edible film can also be applied to jelly candies because, apart from being edible, antioxidant edible films can help

prevent a decrease in the quality of the candy from going rancid (Nuansa et al., 2017).

Effectivity Index

The value of the effectivity index of edible film sodium caseinate with supplementation by sappan wood was shown in Table 6. Determination of the best treatment value with this method begins with assigning a weighted value with the largest value to the observation variable that is the author's goal. Then followed by the weighting of other variables. After the analysis, the largest value indicates the best treatment value. The 1st level of variable value is the antioxidant activity assessed based on this study's urgency and its functional properties when applying edible film to a product, which aims to protect the product from quality degradation. Second, the thickness parameter refers to the characteristics of the edible film in the Japan Industrial Standard (JIS). Solubility, water holding capacity, and color are based on ease of consumption and food products that can be applied using edible films with specific characteristics.

The highest value of effectivity index was the edible film sodium caseinate with supplementation by 10% sappan wood extract (5.767). The physicochemical characteristics of the edible film was thickness (0.20 mm), solubility (24.421%), color L* (79.0) a* (8.3) b* (32.8), water holding capacity (6.863 g/g), and antioxidant activity (24.170%).

CONCLUSION

The best physicochemical characteristics of edible film sodium caseinate with the supplementation of sappan wood extract at a concentration of 10% with characteristics of 0.20 mm thickness, 24.421% solubility, color L* (79.0) a* (8.3) b* (32.8), Water Holding Capacity of 6.863 (g/g), and antioxidant activity of 24.170%.

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Table 1. Edible Film Sodium Caseinate Thickness

Sappan Wood Extract (%)	Thickness (mm)
0	0.14 ± 0.057
2.5	0.15 ± 0.032
5	0.17 ± 0.032
7.5	0.18 ± 0.051
10	0.20 ± 0.025

Table 2. Edible Film Sodium Caseinate Solubility

Sappan Wood Extract (%)	Solubility (%)
0	20.219 ± 0.880[a]
2.5	21.793 ± 2.447[ab]
5	23.037 ± 1.443[ab]
7.5	23.096 ± 2.273[ab]
10	24.421 ± 1.571[b]
HSD 5% = 3.552	

Note: Numbers followed by different letters show a significant difference in the 5% HSD test

Table 3. The Color of Edible Film Sodium Caseinate

Sappan Wood Extract (%)	L*	a*	b*
0	87.2 ± 2.781[a]	1.1 ± 0.273[a]	9.0 ± 0.432[a]
2.5	86.4 ± 1.806[a]	1.6 ± 0.257[b]	10.7 ± 1.547[a]
5	84.9 ± 2.161[a]	2.9 ± 0.751[c]	18.8 ± 1.025[b]
7.5	80.5 ± 2.180[b]	7.0 ± 0.359[d]	31.2 ± 2.409[c]
10	79.0 ± 2.650[b]	8.3 ± 0.091[d]	32.8 ± 4.840[c]
HSD 5% =	4.333	0.758	4.458

Note: Numbers followed by different letters show a significant difference in the 5% HSD test

Table 4. Edible Film Sodium Caseinate Water Holding Capacity

Sappan Wood Extract (%)	WHC (g/g)
0	8.091 ± 0.923
2.5	7.773 ± 0.484
5	7.488 ± 0.407
7.5	7.370 ± 0.423
10	6.863 ± 0.758

Table 5. Antioxidant Activity of Edible Film Sodium Caseinate

Sappan Wood Extract (%)	Antioxidant Activity (%)
0	1.678 ± 0.180[a]
2.5	7.938 ± 0.288[b]
5	18.566 ± 1.384[c]
7.5	22.020 ± 0.516[d]
10	24.170 ± 0.644[e]
HSD 5% = 1.484	

Note: Numbers followed by different letters show a significant difference in the 5% HSD test

Table 6. Effectivity Index of Edible Film Sodium Caseinate

Sappan Wood Extract (%)	NP
0	1.167
2.5	1.923
5	3.463
7.5	5.131
10	5.767

Figure 1. Edible film with different Sappan wood concentrations

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The Refining Capability of Palm Shell Activated Carbon for Waste Cooking Oil

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ABSTRACT

The high level of consumption of cooking oil will have an impact on increasing the waste of cooking oil produced. Using the waste of cooking oil needed purification, including through the adsorption process using activated carbon. This research aimed to study the ability of palm kernel shell activated carbon (PKSAC-AC260) compared to standard activated carbon (DAC or Decolorized Activated Charcoal by Sigma-Aldrich) in refining waste cooking oil at various concentrations of activated carbon. The results showed that the type of activated carbon influenced color, free fatty acid content, peroxide value, and saponification value of purified waste cooking oil, but had no effect on water content, saponification value and iodine value. Activated carbon between PKSAC-AC260 and DAC with a concentration of 2.5% was able to improve the quality of the best waste cooking oil. Purification of used cooking oil using PKSAC-AC260 and DAC was able to increase the color brightness of waste cooking oil (ΔE) by 5.44 and 4.53, reduce the free fatty acid content of waste cooking oil from 1.47% to 0.79% and 0.61%, reduce the peroxide value of waste cooking oil from 163.47 meq/kg to 116.40 meq/kg and 98.82 meq/kg, and increase the saponification value of waste cooking oil from 155.22 mg/g to 180.48 mg/g and 184.48 mg/g, respectively. The ability of PKSAC-AC260 to purify waste cooking oil is lower than that of DAC. The quality of processed oil from waste cooking oil does not meet the quality standards of cooking oil based on SNI 3741: 2013.

Keywords: Palm kernel shells, activated carbon, refining, waste cooking oil

INTRODUCTION

Based on data from the Edible Oil Market Outlook 2022-2026, Indonesia is the world's 5th largest consumer of vegetable oil after India, China, the United States and Brazil (Reportlinker, 2023). The high level of consumption of cooking oil will have an impact on increasing the waste of cooking oil produced.

Using cooking oil repeatedly at high temperatures (170^{o}C – 200^{o}C) causes a decrease in cooking oil quality. Oxidation of cooking oil will change the structure of unsaturated fatty acids (Cis) into (Trans) along with the formation of peroxide and hydroperoxide compounds which are free radicals. The characteristics of waste cooking oil can be seen from the changes in appearance and taste (Ketaren, 2008).

Heating cooking oil can cause changes in its physical and chemical structure. The change in chemical structure is the oxidation

of unsaturated fatty acids to produce peroxide groups which are free radicals and cyclic monomers. Fatty acids will be released from triglycerides so that they are oxidized to aldehydes, ketones, and alcohols resulting in the formation of a rancid flavor and a brown color (Megawati *et al*., 2019). Heating cooking oil for a long time can cause an increase in the temperature of the cooking oil. By heating the oil for 30-40 minutes, the temperature will reach over 190^{o}C and the oil will start to smoke. This results in the process of oxidation, hydrolysis, and polymerization of unsaturated fatty acids, forming form ketone compounds, aldehydes, and polymers (Manurung et al., 2015). Polymers, peroxides, aldehydes, ketones, amines, or diamines that are formed due to the oxidation of heating cooking oil repeatedly have the potential to accumulate in the human body and cause diseases such as cancer, high blood pressure, atherosclerosis, and others (Kamilah *et al*., 2015).

The health effects of repeated heating of cooking oil can cause cancer. Peroxide numbers of palm oil and soybean oil were measured after 5 times of heating, which increased significantly compared to one-time heating or with fresh oil. Fresh soybean oil with five times heating had a higher peroxide value than palm oil with the same treatment. Plasma lipid peroxidation in animals (ovariectomized male and female rats) showed significantly higher thiobarbituric acid reactive substances (TBARS) values in male rats treated orally containing 15% palm oil and soybean oil heated once and five times for four months compared to controls. The TBARS values of the mice fed the oil were five times higher than that of the one-time-heating oil group. Palm oil treatment had a much lower TBARS value than soybean oil (Deshmukh, 2019).

Purification or regeneration of waste cooking oil aims to improve the quality of waste cooking oil. The purification method of waste cooking oil can be done in several ways, including through the adsorption process using activated carbon. Adsorption is a method of purifying waste cooking oil which is considered as an uncomplicated process (Oko *et al*., 2020).

Activated carbon is a carbonaceous material that is chemically or physically activated so that the pores of the carbonaceous material become more open with a surface area ranging from 300 - 2000 m^2/g. An increase in the surface area of activated carbon will cause an increase in the adsorption of activated carbon to gas or liquid (Sudrajat & Soleh, 1994). Activated carbon is a carbon material with an amorphous structure and a large internal surface area with a high level of porosity. Activated carbon contains micropores, mesopores, and macropores in its structure. This structure has an important role in determining the performance of activated carbon as an adsorbent (Lubis *et al*., 2020). Activated carbon adsorption reaches 25-100%, so it is often used by industry.

Palm oil mill biomass waste sources of lignin, hemicellulose, and cellulose-containing carbon elements can be used as precursors of activated carbon, one of which is palm shells. The palm kernel shell contains 31.33% cellulose, 17.94% hemicellulose, and 48.83% lignin (Yanti *et al*., 2017).

The palm kernel shell of the Tenera variety contains 44.74% carbon, 1.12% ash, 0.07% total N, and 5.42% moisture content. Palm shells can be used as activated carbon because of their high carbon content (Ulfah *et al*., 2016). Palm kernel shell activated carbon (PKSAC) which is made through a chemical activation process using a 65% phosphoric acid solution by immersing the palm kernel shell particles in a solution of phosphoric acid (1 g/2 mL) for 60 minutes, has a surface area of 1,395.68 m^2/g, mesopore volume of 0.16 cc/g, with a carbon content of 67.55 ± 0.55% (Ulfah *et al*., 2019). Decolorized activated

carbon (DAC) as standard activated carbon is activated carbon with fine particles that are often used to decolorize colored solutions. According to Ulfah *et al*. (2016), DAC-activated carbon has a surface area of 1068.391 m^2/g.

The process of refining waste cooking oil using palm shell activated carbon compared to standard activated carbon at different concentrations will be studied in this research. This study aims to determine the effect of the type and concentration of activated carbon on the characteristics of waste cooking oil, and to determine the type and concentration of activated carbon capable of producing the best quality oil. The method of refining waste cooking oil to produce oil-based non-food product raw materials is expected to be obtained from this research.

MATERIALS AND METHODS

Tools and Materials

The materials used in this research include palm kernel shell activated carbon (PKSAC-AC260), standard activated carbon namely decolorized activated charcoal or DAC (Sigma-Aldrich), waste cooking oil, control cooking oil (Bimoli Special), alcohol (Merck), chloroform (Merck), CH_3COOH (Merck), ultra-pure distilled water H_2O, KOH (Merck), KI (Merck), amylum (Merck), NaOH (Merck), $Na_2S_2O_3$ (Merck), HCl (Merck), I_2 (Merck), Br (Merck). The tools used in this study include a portable colorimeter (NH310 3nh), vacuum pump (Roker 300), hotplate stirrer (Cimarec SP88857105), oven (Memmert u40), analytical balance (Ohaus CP 214), and other glassware used in this research.

Methods

This study was designed using a Complete Block Design with 2 factors. The first factor is the type of activated carbon with 2 types, namely: A1 = palm shell activated carbon (PKSAC-AC260) and A2 = decolorized activated charcoal (DAC) as standard. The second factor is the concentration of activated charcoal, which consists of: B1 = 1.5%, B2 = 2% and B3 = 2.5%. This study was repeated 2 times which was expressed as a block. The data obtained were analyzed for variance, if there is an influential treatment then it is continued with Duncan's multiple range test with a significance level of 0.05 (Gomez & Gomez, 1984).

Purification of waste cooking oil using PKSAC-AC260 and DAC with concentrations of 1.5%, 2% and 2.5%. Purification was carried out by preparing 100 mL of waste cooking oil into a 250 mL Erlenmeyer for each treatment. Furthermore, activated carbon was added as much as 1.5% (1.5 g), 2% (2 g), 2.5% (2.5 g), respectively. The Erlenmeyer containing waste cooking oil and activated carbon was coated with aluminium foil, then heated and stirred using a hot plate magnetic stirrer for 60 minutes at 70^oC. Furthermore, the filtrate is separated from the residue using a vacuum filter. The filtrate that has been obtained is then filled into bottles that have been coated with aluminium foil for further analysis. The waste cooking oil before and after purification was analyzed for the total color difference (Portable Colorimeter NH310 3nh), the free fatty acid content of the AOCS Ca 5a-40-97 method (AOSC, 2004), the moisture content and volatile compounds using the AOCS Ca 2c-25-97 method. The peroxide value for the AOCS method Cd 8-53-03 (AOCS, 2004), the saponification number for the AOCS method Cd 3-25 (AOCS, 2017), the iodine number for the AOCS method Cd 1d-92 (AOCS, 2017).

RESULTS AND DISCUSSION

Total of Color Difference

Color indicates the level of brightness of a material. PKSAC-AC260 and DAC's

ability to purify waste cooking oil can be seen from the total color difference compared to standard cooking oil. Analysis of color differences was carried out using a portable colorimeter NH310 3nh. The appearance of waste cooking oil after purification using PKSAC-AC260 as well as DAC and standard cooking oil can be seen in Figure 2. The difference of waste cooking oil color after purification between PKSAC-AC260 and DAC can be seen in Table 1.

Table 1 shows that the total color difference of waste cooking oil after being purified using DAC has a smaller value than PKSAC-AC260. This shows that the color of waste cooking oil that has been purified using DAC is similar in color to the control cooking oil, while the total color difference of the control cooking oil can be seen in Table 6. This is due to the higher ability of DAC to absorb the color of used cooking oil compared to PKSAC-AC260. The results of the study (Ulfah *et al*., 2016), showed that the total surface area of DAC was 1068.391 m^2/g, while the total surface area of PKSAC according to (Ulfah et al., 2019) was 1395.69 m^2/g. The mesoporous surface area of the DAC is 275.431 m^2/g with a mesoporous volume of 0.528 cc/g (Ulfah *et al*., 2017), while PKSAC-AC260 has a mesoporous surface area of 139.81 m^2/g with a mesoporous volume of 0.16 cc/g (20%) (Ulfah *et al*., 2019). From the textural properties between DAC and PKSAC activated carbons, it shows that higher mesoporous surface area and mesoporous volume of activated carbon will also adsorb higher dyes.

DAC has the ability to adsorb free fatty acids higher than PKSAC, this is due to the different mesoporous properties of the two types of activated carbon. According to Ulfah *et al.* (2016), palm kernel shell activated carbon (PKSAC) has a lower adsorption ability than DAC. PKSAC-AC260 has a surface area of 1680.877 m^2/g, while DAC has a surface area of 1068.391 m^2/g with a mesoporous volume/total pore volume of 55.4% (Ulfah *et al*., 2017).

DAC has the ability to adsorb free fatty acids higher than PKSAC-AC260, this is also influenced by the composition of surface functional groups of DAC which are non-polar more than polar groups. The surface functional groups of the polar DAC consist of NH (amine), P-H (phosphine), C-N (amine). While the non-polar functional groups consist of C-H (ester), N-H (amine), carbonate ion, C-H (alkyne) and C-I (aliphatic iodo compounds) (Ulfah *et al.*, 2019). Although PKSAC-AC260 has a higher surface area than DAC, the composition of non-polar surface functional groups is less than that of polar functional groups. According to Ulfah *et al.* (2019), PKSAC-AC260 contains a polar functional group consisting of -NH stretch (amine), P-H (phosphine), NO_2 antisym stretch (aromatic nitro compound), organic sulphate, C-N stretch (secondary amine), C-O stretch (alcohol) and O-H out-of-plane bend (alcohol). While the non-polar functional groups of PKSAC-AC260 consist of C-H asym/sym (methyl), C-O-C antisym stretch (aliphatic ether), C-H out-of plane bend (vinyl) and C-H bend (alkyne).

The higher the concentration of activated carbon used for refining waste cooking oil, the free fatty acid content of the purified oil decreased. According to Karabulut *et al*. (2008), increasing the concentration of activated carbon will provide more surface area for adsorption. At a certain concentration, the adsorption ability of activated carbon will slow down and eventually be constant. Activated carbon at a concentration of 1.5% to 2.5% showed an increase in free fatty acid adsorption, this was indicated by the lower free fatty acid content. When compared to the free fatty acid content of standard cooking oil, that is maximum 0.3% based on SNI 3741:2013 (BSN, 2013),

so refined waste cooking oil contains much higher free fatty acids, so the purified oil is not good if used as edible oil because it will have an impact on health.

Free Fatty Acid

Free fatty acid content is a parameter of oil quality due to the hydrolysis process. High levels of free fatty acids indicate low oil quality. The effectiveness of adsorption of waste cooking oil using PKSAC-AC260 and DAC can be seen from the decrease in free fatty acid levels after purification. The levels of free fatty acids after the purification process using PKSAC-AC260 and DAC are shown in Table 2.

Water Content

The water content in cooking oil will contribute to the hydrolysis process which produces free fatty acids, thereby reducing them. The ability of PKSAC-AC260 and DAC to adsorb water from waste cooking oil can be seen from the water content of waste cooking oil after purification which is presented in Table 3.

Based on Table 3, both PKSAC-AC260 and DAC could adsorb waste cooking oil water, which is not significantly different, this is probably due to the low water content of waste cooking oil (1.33%).

The higher concentration of activated carbon did not affect the water content of the waste cooking oil after adsorption. These data support the reason that although the surface area of activated carbon increases with increasing concentration of activated carbon, but because the polar surface functional groups of activated carbon are not strong enough to bind hydrogen ions from water, so the water adsorbed is quite low.

Based on SNI 3741:2013, good quality cooking oil has a maximum moisture content of 0.15% (BSN, 2013). Waste cooking oil after the adsorption process using PKSAC-AC260 and DAC is still in accordance with SNI standards.

Peroxide Value

Peroxide value is a parameter for reducing the quality of cooking oil caused by the oxidation process of unsaturated fatty acids that bind oxygen to the double bonds (Ketaren, 2008). Peroxide value has a positive correlation to the rate of oxidation reaction of cooking oil. The ability of PKSAC-AC 260 and DAC in purifying waste cooking oil based on peroxide value is presented in Table 4.

Table 4 shows that waste cooking oil after refining using DAC produced a lower peroxide value than PKSAC-AC260. The adsorption of waste cooking oil peroxide involves surface functional groups contained in activated carbon. Peroxide compounds are non-polar oxidation products that are soluble in cooking oil, so they are more easily bound to the non-polar surface functional groups of activated carbon.

The higher the concentration of activated carbon used for adsorption, the lower the peroxide value of waste cooking oil. The more activated carbon will increase the surface area of contact with the peroxide compound, so the chance of the peroxide bound to the functional group on the surface of the activated carbon is also higher. Increasing the concentration of activated carbon will increase the adsorption of a compound on the adsorbent particles physically and chemically.

The standard cooking oil peroxide value is 18.84 meq/kg, while based on SNI 3741:2013 the maximum is 10 meq/kg (BSN, 2013). This shows that at concentrations of activated carbon up to 2.5%, it has not been able to produce regenerated waste cooking oil that is suitable for use as edible oil. The regenerated waste cooking oil in this study would be more suitable to be used as an oil-based non-food product.

Saponification Value

The saponification value indicates the mg of KOH required to saponify one gram of oil or fat (Ketaren, 2008). The saponification value is used to estimate the molecular weight of crude oil or fat. A low saponification value indicates a high molecular weight. The ability of PKSAC-AC 260 and DAC for refining waste cooking oil based on saponification value can be seen in Table 5.

Table 5 shows that the saponification value of waste cooking oil after purification using DAC and PKSAC-AC260 is not significantly different, but the saponification value that using DAC is greater than that of PKSAC-AC260. This is because DAC has a higher ability to adsorb free fatty acids, so the oil contains lower free fatty acids and the oil has a higher purity.

The higher concentration of activated carbon for refining waste cooking oil will increase the saponification value. Due to the greater amount of activated carbon used for adsorption, the more surface area for free fatty acid adsorption, so that the purity of the oil is higher. The higher purity of refined oil, it will have properties similar to the quality standards of cooking oil. The saponification value of cooking oil standar is 190.259 mg KOH/g, while according to Hui (1996) the saponification value for palm oil is 190.1 – 201.7 mg/g (Hui, 1996).

The Quality of Refined Oil Using Activated Carbon

The quality of waste cooking oil that was purified using activated carbon PKSAC-AC260 and DAC compared to the quality of waste cooking oil before purification with fresh of cooking oil and standard of cooking oil based on SNI 3741:2013 is presented in Table 6.

From Table 6 it can be seen that activated carbon A1 (PKSAC-A260) and A2 (DAC) have the ability to increase the color brightness of waste cooking oil (ΔE) by 5.44 and 4.53, respectively. PKSAC-A260 and DAC were able to reduce the water content of waste cooking oil from 1.33% to 0.13% and 0.09%, respectively. PKSAC-A260 and DAC were able to reduce the free fatty acid content of waste cooking oil from 1.47% to 0.79% and 0.61%, respectively. PKSAC-A260 and DAC were able to reduce the peroxide value of waste cooking oil from 163.47 meq/kg to 116.40 meq/kg and 98.82 meq/kg, respectively. PKSAC-A260 and DAC were able to increase the saponification value of waste cooking oil from 155.22 mg/g to 180.48 mg/g and 184.48 mg/g, respectively. The ability of PKSAC-AC260 to purify waste cooking oil is lower than that of standard activated carbon (DAC).

CONCLUSION

Activated carbon PKSAC-AC260 or DAC with a concentration of 2.5% was able to improve the quality of the best waste cooking oil. Purification of used cooking oil using PKSAC-AC260 and DAC was able to increase the color brightness of waste cooking oil (ΔE) by 5.44 and 4.53, respectively. PKSAC-A260 and DAC were able to reduce the water content of waste cooking oil from 1.33% to 0.13% and 0.09%, respectively. PKSAC-A260 and DAC were able to reduce the free fatty acid content of waste cooking oil from 1.47% to 0.79% and 0.61%, respectively. PKSAC-A260 and DAC were able to reduce the peroxide value of waste cooking oil from 163.47 meq/kg to 116.40 meq/kg and 98.82 meq/kg, respectively. PKSAC-A260 and DAC were able to increase the saponification value of waste cooking oil from 155.22 mg/g to 180.48 mg/g and 184.48 mg/g, respectively. The ability of PKSAC-AC260 to purify waste cooking oil is lower than that of standard activated carbon (DAC). The quality of processed oil from waste cooking oil does not meet the quality standards of cooking oil based on SNI 3741: 2013. Waste

cooking oil purification products are more suitable used for oil-based non-food products, such as soap, biodiesel, or other non-food products.

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Figure 1. Waste cooking oil after purification using PKSAC-AC260 as well as DAC and control cooking oil. a (DAC 2.5%); b (DAC 2%), c (DAC 1.5%); d (waste cooking oil); e (control cooking oil); f (PKSAC-AC260 1.5%); g (PKSAC-AC260 2%); h (PKSAC-AC260 2.5%)

Table 1. Total difference color from waste cooking oil after purification

Type of Activated Carbon	Concentration of Activated Carbon			Average of A
	B1 (1.5%)	B2 (2%)	B3 (2.5%)	
A1 (PKSAC-A260)	6.35	5.15	4.82	5.44^{x}
A2 (DAC)	5.74	4.86	2.98	4.53^{y}
Average of B	6.04^{c}	$5,01^{d}$	3.90^{f}	

Note: The mean value in the column or row followed by a different letter indicates the difference between treatments at a significance level of 0.05 (Duncan's Test)

Table 2. Fatty acid contents of waste cooking oil after purification

Type of Activated Carbon	Concentration of Activated Carbon			Average of A
	B1 (1.5%)	B2 (2%)	B3 (2.5%)	
A1 (PKSAC-A260)	0.99	0.73	0.66	0.79^{x}
A2 (DAC)	0.71	0.62	0.49	0.60^{y}
Average of B	0.85^{c}	0.68^{d}	0.57^{f}	

Note: The mean value in the column or row followed by a different letter indicates the difference between treatments at a significance level of 0.05 (Duncan's Test). Free fatty acid content of control cooking oil is 0.17%, while based on SNI 3741:2013 a maximum of 0.3% (BSN, 2013)

Table 3. Water content of waste cooking oil after purification (%)

Type of Activated Carbon	Concentration of Activated Carbon			Average of A
	B1 (1.5%)	B2 (2%)	B3 (2.5%)	
A1 (PKSAC-A260)	0.10	0.13	0.16	0.13^{x}
A2 (DAC)	0.10	0.07	0.09	0.08^{x}
Average of B	0.10^{c}	0.10^{c}	0.12^{c}	

Note: The mean value in the column or row followed by a different letter indicates the difference between treatments at a significance level of 0.05 (Duncan's Test). Water content of control cooking oil is 0.02%, while based on SNI 3741:2013 a maximum of 0.15% (BSN, 2013)

Table 4. Peroxide value of waste cooking oil after purification (meq/kg)

Type of Activated Carbon	Concentration of Activated Carbon			Average of A
	B1 (1.5%)	B2 (2%)	B3 (2.5%)	
A1 (PKSAC-A260)	151.51	114.12	83.56	116.40^{x}
A2 (DAC)	120.82	95.08	53.57	89.82^{y}
Average of B	136.16^{c}	104.60^{e}	68.56^{d}	

Note: The mean value in the column or row followed by a different letter indicates the difference between treatments at a significance level of 0.05 (Duncan's Test). Peroxide value of control cooking oil is 18.84 meq/kg, while based on SNI 3741:2013 a maximum of 10 meq/kg (BSN, 2013)

Table 5. Saponification value of waste cooking oil after purification (mg KOH/g)

Type of Activated Carbon	Concentration of Activated Carbon			Average of A
	B1 (1.5%)	B2 (2%)	B3 (2.5%)	
A1 (PKSAC-A260)	174.85	182.00	184.58	180.48^{x}
A2 (DAC)	175.64	177.46	200.33	184.48^{x}
Average of B	175.25^{c}	179.73^{c}	192.45^{c}	

Note: The mean value in the column or row followed by a different letter indicates the difference between treatments at a significance level of 0.05 (Duncan's Test). Saponification value of control cooking oil is 190.259 mg KOH/g, while according to Hui (1996) the saponification value of palm oil is 190.1- 201.7 mg KOH/g (Hui, 1996)

Table 6. The quality of purified waste cooking oil compared to the fresh of cooking oil

Sample	TCD (ΔE)	W (%)	FFA (%)	PV (meq/kg)	SV (mg/g)
Refined oil using PKSAC-A260	5.44	0.13	0.79	116.40	180.48
Refined oil using DAC	4.53	0.09	0.61	89.82	184.48
Waste cooking oil	19.75	1.33	1.47	163.47	155.22
Fresh of cooking oil *	0.00	0.02	0.02	18.84	190.26
SNI 3741:2013	Normal	Max. 0.15	Max. 0.3	Max. 10	190.1 – 201.7**

Note: TCD (Total color difference); W (water content); FFA (free fatty acid content); PV (peroxide value); SV (saponification value); * (Bimoli special); ** (Hui, 1996)

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Assessment of Microbiological Contamination of Branded and Street Vended Ice-Cream: A Comparative Study in Tangail Municipality, Bangladesh

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ABSTRACT

Ice cream is a popular dessert consumed by people of all ages, and its consumption can pose a risk of exposure to various microorganisms, including pathogenic bacteria and viruses. Assessment of the microbiological contamination status of branded and street-vended ice cream is crucial to ensure the public health safety. To identify potential sources of contamination, evaluate the effectiveness of the hygiene practices different microbiological and physicochemical analysis was done. Microbial analysis revealed that total viable bacteria in branded ice-cream ranged from 4.8×10^3 to 1.10×10^5 cfu/ml and in street vended ice-cream ranged from 7.5×10^4 to 1.6×10^8 cfu/ml. Total coliform bacteria present upto 9.20×10^3 cfu/ml in branded ice-cream and 5.3×10^3 to 9.6×10^6 cfu/ml observed in street vended ice-cream. In case of specific pathogen most of the samples contaminated with *E. coli* and *Staphylococcus aureus* were found to be present in branded samples up to 10^4 cfu/ml and 10^6 cfu/ml on street samples. The pH of both type of ice-cream showed acidic to neutral condition. The range of Total soluble solids in several branded ice-creams were 26 to 29% and the value of TSS obtained in street vended ice-creams were ranging from 5 to 10%. These results indicated that, the microbial quality in all street ice-creams exceeded the BSTI standard and exhibited the lower quality than the industrially produced branded ice-creams due to comparatively faulty manufacturing process and poor hygiene practice.

Keywords: Food safety, physicochemical, Ice-cream, foodborne pathogens

INTRODUCTION

Most of the people throughout the world, due to their hectic life style are now increasingly depend on ready-to-eat food. Milk considers as a complete food but as milk is a perishable food so it require immediate consumption but processed into other products like ice-cream, yoghurt, cheese, butter which can also contribute to human nutrition (Rahman *et al*., 2016; Moshood *et al*., 2013). Among these processed dairy products, ice-cream is undoubtedly the most popular dairy product in whole world and continues to be a dominant interest of large segments of the population (Rahman *et al*., 2016). Ice-cream is a congealed and nutritionally enriched dairy product combined of milk fat (about 10-16%), sugar

(9-12%), non-fat milk solids (about 9-12%), (0.20- 0.50%) stabilizer and /or emulsifier, flavoring agents, coloring materials and thickeners (Armnanios *et al*. 2017; Aaku *et al*., 2004; Potter and Hotchkiss 1995).

Physical, chemical and microbial quality of products are mainly affected by the methods of handling and storage conditions of ice cream (Akter *et al*., 2019; Rahman *et al*., 2016; Park *et al*. 2020). Most of the ice-creams become contaminated with microbes during production, transit, and preservation (Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019). Primary sources of contamination include the use of contaminated raw material like defiled water, contaminated milk and milk powder and secondary sources of contamination may include flavoring, coloring agents, utensils, stabilizers, handling and also from air during processing (Velázquez-Ordoñez *et al*., 2019; Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019). Generally, sterilization method like, pasteurization followed by freezing and hardening can eliminate the most of pathogenic organisms; however a handful of hazards can still retain in the finished products (Rahman *et al*., 2016; Akter *et al*., 2019). Normally microorganisms cannot grow in frozen mixes and it occurs only when there is prolongation between pasteurization and freezing process responsible for that spoilage by microorganisms (Rahman *et al*., 2016; Akter *et al*., 2019). In the last few decades, several reports have been exhibited the manifestation of gastrointestinal diseases by contaminated ice creams in Asia, Europe and North America (Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019). In other prior reports, high microbial loads in ice-cream samples found from different city of Nigeria (Edward *et al*., 2017; Mohammed *et al*., 2019). Studies conducted in Iran investigated that the main bacteria which causes food poisoning in this country include *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus*, which are transferred to humans through the consumption of milk and dairy products, thereby leading to zoonotic diseases with a high rate of mortality (Ghorbani-Ranjbary *et al*., 2011; Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019).

The consumption of ice-cream is higher in vulnerable age groups like children and these are also consumed by hospital patient in case of throat and mouth operation which raises the necessity to maintain a high microbiological safe standard of ice-creams throughout the world (Rahman *et al*., 2016; Jadhav and Raut, 2014). The Centre for Food Safety (CFS) betokened the findings of a targeted food surveillance project to assess the microbiological quality of ice-creams and frozen confections today. In developed countries, quality control measures are taken to increase the shelf life of ice-creams as well as to prevent potential threat of public health now a days (Rahman *et al*., 2016; Akter *et al*., 2019). Unfortunately, Bangladesh is still behindhand in this respect. Due to improper standard hygiene practices, non- enforcement of routine inspection act and lack of awareness the standard quality of ice-cream is badly affected (Kells and Gilmour 2004; Rahman *et al*., 2016; Akter *et al*., 2019). Thus, the consumers are deprived of getting standard quality ice-cream and suffer from several food borne diseases in our country. Especially, street vended ice-creams are most popular among school children in rural area as it is the cheapest but pose great threat to public health. Being a popular ready-to-eat food among all age groups, ice-creams warrant the maintenance of sound microbiological quality to ensuring the public health safety (Rahman *et al*., 2016; Akter *et al*., 2019).

Considering all these facts, the present study was conducted to determine the

bacterial load in branded and street vended ice-creams in our country to ensure the public health safety and find out which one is more microbiologically safe.

MATERIALS AND METHODS

Study area, duration, collection of sample

The study was conducted at Tangail municipality, Dhaka, Bangladesh**.** In total 24 ice-cream samples were randomly taken out from three stations namely New bus stand, Old bus stand, Santosh at Tangail. Among them 15 samples were of branded ice-creams and 9 samples were of street ice-creams. All samples were transported in an insulated container packed in ice. After bringing the samples into the laboratory, these were moved into refrigerator immediately for analyzing and subsequent studies. The experimental study was carried out during the month of January-August in 2019.

Preparation of samples

1mL of each sample will be taken aseptically with a sterile micropipette and transferred carefully into the test tube having 9mL sterile saline. Thus 1:10 dilution of the samples will be obtained. The mixture will be mixed properly and serially diluted up to 10^{-5} according to the standard method. Thus, the samples will be studied in quantitative and qualitative method (Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019).

Microbial analysis

The Spread Plate technique was performed for bacterial total plate count with serial dilution by following the standard procedure (APHA, 2004; Cappuccino and Sherman, 2001; Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019). Plate count agar, MacConkey agar, Eosine Methyle Blue agar, Mannitol salt agar were used for the growth of total viable bacteria, total coliform, total *E. coli*, *Staphylococcus aureus* respectively. Colonies formed in the plates were counted by using digital colony counter after incubation at 37 ^{o}C for 24 hours (Rahman *et al*., 2016; Marjan *et al*., 2014; Banik *et al*., 2014; Akter *et al*., 2019). The actual numbers of bacteria were estimated as colony forming unit (cfu/ml) and the counted results were recorded by the standard equation.

Physico-chemical analysis

pH meter was used for determination of pH of ice-cream and Total soluble solids content of ice-cream was determined by using a refractometer whereby a drop of solution was placed on its prism.

Statistical analysis

MS Excel and SPSS were used for the statistical analysis of data. Results were presented in tabular form. Findings of the analyzed data were represented by different column charts.

RESULTS AND DISCUSSION

Microbial analysis

The range of total viable count (TVC) among different brands of ice-cream samples ranged from 4.8×10^3 cfu/ml to 1.10×10^5 cfu/ml at different locations (Figure 1). Among different brands of ice-cream samples, the highest value of TVC found in sample 5 which was 1.10×10^5 cfu/ml (log value 5.04 cfu/ml) at Santosh and the lowest value of TVC found in sample 1 which was 4.8×10^3 cfu/ml (log value 3.6 cfu/ml) at New bus stand.

According to Farah *et al.*, 2010, the highest microbial load was observed in Cornetto cone ice-cream (1.6×10^3cfu/g) and lowest value found for Mango cup ice-cream 6.3×10^2cfu/g in Dhaka city which value is lower than the present study. Moshood *et al*., 2013, found that the total bacteria count per ml of the industrially produced ice cream sample range between 3.0×10^3 - 8.8×10^3cfu/ml with a mean count of 5.3×10^3 in

Bauchi, Nigeria which indicates the lower value from this study.

According to Bureau of Indian Statistics (BIS) 1998, acceptable range of total viable count in ice-cream is 2.5×10^5 cfu/g. In the present study, all brands of ice-cream did not exceed the cutoff level of standard value but all brands of ice-cream sample were more or less contaminated with pathogenic bacteria. But in different stations the value of branded ice-cream varied due to the contamination during transportation and the source of contamination could be occurred from retailers, most of them do not store ice-cream at the appropriate storage temperature. This unappropriated temperature may favors and encourage bacterial growth which eventually causes spoilage.

On the other hand, the range of TVC in street vended ice-cream was 7.5×10^4cfu/ml to 1.6×10^8cfu/ml at different locations (Figure 2). The highest value of total viable count was 1.6×10^8cfu/ml (log value 8.2 cfu/ml) which was found in lolly ice-cream at New bus stand and the lowest value was 7.5×10^4 cfu/ml (log value 4.8 cfu/ml) found in mango ice-cream at Santosh.

According to Jannat *et al.,* 2016, Total viable count in street vended ice-cream samples was in the range of 7.0×10^3 cfu/ml to 2.7×10^5 cfu/ml in different locations of Dhaka city. This value was comparatively lower than our findings. Moshood *et al*., 2013, found that the locally produced ice-cream sample had the higher mean count of 2.0×10^4 cfu/ml and industrially produced ice-cream with a mean count of 5.3×10^3 cfu/ml in Bauchi.

From this finding, it was confirmed that all the street vended ice-creams crossed the acceptable limit of BIS. The value of TVC of street vended ice-creams was comparatively higher than the branded ice-cream products. These are mainly due to faulty manufacturing process, improper pasteurization and storage temperature and poor environmental hygiene practice in production and handling process. So, it is observed that, comparatively branded ice-creams were more hygienic than the street ice-creams (Rahman *et al*., 2016).

According to Food Safety and Standard Authority of India (FSSAI), Total Coliform Count (TCC) of ice-cream should not exceed 100cfu/ml. According to Bureau of Indian Statistics 1998, no coliform bacteria should be present in ice-cream. From this finding, the mean value of all branded of ice-cream except sample 1 exceeded the Indian Food Safety Standard and regulation (2011).

According to Jannat *et al.,* 2016, the average number of coliform count in branded ice-cream samples was 4 cfu/ml in Dhaka city which value was lower than this study.
On the other hand, the range of TCC in street vended ice-cream was 5.3×10^3cfu/ml to 9.6×10^6cfu/ml at different locations (figure 4). The highest value of total coliform count was 9.6×10^6cfu/ml (log value was 6.9 cfu/ml) found in lolly ice-cream at new bus stand and the lowest value was 5.3×10^3cfu/ml (log value was 3.7cfu/ml) observed in mango ice-cream at Santosh.

Mokbul *et al*, 2016, investigated bacteriological profile of two street of ice-cream, kulfi and lolly, collected from four different zones of Dhaka city. For kulfi, the highest coliform count was found in zone 3 (5.7×10^5 CFU/ml) and lowest in zone 1 (7×10^3 CFU/ml). For lolly, the highest count was found in zone 2 (5.1×10^7 CFU/ml) and lowest count was found in zone 4 (5.2×10^5 CFU/ml). Comparing this result with the previous study, it was found that the value of both studies almost similar or there is a slight higher extent of coliform found in their study. Elango *et al*., 2010, studied 24 samples of Kulfi ice-cream sold in Chennai. In their study, the highest level of coliform count was found in local vendor samples (1.7×10^3cfu /

g) followed by that of small-scale producer (7.7×10^1cfu /g) and organized sector (2.4×10^1cfu / g). From this investigation of street ice-creams, it was confirmed that all the studied ice-cream samples exceeded the standard value (BSTI 2005, Rahman et al., 2016).

Coliform organisms are the main microorganisms which reflect hygienic status of final product and effectiveness of hygienic practices in ice-cream production and their presence in any product indicates the bad hygiene practice. In both branded and street vended ice-cream exceeded the standard limit for total coliform count and comparatively street vended ice-cream showed higher total coliform count than branded ice-cream. These mainly occurred as coliform contamination were susceptible to pasteurization and other reasons for contamination might be the lack of personal hygiene of the workers of ice-cream factories.

In this research work, Total *Escherichia coli* count (TEC) among different brands of ice-cream samples were ranging from 0cfu/ml to 4.5×10^3cfu/ml at different locations (Figure 5). The highest value of TEC was found in sample 5 which was 4.5×10^3 cfu/ml (log value 3.6cfu/ml) at Santosh and the lowest value found in sample 1 which was 0 cfu/ml (log 0) at New and Old bus stand.

The presence of *E. coli* in food indicated fecal contamination. Food Safety and Standard Authority of India (FSSAI) stipulates that *E. coli* should be absent in one gram of ice cream. In the other hand according to BSTI, acceptable limit of *E. coli* is 0 cfu/ml. The obtained value of the research work clearly revealed that most of the branded ice-cream samples exceeded the standard limit for TEC except sample 1.

Hassan *et al*., 2015, the average *E. coli* counts obtained from the study was in Igloo 9.26×10^3 CFU/ml (log 4.0), in Polar 1.14×10^3 CFU/ml (log 3.0) and in Kwality 7.95×10^2CFU/ml (log 2.9) in Dinajpur district. In comparing with this study, it is shown that present study result is quite similar with their study. In another study of Edward *et al.,* 2017, found zero count of *E. coli* in industrially produced ice-cream.

On the other hand, the range of TEC in street vended ice-cream was 4.1×10^2cfu/ml to 7.5×10^4cfu/ml at different locations (figure 6). The highest value of Total *Escherichia coli* count was 7.5×10^4cfu/ml (log value 4.8cfu/ml) found in lolly ice-cream at New bus stand and the lowest value was 4.1×10^2cfu/ml (log value 2.6 cfu/ml) observed in mango ice-cream at Santosh.

Edward *et al.,* 2017, found *Escherichia coli* in locally produced ice-cream ranged from 4.00×10^3, to 1.20×10^4cfu/g which is almost closure to this study. Moshood *et al.,* 2013, studied locally produced ice-cream where the presence of *Escherichia coli* found in 7 samples. *Escherichia coli* had the highest frequency with the percentage occurrence of 35%.

The result of TEC found in analysis of street ice-cream clearly showed that the obtained value was much higher than the standard limit. In comparison with the industrially produced ice-creams the value of studied street ice-cream was fairly high. The use of comparatively more contaminated water may be from polluted river for production of ice-cream, improper heating and storage and poor hygiene practice are responsible for such lower quality of street ice-cream.

The range of *Staphylococcus aureas* count (SC) among different brands of ice-cream samples were ranging from 1.8×10^2cfu/ml to 2.9×10^4cfu/ml at different locations (Figure 7). The highest value of SC found in sample 5 which was 2.9×10^4cfu/ml (log value 4.5 cfu/ml) at Santosh and the lowest value of SC found in sample 1 which

was 1.8×10^2cfu/ml (log value 2.2 cfu/ml) at New bus stand.

In the other hand according to BSTI, the acceptable limit for *Staphylococcus aureus* count for ice-cream product is 10 cfu/ml. From the present findings, the mean value for each branded ice-cream showed that they exceeded the standard value for SC and lowest bacterial count observed in sample 1 and highest count observed in sample 5.

Study conducted by El-Ansary & Maria in Egypt, the average *Staphylococcus aureus* count was $1.10 \times 10^3 \pm 2.45 \times 10^2$ cfu/ml, which could be associated with potential food poisoning hazards. Edwar *et al*., 2017, studied the industrially and locally produced ice-cream sample in Nigeria. Where the least Staphylococcal mean count of 2.00×10^3 cfu/g was recorded in the industrially produced samples.

On the other hand, the range of SC in street vended ice-cream was 3.9×10^4 cfu/ml to 7.9×10^6 cfu/ml at different locations (Figure 8). The highest value of *Staphylococcus aureas* count was 7.9×10^6cfu/ml (log value 6.8cfu/ml) found in lolly ice-cream at New bus stand and the lowest value was 3.9×10^4cfu/ml (log value 4.6 cfu/ml) observed in mango ice-cream at Santosh.

Jannat *et al*., 2016, studied that 71 % of street vended samples were contaminated with *Staphylococcus aureus in* Dhaka city. Edwar *et al.,* 2017, recorded in the locally produced ice cream samples (1.50×10^4 cfu/g). The absence of *S. aureus* has been rarely reported in several studies. In this study the value is much higher than the both study.

In this research work, it was observed that the value of SC in all street ice-creams were fairly high than the standard value of SC and also from industrially produced branded ice-creams. Staphylococcal species are widespread in the environment where the human nasal cavity and skin is the largest reservoir of this bacterium. Comparatively poor maintenance of proper hygienic environment during production system of street ice-cream was the main reason for higher contamination of such type of bacteria than the industrially produced branded ice-creams.

Physico-chemical parameter analysis

In this study, the concentration of pH for different branded ice-cream in different locations ranged from 5.5 t0 6.9 (figure 9). The highest pH value was observed in sample 5 that was 6.9 and the lowest value of pH observed in sample 1 which was 5.5. In this study almost all the brands showed quite similar pH and the values are closer to pH value 7 which indicated the neutral condition according to pH scale. Commonly the pH value in ice-cream ranges from 6-7 which indicates neutral value of pH according to pH scale (Naim *et al.,* 2014). According to Marshall *et al.,* 2003, the pH of ice-cream varies with the composition of the product, in general it is in the range of 6.3 to 6.5. All the value of pH of branded ice-creams that were studied are quite similar as the value of pH depends on the composition of ice-cream and also within the range of neutral value. In comparison with the previous literatures the values obtained from this study were much closer to them.

On the other hand, the value of pH in street ice-cream that found from this research were ranged from 6.0 to 7.0 at different locations (figure 10). The highest value of pH was 7 observed in Kulfi ice-cream and the lowest concentration of pH observed in mango ice-cream which was 6. All the values were close to neutral value of pH.

The TSS in different branded ice-cream were ranging from 26 to 29% (Figure 11). The highest value of TSS was 29% observed in sample 5 and the lowest value of TSS was 26% observed in sample 1. The

values in different brands of ice-cream were almost closer to them.

On the other hand, the value of TSS observed in street vended ice-cream were ranging from 5% to 10% (Figure 12). The highest value was 10% observed in Lolly ice-cream and the lowest value was 5% observed in mango ice-cream. The highest average found in lolly which was 8.6% and the lowest average observed in mango which was 6%.

Findings from this work showed that though ice-cream is high fat and sugar content, it enhances the microbial growth also serves as a vehicle for transmission of pathogens. The results obtained from this study showed that both industrially manufactured branded and locally produced street vended ice-cream samples were contaminated with potential pathogenic microorganisms. Among them the industrially produced branded ice-creams offered comparatively better-quality products in respect of production system and sanitary condition than the street ice-creams. But in the case of branded ice-creams, the microbial quality was not satisfactory at all since every sample indicated the bacterial proliferation. Several steps during production can cause bacteriological hazards. Though pasteurization of milk can destroy most of the pathogens posing risk to public health, yet, potential bacteriological hazards can still be found in the final products after pasteurization through cross contamination or improper handling. At retail shops, improper storage temperature and prolonged storage time affects the microbiological quality of ice-cream. The high level of bacterial contamination can be attributed to poor hygienic conditions under which street ice-creams are produced, stored, transported and distributed, as it is largely in the hands of the roadside/ small local vendors. In this study work, some chemical parameters like pH and TSS were also observed. In both categories of ice-creams, the pH and TSS were in satisfactory level.

CONCLUSION

Considering the bacterial load in both studied ice-cream, it can be recommended that much attention is still needed to apply in aspects of microbiological quality control at each level of the production, handling, processing, distribution and storage of ice cream for attaining desired safety margins and giving assurance that the ice-cream product received by the consumer will be pure, healthful and of the quality claimed. The government authorized institute (like BSTI) should take intensive investigation to control the microbial and chemical quality of the ice creams as well as the public awareness about the adulterated ice creams should be increased.

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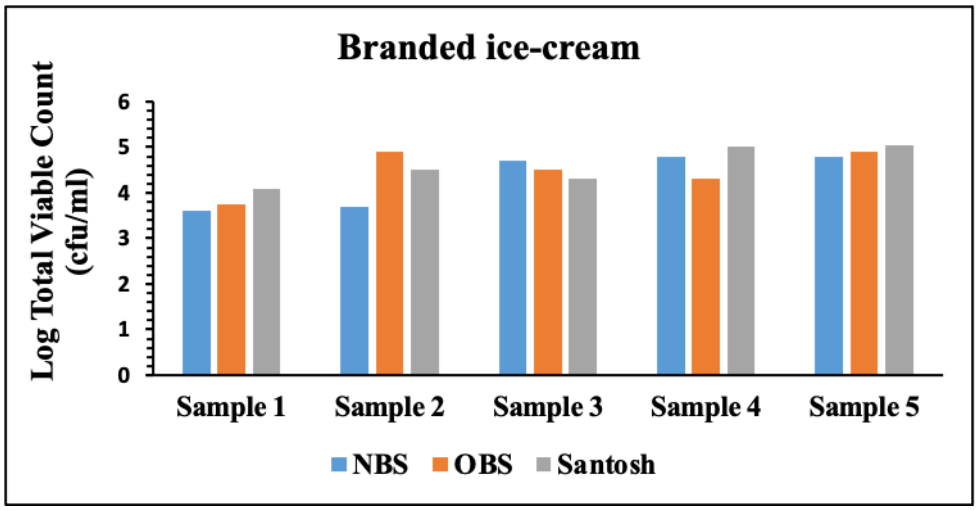

Figure 1. Status of Total Viable Count (TVC) in different branded ice-creams and locations. [Note: NBS= New bus stand, OBS= Old bus stand]

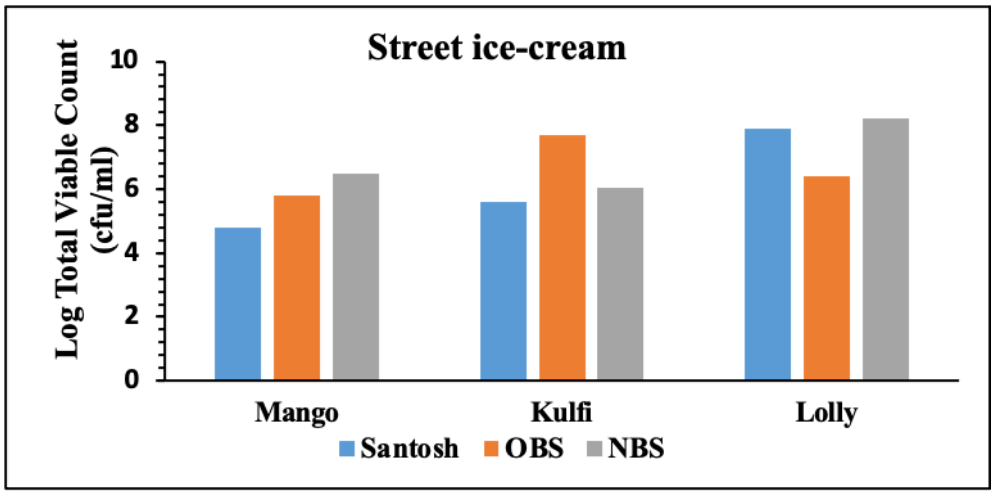

Figure 2. Total Viable Count of bacteria in different street vended ice-cream. [Note: NBS= New bus stand, OBS= Old bus stand]

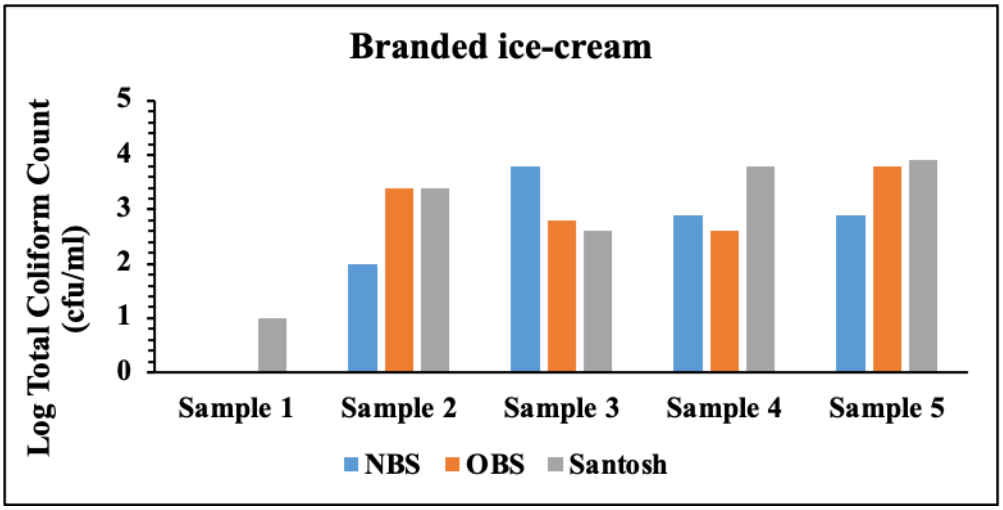

Figure 3. Total coliform count in different branded ice-cream in different locations. [Note: NBS=New bus stand, OBS=Old bus stand]

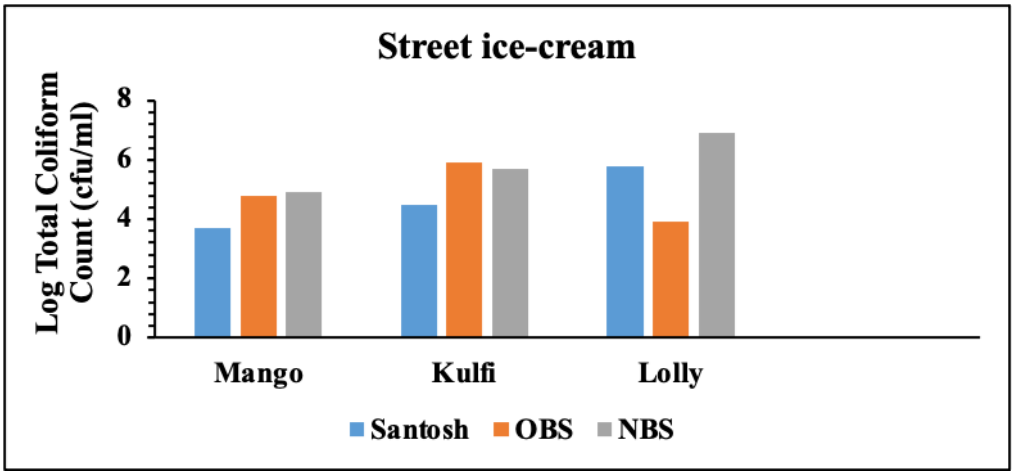

Figure 4. The concentration of Total Coliform count in different street vended ice-cream in different locations. [Note: NBS=New bus stand, OBS= Old bus stand]

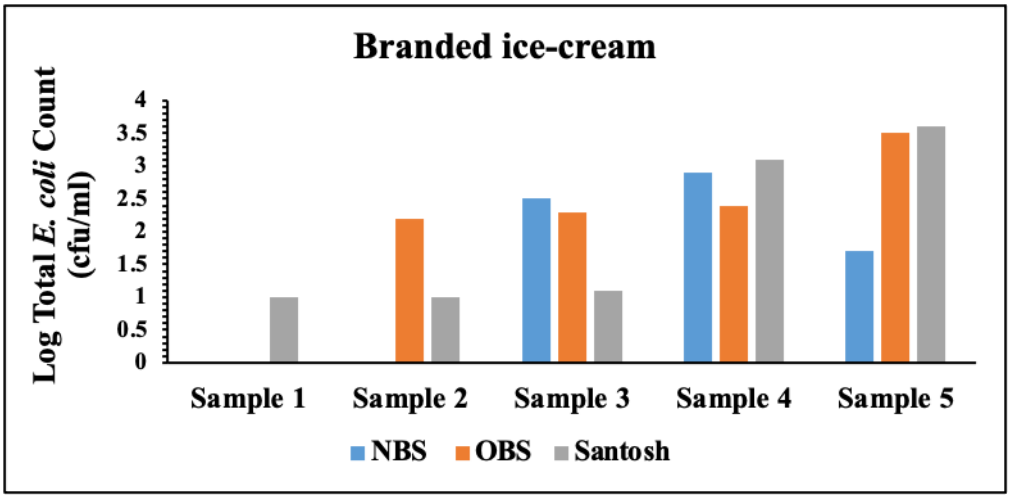

Figure 5. The concentration of Total *E. coli* Count in different branded ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

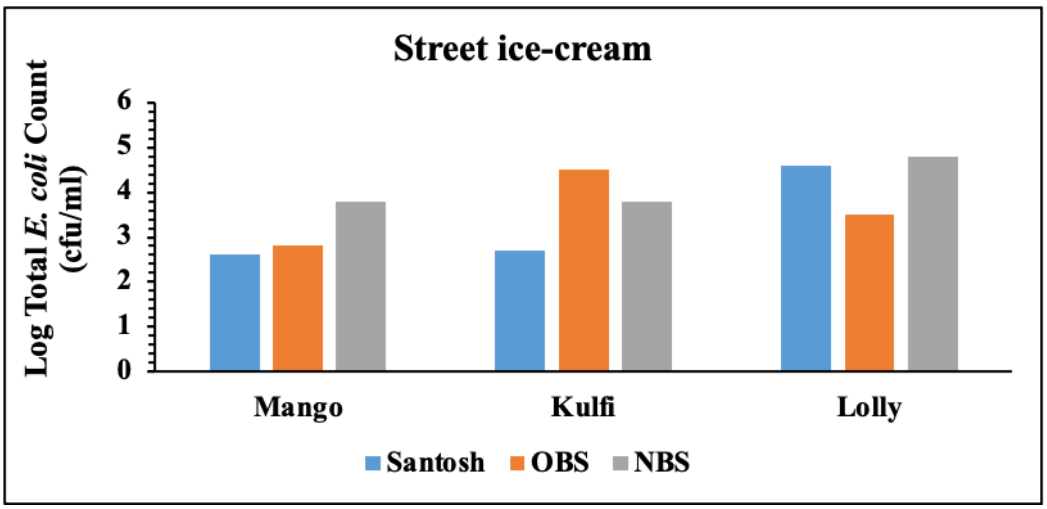

Figure 6. Total *E. coli* count in different street vended ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

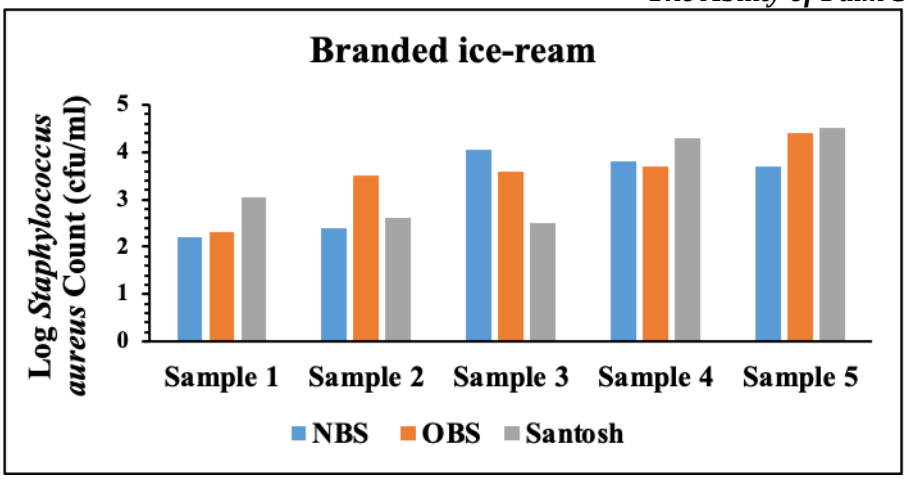

Figure 7. The concentration of *Staphylococcus aureus* count in different branded ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

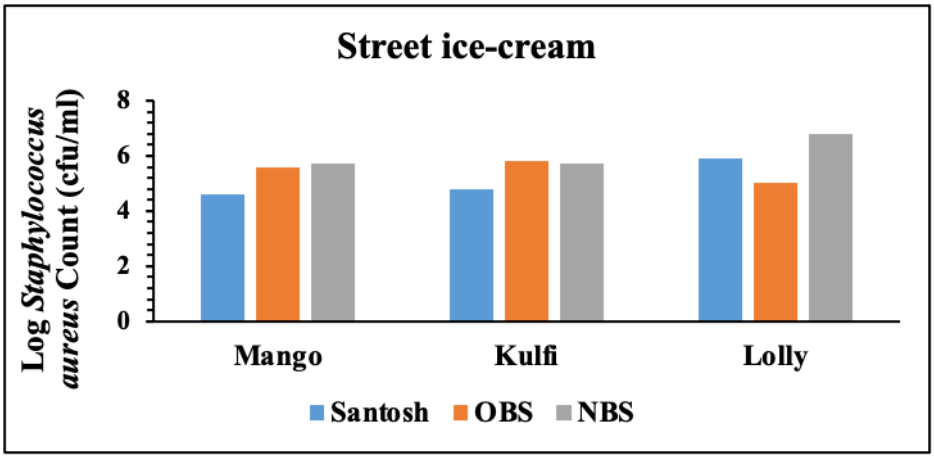

Figure 8. The concentration of *Staphylococcus aureus* count in different street vended ice-cream in different locations. [Note: NBS= New bus stand, OBS= Old bus stand]

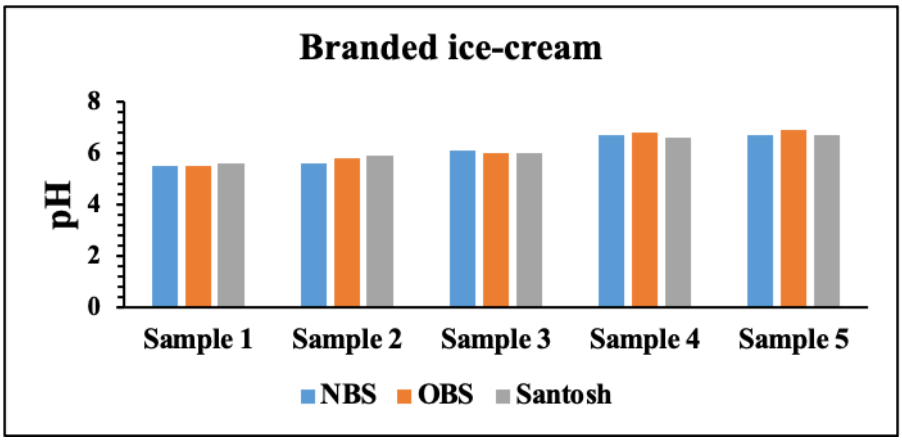

Figure 9. Concentration of pH in different branded ice-cream and locations. [Note: NBS=New bus stand, OBS= Old bus stand]

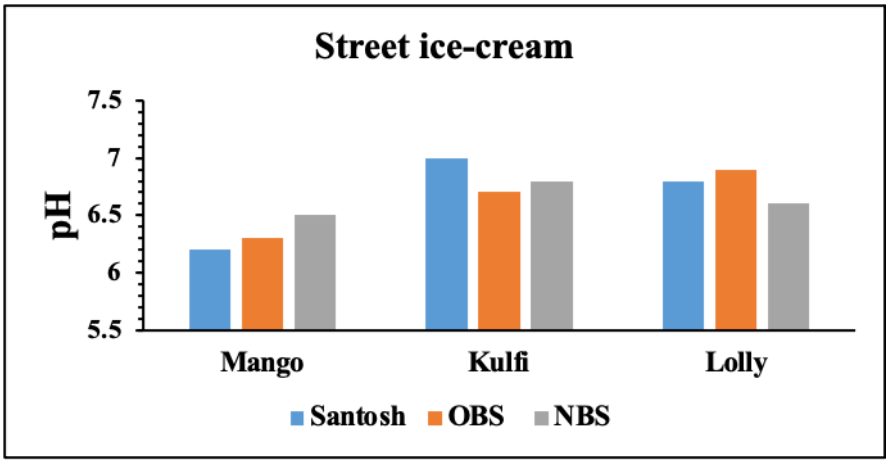

Figure 10. pH in different street vended ice-cream and locations. [Note: NBS=New bus stand, OBS= Old bus stand]

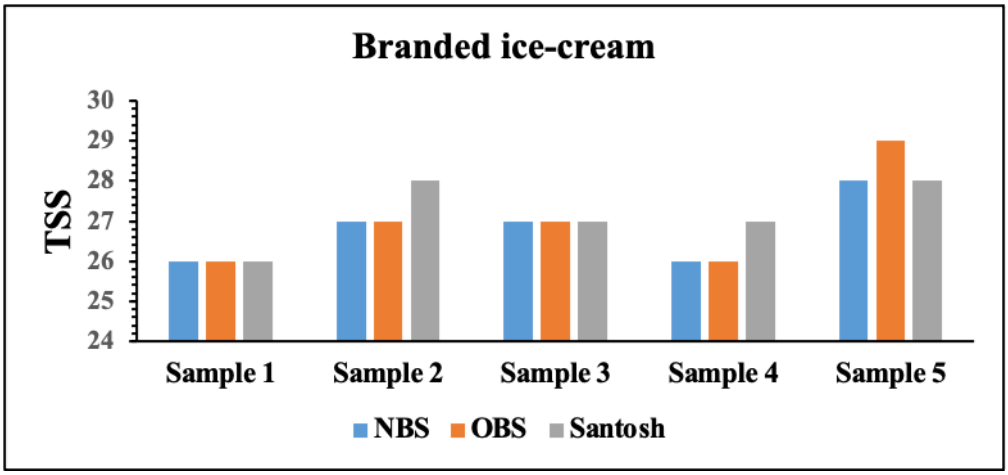

Figure 11. Total soluble solid in different branded ice-cream and locations. [Note: NBS=New bus stand, OBS= Old bus stand]

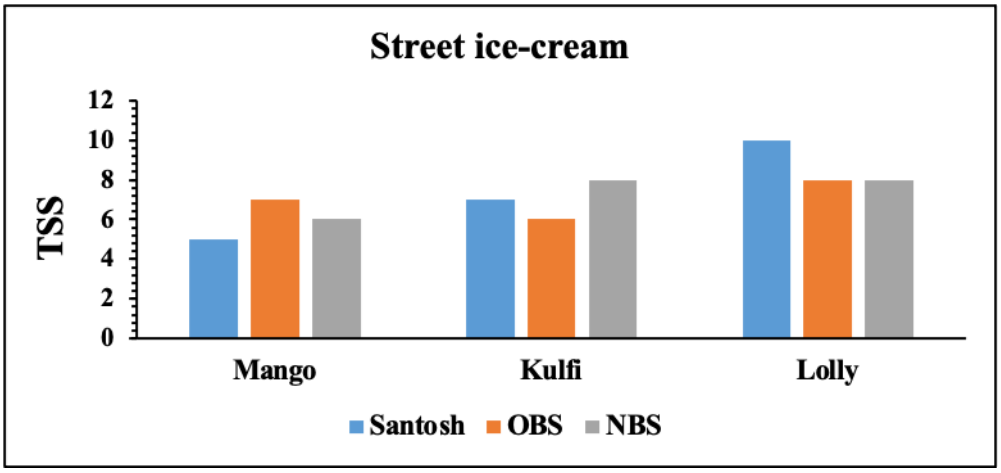

Figure 12. Total soluble solid in different street vended ice-cream and locations. . [Note: NBS=New bus stand, OBS= Old bus stand]

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Formulation of Snack Bar Based on White Mussel as TFA (Therapeutic Food for Anemia) To Improve Adolescents Nutrition

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ABSTRACT

The purpose of this study is to enhance adolescent nutrition, specifically anemia and stunting, in accordance with the National Research Master Plan's public health and nutrition theme. In order to break the chain of stunting, giving TFA (Therapeutic Food for Anemia) to adolescent females at school is one of the measures taken. This study employed four formulation groups (P1) 0:80:20; (P2) 15:65:20; (P3) 25:55:20; (P4) 35:45:20 (White Mussel Powder: Moringa Flour: Brown Rice Flour) to create the refreshment bar. This study's objective was to evaluate the TFA food formula based on organoleptic and nutrient content tests prior to administering it to panelists. This is experimental research in the form of a formulation of a local seafood product with white mussel (*Corbula faba* Hinds) as the primary ingredient. The obtained data were then analyzed using ANOVA (Analysis of Variance) to determine which TFA formula was the panelist team's organoleptic preference. The P4 formulation contained the maximum levels of protein (11.31%) and iron (87.42 mg/100 g), as determined by the results. In addition, the organoleptic test revealed that formulation P3 was the most preferred treatment. In conclusion, TFA with formulation P3 can be used to enhance the nutrition of adolescents.

Keywords: adolescent, anemia, recovery food, malnutrition, iron

INTRODUCTION

Nutritional problems in adolescence will increase susceptibility to disease in adulthood and are at risk of giving birth to a generation with nutritional problems. Anemia is a condition when the number of red blood cells or hemoglobin is lower than the normal number. Adolescents who have poor nutritional status or are often called malnutrition, if it has been too long, there will be a chronic energy deficiency. Chronic energy deficiency is when a person suffers from a long or chronic lack of energy and protein nutrition. Chronic energy deficiency in adolescents if not addressed quickly will have a major and sustainable impact when these young women become pregnant later.

UNICEF revealed data in 2017, the prevalence of anemia is the most common worldwide in women aged over 15 years as

much as 23% and 37% of pregnant women. A study by Mengistu, et al (2019) stated that adolescent girls with Hb between 11 to 11.9 g/dl and 8 to 10.9 g/dl showed mild and moderate anemia, respectively. The prevalence of anemia in Indonesian adolescents continues to increase from 6.9% in 2007, 18.10% in 2013 and 32% in 2018. The prevalence of anemia in adolescent girls in the city of Surabaya is 26%. While anemia during pregnancy is very high, reaching a prevalence of 48.9% in 2018.

White Mussels (*Corbula faba*), contain nutrients useful for humans, especially fresh mussels. Fresh mussels contain quite a lot of nutrients, especially protein content. The nutritional content of mussels is much higher when compared to other people's foods, such as crackers and tofu. The nutritional components of white mussel meat (*Corbula faba*) include a water content of 75.70%, ash content of 3.09%, protein content of 10.85%, fat content of 2.68%, and carbohydrate content of 1.02%. In addition, mussels contain 133,800 ppm Fe, 14,836 Zn, 12.31% linolenic acid, 6.25% EPA, and 6.61% DHA (Baswardono in Yuniar, 2019). Moringa leaf flour can be added to any food as a nutritional supplement, such as iron (6). The nutritional content of Moringa leaves is 5.1 g/100 g protein, 6 mg/100 g iron, 1.077 mg/100 g calcium, and 1.6 g/100 g fat. Chocolate (contains non-heme iron), milk (contains protein), and dates (contains iron and low in fibre) (Rahmayanti, 2020).

The snack bar is a practical form of snack, with complete nutritional value and includes durable food for storage. Every 100 grams of Snack Bar is estimated to contain 3.7 mg of iron and 24.2 grams of protein, so it can meet 23.56% of the adequacy of Fe and 38.61% of the adequacy of protein. Enough eight grams of Moringa leaf flour daily can contribute nutrients to adolescents (16-18 years), namely 14% protein, 40% calcium, 23% iron and almost all the needs of vitamin A. As much as 100 grams of Moringa leaf flour can provide more than a third of the need for calcium, iron, protein, copper, sulfur and B vitamins (Syahwal, 2018).

To improve nutrition for young women as prospective mothers, a TFA (Therapeutic Food for Anemia) formula was formed. This TFA is in the form of snack bar covered chocolate so that it attracts young women to consume it. The TFA formula is prepared based on the Nutrient Adequacy Number recommended in Permenkes No. 28 of 2019. Therefore, this research was conducted to develop a snack bar product made from white mussel (*Corbula faba*), moringa leaf flour and brown rice flour.

MATERIALS AND METHODS

Tools and Materials

The snack bar's production begins with white mussels (*Corbula faba*) sourced from Surabaya's Kenjeran District. The moringa plant is native to Sumenep in East Java. The Java Rice Organic Farmers Group in Wates Village in Tulungagung's Sumbergempol District supplied the brown rice used in this dish. Dried pineapple, almonds, chocolate, dates, butter, sugar, and vanilla extract are also added. The necessary equipment for creating snack bars includes: kitchen scale, washbasin, baking sheet, pan, Teflon, knife, cutting board, tablespoon, 80 mesh sieve, gas stove and oven. The tools used for the analysis of nutrient content, namely: aluminum dish, porcelain dish, oven kiln, desiccator, condenser, soxhlet, Kjeldhal flask, distillation apparatus, Erlenmayer flask, upright cooler, measuring flask, measuring cup, hotplate, burette, pipette, filter paper, muscles and Thermo Scientific Genesys 10S UV-Vis spectrophotometer. The instrument used in the organoleptic test was an organoleptic form with 5 hedonic scales covering taste, aroma, color and texture to measure the level of acceptance of

the snack bar, which was carried out by 30 moderately trained panelists.

Method

This research consists of two stages, namely preliminary research and main research. Preliminary research aims to 1) Develop a formula and process for making white mussel snack bars, Moringa leaves, and brown rice; 2) Proximate analysis and Fe content and organoleptic test on snack bars. The formulation of the snack bar is based on Table 1.

The data collected was processed quantitatively using statistical analysis to determine the formulation of white mussel flour, Moringa leaf flour, and brown rice flour on the characteristics and nutritional content of the snack bar. Data on nutrient content, chemical characteristics and glycemic index values were analyzed descriptively. Data from organoleptic test results with 26 panelists (hedonic test and hedonic quality test) were analyzed using One Way ANOVA and Kruskal-Wallis nonparametric test according to the results of data normality. If the analysis results are $p<0.05$, then the difference is considered statistically significant, and Duncan's further test was carried out.

RESULTS AND DISCUSSION

Nutritional Content Test Results

The results of the nutrient content test showed that the treatment with the highest nutrient content (fats, proteins, and iron/Fe) was in the P4 formulation and the lowest was the P1 formulation. The complete results of the nutrient content test are presented in Table 3.

The results of the carbohydrate analysis on the snack bar were the highest in the P2 treatment group with a value of 67.19%. This value indicates the biscuit formulation has met the biscuit quality requirements according to SNI 01-2973-199. The white kupang, moringa leaf and brown rice snack bars have carbohydrate content of 64.12% - 67.19%. The carbohydrate content in white shellfish, Moringa leaves, and brown rice is quite high, this makes the snack bar produce sufficient carbohydrate content. The results of this study differ from studies which show that there is a reduction in carbohydrate content due to partial replacement of wheat flour which is the main source of carbohydrates with seaweed flour (Wiranata et al, 2017).

The results of the carbohydrate content test in each snack bar formulation were in accordance with the requirements for the nutritional content of the snack. Snack bars are generally in small portions with sufficient carbohydrate content ranging from 60% of daily energy needs, which is 20-40 g. The addition of white mussel flour reduces carbohydrate content because it contains high protein (Ferazuma 2009, Kusharto et al. 2012). Likewise, with Moringa leaf flour which contains high protein and minerals (Broin 2010) so that the carbohydrate content decreases.

The results of the analysis of the nutritional content showed that the protein content of the snack bar ranged from 5.59% - 11.31%. Based on the protein requirement of 10% of the total daily energy, each serving of the three snack bar formulas (35 g) contributes 4.4 to 5% of the total protein requirement per day. White Mussels as a source of animal protein because of its relatively high protein content. This supports the community's nutritional needs for animal protein needs, where the price is affordable. As an alternative source of protein. Research on the protein content of white mussels is quite high, from the results of research by Subani it was reported that the proximate content of white mussels was 24.24%, while the total protein content by adding up the amino acid levels studied by PKMT-Lemlit Unair (2000) in Yuniar (2019) found the

protein of rice mussels at 9.054%, and mussels wasps 10.854%. Protein plays a role in preventing malnutrition and hypoalbuminemia. Protein is known to play an important role in the transportation of iron in the body, if there is not enough protein in the body, the iron that is consumed cannot be distributed to the organs. The protein that functions to transport iron is transferrin. Transferrin is a glycoprotein synthesized in the liver. This protein plays a central role in the body's iron metabolism because transferrin transports circulating iron to places that need iron, such as from the intestines to the bone marrow to form new hemoglobin.

The results of the analysis of the nutritional content showed that the fat content of the snack bar ranged from 22.32% - 23.92%. Mussles also contains fatty acids that the human body needs. Red Mussles contains 8.97% LA (Linoleic Acid), 2.77% EPA (Eicosapentanoic), 3.65% DHA (Docosa-hexanoic Acid) while Mussles contains 12.31% LNA (Linolenic Acid), 6.52 % EPA, 6.61% DHA (Baswardono 1983) in Yuniar (2019). Omega 3 essential fatty acids form components that facilitate the transportation of oxygen and macronutrients (proteins, fats, and carbohydrates) into body cells so that they can help remove metabolic waste products such as carbon dioxide from body cells. Fat is the most important raw material in the manufacture of biscuits. The more fat you add to the dough, the crumblier the biscuit will turn out. Utami *et. al* (2021) revealed that fat is a food substance that is important to maintain the health of the human body. In the body, fat functions primarily as an energy reserve in the form of fat tissue. The function of fat in food is to provide a savory taste, crunchy quality and give soft and soft properties to baked cakes.

The moisture content of the white mussel snack bar, Moringa leaves and brown rice in this study was influenced by the roasting process (temperature and roasting time). The water content of the snack bar in this study ranged from 6.39% - 10.12%, which was lower when compared to commercial snack bar products which contained 8.7 - 11.4% water content. Moisture content is an important analysis during processing and testing of food products. According to Septiani (2016) stated the water content of foodstuffs that are safe for storage is less than 14% so that snack bars with low water content are sufficient to prevent the growth of bacteria and molds. The drying process is strongly influenced by temperature and drying time. Analysis of water content as the dominant component in food products because water affects the stability and quality of the ingredients. In addition, the reduction and reduction of water content in certain products can simplify the process of packaging and storing products. Water content analysis is also used to determine the percentage standard of water content in a food ingredient (Utami, 2021). The water content in biscuits will affect consumer acceptance, especially on texture (crispy). All biological activity is possible only in the presence of water. The main causes of food spoilage are microbial growth, enzyme activity and chemical changes. This reaction takes place most rapidly at high water activity and is supported by environmental factors that can cause bacterial contamination so that damage occurs more quickly (Yuniar, 2019).

The ash content of this research snack bar ranged from 1.04% - 1.87%. The ash content exceeds the range of cookie quality requirements according to SNI 01-2973-1992. Measurement of ash content aims to determine the amount of mineral content contained in food. Ash content that exceeds the quality requirements of this SNI can indicate the minerals contained in the food. Mineral elements and inorganic substances are not destroyed and do not evaporate so as

to produce greater ash content (Krisyanella et al. 2013).

The highest Fe content value obtained was 87.42%. This is sufficient to meet the requirements for Iron/Fe needs per day based on RDA (2013) for adolescent girls, a maximum of 20-30 mcg per day and which is almost close to the Fe needs of adolescents. The high content of Vitamin B-12 in mussel is efficacious for the formation of a person's DNA which can maintain nerve function. Consuming mussels regularly can avoid feeling tired and lethargic due to anemia. The high iron content in mussles can also help improve blood circulation, so energy will be formed quickly to prevent fatigue and anemia. High levels of iron are needed to form hemoglobin, a special protein that carries oxygen in your blood throughout the body.

Iron is one of the minerals that plays a role in the formation of hemoglobin by forming a heme which is able to bind and transport oxygen to all body tissues (Suryani, et al., 2019). Iron (Fe) is an important mineral for the body because of its function in hematopoiesis, namely in the synthesis of hemoglobin. Iron (Fe) is an essential microelement for the body. Iron is mainly needed in hematopoiesis (blood formation), namely in the synthesis of hemoglobin. The body cannot produce iron itself, the fulfillment of iron in the body is obtained from intake. The amount of iron intake will affect the increase in hemoglobin production. Several studies have shown that substitution of biscuits with high-iron ingredients can increase the Hb value of experimental animals. Mahmoed et al (2009) also stated that wheat biscuits supplemented with fenugreek seed flour which has a high iron content can increase the hemoglobin levels of anemic rats, further Sari et al (2018) also stated that iron deficiency anemia can be treated by providing food. such as in his research using Moringa leaf flour.

Organoleptic Test Results

The results of the organoleptic test showed that the most preferred treatment was formulation P3 (White mussels flour: moringa leaf flour: brown rice flour = 25:55:20%) and the least preferred was formulation P4 (Mussel flour: Moringa leaf flour: flour brown rice = 35:45:20%). These results were obtained based on four parameters of taste, aroma, color and texture. The results be shown in Table 2.

Within the color attribute, treatment P1 achieves the highest value, while treatment P4 obtains the lowest value. This disparity is attributed to the panelists' growing disapproval of snack bar products as the quantities of white mussel flour, Moringa leaves, and brown rice increase, primarily due to the resulting darker color. Based on the results of the Anova test analysis, it was found that the p value = 0.019 < = 0.05, indicating a significantly different value between each treatment, meaning that the addition of white mussels affected the panelists' acceptance of the snack bar color parameter.

Color in food is the first appearance that greatly influences consumers to choose a product. An ingredient that is considered nutritious, delicious, and has a very good texture will not be eaten if it has an unattractive color or gives the impression that it has deviated from the proper color (Larasati, 2017). The function of the color of a food product is very important because it can affect consumer tastes and is able to arouse appetite. The decrease in color preference of the panelists along with the addition of white mussel flour, moringa leaves, and brown rice was in accordance with previous research, which stated that the more Moringa leaf flour was mixed into the dough, the darker the color of the snack bar and the lower the level of preference for color (Hasniar, 2019). Green vegetables contain a lot of chlorophyll pigment, usually found on

the leaves and stem surfaces of plants. Therefore, it is necessary to pay attention to the proportion of adding Moringa leaf flour to the snack bar to make it look more attractive, namely the ratio between the amount of white mussel flour and Moringa leaves. The color is obtained from the use of sugar, the brown color of mussel jerky is also obtained from the caramelization reaction of sugar and the Maillard reaction between sugar and protein when heating. The use of sugar accelerates the non-enzymatic browning process which is supported by heating in the drying process plus the frying process. Cooking with heat repeatedly will add a brown color to the results of the snack bar (Rahmayanti, 2020).

The aroma attribute indicates the treatment with the highest value is P2 and the lowest value is treatment P4. This is because The snack bar prepared in treatment P2, which includes white mussel flour and Moringa leaves, possesses a harmonious scent combining both the white mussel and moringa. Conversely, treatment P4 exhibits a predominant aroma of white mussel. The results of the ANOVA test analysis indicate a significant difference in values among the treatments, as the calculated p-value of 0.018 is less than or equal to the significance level of 0.05. This suggests that altering the concentration of mussels in the snack bar formulation significantly impacts the panelists perception of the aroma parameter in the mussel snack bars. The flavor of a food ingredient plays a crucial role in its overall appeal. The aroma aspect, which is closely tied to our sense of smell, holds significant importance. An identifiable and appealing aroma has the potential to enhance consumer preference for food products, thus necessitating careful consideration during ingredient processing. In the case of the snack bar, the panelists expressed a growing aversion to the scent as the quantity of white mussels increased. This is primarily since the aroma of mussels contributes significantly to the snack bar's distinctive fragrance, typically characterized by a marine animal scent.

This study aligns with prior research conducted on the development of functional sausage products containing mackerel fish and Moringa leaf flour. Consistent with previous findings, it has been observed that an excessive amount of Moringa flour diminishes the panelists' liking for the aroma of the sausage (Nurlaila et. al, 2018)). The distinctive aroma of excessive Moringa leaves can be one of the factors that affect the aroma of tempeh sausage with the addition of too much Moringa leaf flour. Green vegetables contain lipoxidase enzymes which if the cooking process is not perfect it will cause a quite unpleasant aroma that is less pleasant (Rahmayanti, 2020).

The taste of a product plays a significant role in determining consumer preference and acceptance. According to the findings presented in Table 2, the organoleptic quality test results for taste attributes indicate that the P4 snack bar has a slightly bitter taste. The analysis of the ANOVA test further reveals that the p-value of 0.010 is less than or equal to the significance level of 0.05, indicating a significant difference in hedonic quality among the taste attributes. Duncan's further test results confirm that the different formulations used in the snack bar exhibit significantly different values between each treatment ($p<0.05$). It can be concluded that the addition and increase in the concentration of Moringa leaf flour in the snack bar formulation result in a bitter taste. This finding is consistent with multiple studies conducted on this topic such as research by Pratiwi, K (2018) regarding the manufacture of cookies, and Darmawan (2017) regarding Moringa leaf-based buns. The presence of saponins in Moringa leaves imparts a bitter taste, as well as specific properties such as foaming and easy solubility in water

(Indriasari et al., 2016). The presence of glutamic acid in white mussel flour influences the taste of the snack bar. Glutamic acid plays a crucial role in food processing as it contributes to the development of a savory taste and enhances the overall flavor balance in processed foods. White mussel flour contains approximately 1.443% glutamic acid per 100 grams, while Moringa leaves have a glutamic acid content of 30106.87 ppm. The findings from the organoleptic test revealed that the P1 snack bar was the most preferred in terms of texture. The results of the ANOVA test indicated that the p-value of 0.010 indicated a significant effect ($p<0.05$) of the varying levels of Moringa leaf flour substitution on the texture attributes assessed in the organoleptic test. However, Duncan's further test results showed no significant differences in texture attributes among the formulations of P1, P2, P3, and P4 snack bars. Texture is an essential component evaluated in sensory tests, utilizing the oral cavity and the sense of touch, such as using fingers. These findings are consistent with previous research conducted by Pratiwi, F (2018), which concluded that the formulation of Moringa leaf flour in snack bar products does not significantly impact texture attributes. The texture of each food form varies and can be assessed in terms of hardness, elasticity, or crunch, depending on its physical characteristics. (Hariyani, 2017).

CONCLUSION

The sensory evaluation conducted on the snack bar formulations containing white mussel, Moringa leaf, and brown rice assessed attributes such as color, aroma, taste, and texture. Among the formulations, P3 (25:55:20%) was found to be the most preferred in terms of overall organoleptic acceptability. Moreover, the analysis of nutrient content, including carbohydrates, protein, fat, ash, water, and iron, in the snack bar formulation P2 met the quality standards for snack bar biscuits. Therefore, this snack bar formulation holds potential as a healthy snack option based on locally sourced ingredients.

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Table 1. Formulation of snack bar therapeutic for anemia

Ingredients	Unit	Recipe Groups			
		P1	P2	P3	P4
White mussel powder	%	0	15	25	35
Moringa Flour	%	80	65	55	45
Brown Rice Flour	%	20	20	20	20
Dates	g	15	15	15	15
Pineapple	g	15	15	15	15
Almond	g	15	15	15	15
Chocolate	g	20	20	20	20
Margarine	g	15	15	15	15
Sugar	g	10	10	10	10
Vanilla flavor	g	5	5	5	5

Table 2. Organoleptic test results

Sample	Mean Rank			
	Colour	Aroma	Flavor	Texture
P1 (0:80:20%)	$3,97 \pm 0,12^{a}$	$3,68 \pm 0,57^{b}$	$3,96 \pm 0,72^{a}$	$3,59 \pm 0,28^{b}$
P2 (15:65:20%)	$3,72 \pm 0,14^{a}$	$3,89 \pm 0,85^{b}$	$3,73 \pm 0,21^{a}$	$3,50 \pm 0,17^{c}$
P3 (25:55:20%)	$3,62 \pm 0,35^{a}$	$2,58 \pm 0,30^{b}$	$2,62 \pm 0,35^{c}$	$2,58 \pm 0,12^{b}$
P4 (35:45:20%)	$2,54 \pm 0,84^{a}$	$2,19 \pm 0,76^{b}$	$2,15 \pm 1,26^{c}$	$2,19 \pm 0,51^{b}$

Values with different letters in the same row are demonstrate significantly different ($p<0.05$)

Table 3. Proximate test results of white mussle snackbar

Sample	Parameters					
	Protein (%)	Carbohydrate (%)	Fat (%)	Water (%)	Ash (%)	Iron (mg/100g)
P1	5,59	66,95	22,72	6,39	1,25	15,36
P2	6,01	67,19	22,89	6,14	1,87	35,61
P3	9,19	66,81	23,44	8,52	1,04	66,33
P4	11,31	64,12	23,92	10,12	1,53	87,42

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Phytochemicals, Heavy Metals, and Antioxidant Vitamins Assessment in Tomatoes (*Solanum lycopersicum*) Cultivated Near Cement Company Firm of Sokoto, Nigeria

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ABSTRACT

Even at low concentrations, it is well known that vegetables can readily absorb metals from contaminated soil or deposits on air-exposed plant parts. These metals then build up at high levels in the edible parts of the vegetables. Vegetables are a significant part of the human diet; hence heavy metal poisoning of these food items cannot be understated. As a result, this study sought to quantify the amounts of phytochemicals, heavy metals, and antioxidant vitamins in tomatoes grown close to the Cement Company of Northern Nigeria (Sokoto Cement). Phytochemicals, heavy metals, and antioxidant vitamins were determined using standard analytical procedures. The study confirmed the presence of flavonoids, saponins, alkaloids tannins, steroids, and volatile oils. The heavy metals detected (Cu, Zn, Cd and Cr) were found below the WHO safe limits guidelines apart from Cr which exceed WHO safe limit guidelines. Substantial amounts of vitamin A, C and E were also observed. It was concluded that tomatoes cultivated in Kalambaina area are safe for consumption in terms of heavy metals.
Keywords: Antioxidant, Heavy metals, Phytochemicals, Pollution, Tomato, Vitamin

INTRODUCTION

Plants such as *Psidium guajava* and *Carica papaya* have long been our forefathers' first line of defense against diseases such as malaria, cholera, and diarrhea (Kar & Borthakur, 2008). They can easily be divided into groups based on their uses, such as those that are edible, those that are used as a source of spices or medicines, those that have ornamental value, etc. (Dalhat *et al.*, 2017). Tomato (*Solanum lycopersicum*) is a savory, red, edible fruit that originated in South America and spread around the world after the Spanish colonized America. Nowadays, it is widely farmed, often in greenhouses in kinder climates. The tomato fruit is frequently consumed in many forms, including as fresh, as a component of salads and sauces, or after being processed into purees, ketchups, and tomato soups (Aremu *et al.*, 2017). Tomatoes are high in minerals, vitamins, proteins, essential amino acids (leucine, threonine, valine, histidine, lysine, and arginine), monounsaturated fatty

acids (linoleic and linolenic acids), carotenoids (lycopene and -carotenoids), and phytosterols (β-sitosterol, campesterol, and β-stigmasterol) (Ali *et al.,* 2021). In Nigeria, tomatoes are mainly cultivated in the Northern region, precisely in the area called Sudan Savannah. This zone is affected by some level of environmental degradation from industrial activities, mostly Cement industries.

Environmental contamination from anthropogenic activity that is mostly caused by industrialization is a major problem. Different environmental elements like water, air, soil, and plants are harmed because of industrial and human activity (Singh *et al.,* 1980).

Heavy metals and other chemicals are released into the environment in significant quantities, particularly by industries amongst which the Cement Industry is a major player (Yaşar *et al*., 2010). Cement factories are known to emit a variety of pollutants, including dust, particulate matter, gases, and various heavy metals, all of which are harmful to the biotic environment, manifesting themselves in soil, plants, and terrestrial and aquatic flora and fauna (Akinci & Caliskan, 2010, Dalhat *et al*., 2017).

Food safety concerns caused by pollution have been recently reported worldwide (Li *et al.,* 2021). Food safety issues are mostly caused by biological agents such as viruses, bacteria, molds, protozoa, helminths (worms), and/or chemicals, which can cause both acute ailments (especially diarrheal diseases) and increase the risk of long-term illnesses for example, aflatoxins and heavy metals. Heavy metals are thought to pose the greatest threat to food safety of all the pollutants that have been reported. For instance, it was discovered that the levels of cadmium (Cd), lead (Pb), and arsenic (As) in vegetables and rice grown in polluted areas were significantly higher than the established safety limits (Odai *et al.,* 2008, Khan *et al.,* 2010).

Heavy metals are naturally occurring earth elements with densities more than 5 g/cm^3 and long biological half-lives. They cannot decompose biologically. In trace levels, several heavy metals are required to maintain the body's metabolism. On the other hand, toxic levels could happen. Heavy metals can enter our bodies in trace levels via food, water, and air. It is well-known that metals, even at low concentrations, can be readily absorbed by vegetables from contaminated soil or deposits on plant parts exposed to the air. These metals then build up at high levels in the edible parts of the vegetables. It is possible for heavy metals to contaminate crops when they are irrigated with tainted water, fertilized, or treated with pesticides that include metals. Contamination with heavy metals is also possible during crops transportation, harvesting, or storage (Obadahun *et al.,* 2021). Vegetables are a significant part of the human diet, hence heavy metal poisoning of these food items cannot be understated (Abdulmojeed and Abdulrahman, 2011).

A range of illnesses, particularly those affecting the heart, kidneys, neurological system, and bones, may result from eating heavy metal-contaminated fruits and vegetables. As a result, this study sought to quantify the amounts of phytochemicals, heavy metals, and antioxidant vitamins in tomatoes grown close to the Cement Company of Northern Nigeria (Sokoto Cement).

MATERIALS AND METHODS

Chemicals and Reagents

The reagents used for the study included sulphuric acid, hydrochloric acid, n-hexane, trioxonitrate acid, trichloroacetic acid (TCA), sodium hydroxide, acetic acid, ferric chloride, Wagners reagent, α^1, α^1-dipyridyl, potassium hydroxide, sodium chloride,

metaphosphoric acid, acetone, perchloric acid, chloroform, methanol, ethanol, indophenol, and distilled water. All chemicals used were of analytical grade. The tools used include Buck Scientific 210 Atomic Absorption Spectrophotometer and UV-Vis Spectrophotometer (752N).

Sample collection and preparation

Fresh tomato sample were collected in a clean plastic bag from Kalambaina area which is just less than one kilometer away from the main deport of Cement Company of Northern Nigeria in October 2021. The sample was identified and authenticated by the Department of Biological Science Sokoto State University, Sokoto. The collected sample was washed under a running tap, cut into smaller pieces using knife and then dried at 37^0C in an oven for approximately 2 weeks. After drying, the sample was crushed using clean mortar and pestle into a powder which was then stored at room temperature for the duration of the research.

Phytochemicals Screening

Alkaloids

The presence of alkaloids was investigated using the methods described by Wagner's (Wagners, 1991). 1 ml of the extract was treated with 2 drops of Wagner's reagent (2 g of iodine and 3 g of potassium iodine were dissolved in 20 ml of distilled water and made up to 100 ml with distilled water). Formation of brown precipitate indicates the presence of alkaloids in the extracts.

Flavanoids

The determination for the presence of Flavanoids in the sample was conducted using alkaline reagent test by Okerulu *et al* (2017). 3 ml of each extract were treated with 1 ml of 10% NaOH solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids in the extracts.

Tannins

The determination of the presence of tannins in the test sample was carried out using Ferric chloride test described by Harbone (1973). 2 drops of 5% $FeCl_3$ was added to 1 ml of the extract. A greenish precipitate indicated the presence of tannins in the extract.

Test for cardiac glycosides (Keller-Killiani Test)

One milliliters (1 ml) of the filtrate was added to a test tube and then 2 ml of 3.5% $FeCl_3$ was added. The mixture was shaken for 1 minute and then 1 ml of concentrated H_2SO_4 was poured down the wall of the test tube so as to form a lower layer. A reddish brown ring at the interface indicates the presence of Cardiac Glycosides (Okerulu *et al*., 2017).

Saponins

The presence of saponins in the sample was done using Harbone (1973). 0.5 g of the extract was treated with 5 ml of distilled water and mixture was shaken vigorously, the production of foam which persisted in few minutes indicated the presence of saponins in the extracts.

Test for steroids (Lieber Mann Burchard Reaction)

Two milliliters (2 ml) of chloroform was added to 2 ml of plant extract and the mixture was shaken vigorously. The mixture was allowed to settle until two layers are formed. 1 ml of concentrated H_2SO_4 was carefully added alongside of the test tube. A reddish-brown ring at the interface indicates the presence of Steroids (Bashiru *et al*., 2017).

Test for Anthraquinones (Borntragers test)

Two milliliters (2 ml) of chloroform was added to 2 ml of plant extract in a test tube and shaken vigorously. 5 ml of 10% NH_3 was then added to the chloroform layer. The mixture was allowed to settle and observation was made. A bright pink color at the upper part of 2 layers formed indicates the presence

of free Anthraquinones (Okerulu *et al*., 2017).

Test for volatile oils

Two milliliters (2 ml) of the filtrate was added to a test tube and 2 ml of 10% HCl was added. A white precipitation indicates the presence of Volatile oils (Harbone, 1973).

Determination of Heavy Metals

A sample of 0.5 g of the sample was digested for two hours at 95°C with 0.5 ml H_2SO_4, 0.6 ml concentrated HNO_3, and 1.8 ml concentrated HCl. The sample was chilled and made into a volume of 10 ml using deionized water, after which it was tested for the presence of Copper (Cu), Lead (Pd), Zinc (Zn), Cadmium (Cd) and Chromium (Cr) using a Buck Scientific 210 Atomic Absorption Spectrophotometer (Adekiya *et al.,* 2018). The mean and standard error of the mean were computed, each measurement was repeated three times (n=3).

Determination of antioxidant vitamins

Determination of Vitamin A

Five grams (5 g) of the sample was ground to fine paste and 1ml of saponification mixture (2 N KOH in 90% alcohol) was added. The tube was gently refluxed for 20 minutes at 60^0C and then cooled at room temperature followed addition of 20 ml of distilled water. Vitamin A was extracted with10 ml of petroleum ether in a separating funnel, twice. The organic extract was pooled and sodium sulphate (anhydrous) was added to remove the moisture for 30 minutes. Aliquot of ether was evaporated to dryness at 60^0C and the residue was dissolved in 1 ml chloroform.

Aliquot of the standard (Vitamin A acetate) was pipetted into a series of clean test tubes in the concentration range 1.5-7.5µg and the volume in each test tube was made up to 1 ml with chloroform. A 2 ml of Trichloroacetic acid (TCA) solution (prepared by dissolving 15 g TCA in 25 ml chloroform and stored in the dark) was added from fast delivery pipette, rapidly mixing the contents of the tube and the absorbance was immediately measured at 620nm in UV-Vis spectrophotometer (752N). Absorbance of the sample was also determined in triplicate in a similar manner. Standard graph was constructed by plotting the A_{620} in Y-axis and vitamin concentration in the X-axis and the amount of vitamin A/g in the sample was determined from the standard graph (Bayfield and Cole, 1980).

Determination Vitamin C

Vitamin C (Ascorbic acid) concentration of the sample was determined according to the procedure describe by Sadasivam and Balasubramanian (1987). A dye solution was prepared by dissolving 42 mg of sodium bicarbonate into small volume of distilled water and 52 mg of 2, 6-dichlorophenol indophenol was dissolved in it and the solution was made up to 200 ml with distilled water.

A 5 ml of working standard solution (100 µg/ml ascorbic acid prepared using 4% oxalic acid) was pipetted in to 100 ml conical flask. Ten milliliters (10 ml) of 4% oxalic acid was added and titrated against the dye (V_1). The end point was noted by the appearance of pink color which persists for few minutes.

A 5 g of ground sample was extracted in 4% oxalic acid and made up to 100 ml and centrifuged. To the supernatant (5 ml), 10 ml of oxalic acid was added and titrated against the dye (V_2 ml). Amount of Ascorbic acid mg/100g sample =

$$\frac{0.5\text{mg}}{\text{V1ml}} \times \frac{\text{V2}}{5\text{ml}} \times \frac{100\text{ml}}{\text{Wt. of sample}} \times 100$$

Determination of Vitamin E

A 0.5 g sample was weighed into stopper tube and 10 ml of 0.1N sulphuric acid was slowly added without shaking. The tube was stopped and allowed to stand overnight. Afterward, the contents of the tube was

vigorously shaken and filtered through Whatman No. 1 filter paper.

A 1.5 ml of the filtrate (in triplicate) was pipetted into centrifuge tubes labeled as test and 1.5 ml each of standard and distilled water was pipetted into centrifuge tubes labeled as standard and blank respectively. To the test and blank, 1.5 ml of ethanol was added and to the standard 1.5 ml of distilled water and then centrifuged. Xylene (1.5 ml) was added into all the test tubes, capped, mixed and centrifuged again. Carefully, 1 ml of xylene layer from each test tubes was transferred into another stopper tubes followed by 1 ml of 2, 2-dipyridyl reagent, stoppered and mixed. The extinction of the resulting mixture for the test and standard was read at 460nm against the blank. Then, 0.33 ml of ferric chloride solution was added, mixed and the absorbance of the test and standard was read against blank after 15 minutes at 520nm (Rosenberg, 1992).

The amount of vitamin E (µg/g) in the sample was calculated using the following formula:

$$\frac{\text{Ax520} - \text{Ax460}}{\text{As520}} \times 0.29 \times 15 \frac{\text{Vt}}{\text{Vu} \times \text{g}}$$

Where:
Ax520 = Absorbance of test at 520nm
Ax460 = Absorbance of test at 460nm
As520 = Absorbance of standard at 520nm
Vt = Total volume of homogenate
Vu = volume used and g = Weight of the sample.

Statistical Analysis

Results are expressed as mean ± standard error of mean, and the calculation was done using SPSS (Version 20). The results were compared with World Health Organization (WHO).

RESULTS AND DISCUSSION

Phytochemicals are important in the food, pharmaceutical, and dye industries. Some of them have pharmacological effects; flavones and tannins, for instance, are crucial components of many laxatives, medications, and colors (Dalhat *et al.,* 2017). The study sample's phytochemical analysis revealed a high content of flavonoids and saponins (Table 1). There is evidence that flavonoids and saponins have anti-inflammatory, cytostatic, analgesic, antibacterial, and analgesic properties (González-Madariaga *et al.,* 2020, Ullah *et al.,* 2020). Tannins, volatile oils, and alkaloids were found in moderate amounts (Table 1). Alkaloids are significant secondary metabolites that have considerable anti-cancer, analgesic, and cytotoxic potential (Nobori *et al.,* 1994, Pietta, 2000). While anthaquinones and cardiac glycoside were not found, a little amount of steroids were (Table 1).

The level of heavy metal concentration was shown in Table 2. Only chromium (Cr) was found to be slightly above the World Health Organization's (WHO) recommended limit, according to the current investigation. According to Dalhat *et al.,* (2017) the high level of Cr in the study sample may be related to its high level in the soil. However, the results are consistent with those of Bashiru *et al*. (2017), who found significant levels of Cr in *Sapinacea olaracea, Allium cepa*, and *Amarantus species* grown in the Kalambaina region. Cr is a necessary trace element that regulates the metabolism of carbohydrates, lipids, and proteins in its trivalent state (Cr^{3+}), but when it is in its hexavalent state (Cr^{6+}), it is poisonous, mutagenic, and carcinogenic (NIH, 2019, Wakeman *et al.,* 2017). The present investigation did not reveal the presence of lead (Pb). However, *Lactuca sativa* grown close to the cement mill was found to contain high levels of Pb, according to Dalhat *et al.,* (2017). This can be due to the plant's different capacity to absorb heavy metals from the soil or its close proximity to the facility. Although copper (Cu) is necessary for human existence, excessive concentrations of Cu have been linked to an

increased risk of cardiovascular illness, metal fume fever, dermatitis, hair and skin discolorations, respiratory tract infections, and certain other fatal conditions in people (Badaloni *et al.,* 2017, Lavigne *et al.,* 2019, Wang *et al.,* 2020). It has been demonstrated that, zinc (Zn) is involved in the production and breakdown of lipids, proteins, nucleic acids, and carbohydrates, is crucial for polynucleotide transcription and translation and, consequently, for the process of genetic expression (Dalhat *et al.,* 2017). Due to the high rate of soil-to-plant transfer of cadmium (Cd), these foods are the main sources of cadmium (Cd) contamination (Vergine *et al.,* 2017). It is a very toxic, non-essential heavy metal that is widely known for having a negative impact on cellular enzymatic systems, oxidative stress, and for making plants nutritionally deficient (Hassan *et al.,* 2020). It is important to note that the study plant's levels of Cu, Zn, and Cd are within the WHO-acceptable ingestion limits.

The antioxidant vitamins results in the research sample are shown in Table 3. Vitamin A is essential for normal gene expression, growth, and immune function as well as for maintaining epithelial cell processes (Lukaski, 2004). Vitamin C contains anti-inflammatory and immune-boosting characteristics, functions as a cofactor for mono- and di-oxygenases, and is needed for the synthesis of biological components such as collagen and catecholamines (Fujii, 2021, Spoelstra-de Man *et al.,* 2018).

Vitamin E is a powerful antioxidant that helps to protect cells from free radical damage. Red blood cells and muscles both require it for growth and optimal function (Lukaski, 2004). Vitamin A, C, and E concentrations were found to be 85.043, 234.062, and 19.086 µg/g in the sample, respectively (Table 3).

CONCLUSION

This study confirmed the presence of flavonoids, saponins, alkaloids, tannins, steroids and volatile oils. The presence of the heavy metals was also revealed (Cu, Zn, Cd and Cr). The amounts of heavy metals were currently below the WHO safe limits criteria, apart from Cr. Vitamins A, C, and E, which are antioxidant vitamins, were also found in significant amounts. In conclusion, tomatoes grown in the Kalambaina region are safe to eat in terms of heavy metals.

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Table 1. Result of phytochemical screening of *Solanum lycopersicum*

Parameter	Result
Flavonoids	+++
Saponins	+++
Alkaloids	++
Anthraquinones	ND
Tannins	++
Steroids	+
Volatile oils	++
Cardiac glycosides	ND

Keys: ND not detected, + slightly present, ++ moderately present, +++ abundantly present

Table 2. Heavy metal concentration in the *Solanum lycopersicum* sample

Heavy metal	Concentration (mg/kg)	FAO/WHO (mg/kg)
Cu	0.125±0.025	73.3
Pd	BDL	0.3
Zn	0.228±0.022	99.4
Cd	0.010±0.005	0.2
Cr	0.058±0.240	0.05

The results represent mean ± standard error of mean of triplicate experiment (n=3)
WHO STD = World Health Organization Standard (2011)

Table 3. Antioxidant vitamins concentration in *Solanum lycopersicum* sample

Parameter	Concentration (µg/g)
Vitamin A	85.043±5.77
Vitamin C	234.062±0.21
Vitamin E	19.086±1.73

The results represent mean ± standard error of mean of triplicate experiment (n=3)

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Rehydration Kinetics of Dehydrated Vegetables Pre-Treated By Ohmic-Blanching

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ABSTRACT

Dehydration is an irreversible process resulting in the loss of structural integrity and rehydration capacity (RC) of food products. Pretreatment methods are used to condition the tissue of vegetables for dehydration to reduce its effect on the structural integrity of the products. In this study, we investigated the effect of ohmic blanching as a pretreatment method and compared it with water blanching and microwave blanching. The Peleg model was used to evaluate the rehydration properties through regression analysis. The model was satisfactorily fitted with the data. However, there was a model deviation with water-blanched potato and yam. Dehydrated products pretreated by ohmic blanching compared favorably with microwave-blanching in carrots, potatoes, and yams. The RC ranged between 264.04% to 449%, 141.40% to 274.32%, and 70.46% to 155.54% in ohmic-blanched carrots, potatoes, and yams respectively. The application of ohmic blanching in the pretreatment of vegetables showed the potential of producing dehydrated products with better rehydration properties. We have suggested through this study, an improved method of hot air dehydration which was of lower cost compared to freeze-drying. The design and model of a bench-top ohmic heating device provided a portable, simple, and low-cost alternative to the otherwise more capital-intensive equipment designs.

Keywords: Ohmic-blanching, rehydration-kinetics, kinetic-model, moisture absorption

INTRODUCTION

The purpose of dehydration is to reduce the moisture content of food products to low enough levels for the prevention of spoilage due to microbial activities as well as biochemical and physical changes. Dehydration is also applied in minimally processed foods which have become important not only in food preservation given the recent demand for convenience in food preparations. However, studies (Lopez-Quiroga et al., 2019; Okpala and Ekechi, 2014; Rana et al., 2021) have underlined the difficulty of rehydration, given the need for the rehydration of food products before consumption by the final consumer. This is a result of microstructural damage due to modified cell crystallinity, middle lamella, and clumped microfibrils during dehydration. As a result, research attention has been given to drying pretreatment methods in a bid to improve the rehydration properties of

dehydrated food products as well as to conserve energy consumption and reduce drying time (Rana et al., 2021).

Blanching is a minimal heat treatment of primarily solid food products (fruits and vegetables) to inactivate enzymes (Xiao et al., 2017). Blanching is also applied as a drying pretreatment. The moist heat opens up the pores of the food material thereby conditioning the tissue for moisture absorption by capillary action (Xiao et al., 2017). Steam, water, ultrasonic, and microwave blanching (Cheng et al., 2015; Rana et al., 2021) has been used applied in drying pretreatment. However, no literature has reported the effect of ohmic blanching as a drying pretreatment method on the rehydration properties of vegetables.

Ohmic heating is the passage of alternating current through a food material. The process ignores the effect of temperature gradient in convectional heat transfer resulting in rapid and uniform heating due to reactor fluctuation of electric fields (Indiarto and Rezaharsamto, 2020). The equipment design varies but consists fundamentally of a heating chamber, a pair of electrodes, and an alternating current source (Indiarto and Rezaharsamto, 2020). Ohmic blanching of solid food is carried out by dispersing the food materials in a carrier fluid usually containing 0.15 – 1.5% w/w NaCl (Zhu et al., 2010). (Rao et al., 2014) observed that dispersed food products pretreated in this method heated more uniformly during ohmic heating. The choice of ohmic heating in this experiment is due to its ability to heat the food materials rapidly, and uniformly (Deng et al., 2019; Sakr and Liu, 2014), ensuring that the product is thoroughly blanched. As a result, there would be minimal damage to the tissues of the food products. Ohmic heating is rapid and efficient which could reduce the processing time, cut down processing costs and increase efficiency (Xiao et al., 2017).

Guida et al. (2013) investigated the effect of ohmic and conventional blanching on the nutritional, bioactive compounds and quality parameters of artichoke heads. It was observed that ohmic blanching inactivated spoilage enzymes at a lower blanching time and preserved the colour and texture of the products while also retaining more protein and phenolic compounds (Guida et al., 2013). Ohmic blanching of carrots, red beet and golden carrots resulted in greater softening rates and weight losses compared to those blanched in hot water and microwave (Xiao et al., 2017). The combination of ohmic heating and vacuum impregnation was found to improve mass transfer during the osmotic dehydration of strawberries (Xiao et al., 2017).

The objective of this work was to study the effect of ohmic blanching as a drying pretreatment on the rehydration properties of carrots, potatoes, and yams. We applied the Peleg model to describe the moisture absorption process. Previous reports (Lopez-Quiroga et al., 2019) have shown the suitability of the model in predicting the equilibrium moisture content (X_{eq}) over short soaking periods.

MATERIALS AND METHODS

The food materials (yam, potato, and carrot) were purchased from a local market in Abuja, Nigeria. The food materials were washed thoroughly to remove adhering soils. Yams were peeled and cut into slices, potatoes were peeled and cut into the shape of french fries while carrots were cut into cylindrical shapes. All slices and cuts were 20mm thick. All food samples were grouped into five. A group was left unblanched to serve as the control. The moisture content of the fresh food materials was determined according to the AOAC described in (Okpala and Ekechi, 2014).

Ohmic heating device

A table-top ohmic heating device for batch processing applications was designed. Using the isometric grid system described by (Otukoya, 2022) a 3D model was developed. A prototype of the design was fabricated with a wooden frame surrounding a plastic heating vessel and a pair of aluminum plate electrodes. The device was equipped with a digital kitchen thermometer (Model KBT010001, China) having a stainless-steel probe capable of measuring -50°C to + 300°C as shown in Figure 1.

Water-blanching

The food materials were submerged in hot water in a 1:10 material-to-water ratio for 2 minutes after heating to a temperature of 90°C on a hot plate (Rana et al., 2021).

Microwave-blanching

Food materials were placed in a polyethylene terephthalate plate in a microwave oven (Sony-25 Liters, China) at 650 watts for 2 minutes as previously described by (Rana et al., 2021).

Ohmic blanching

Ohmic blanching was carried out according to previously reported methods Zhu *et al.*, 2010). Briefly, the food materials were dispersed in 1% NaCl solution (carrier fluid) in the heating chamber of the ohmic heating device. AC voltage of 230 volts was applied for test periods of 1 minute and 2 minutes.

Dehydration

Adopting the procedures followed by (Lopez-Quiroga et al., 2019; Lopez-Quiroga et al., 2020) with few modifications, all samples were dried at 70°C in a vacuum oven (Model No. DHG-9101-0SA, China) to a constant weight. The samples were spread uniformly on an aluminum drying tray and placed in the oven after preheating to 70°C.

Rehydration

The initial moisture contents of dehydrated vegetable samples were determined according to the AOAC as the method described in (Chen et al., 2016) followed by a series of rehydration experiments according to previously reported methods (Lopez-Quiroga et al., 2019; Rana et al., 2021). The food samples were immersed in distilled water in a beaker at 40, 60, and 80°C and removed at intervals (30 minutes for the first 3 hours followed by an hourly interval until equilibrium), blotted with tissue paper to dry out surface water, and reweighed. High rehydration temperatures were chosen to sufficiently examine the effect of pretreatment methods on the structural integrity of the vegetable samples.

Rehydration modelling

The Peleg model was chosen due to its suitability in predicting the equilibrium moisture contents over short rehydration periods. The Peleg model (Lopez-Quiroga et al., 2020) describes the moisture content (dry basis) as follows;

$$X(t) = X_0 + \frac{t}{k_1 - k_2 t} \qquad (1)$$

where *t* (in minutes) is time, X_0 is the initial moisture content on dry basis (d.b), k_1 is the Peleg rate constant and k_2 is the Peleg capacity constant. Peleg capacity constant k_2 (Lopez-Quiroga et al., 2020) is related to the equilibrium moisture content;

$$X_{eq} = X_0 + \frac{1}{k_2} \qquad (2)$$

Rehydration capacity (RC) (Lopez-Quiroga et al., 2020) was calculated as;

$$RC(\%) = \frac{X_f - X_i}{X_f} \times 100\% \qquad (3)$$

Where X_f is the moisture content at saturation and X_i is the initial moisture content of the dried samples (Lopez-Quiroga et al., 2020).

All experiments were performed in triplicates. Mean moisture contents were

used to fit the Peleg kinetic model. R^2 and mean moisture contents and standard deviations were calculated in Microsoft Excel 2016.

RESULTS AND DISCUSSION

Moisture Content

The moisture contents of carrots, potatoes, and yams before and after dehydration are shown in Table 1. The pretreatment with microwave and ohmic blanching resulted in relatively higher moisture reduction at the end of the drying process compared to unblanched and water-blanched food materials. This observation supports the previous report of (Rana et al., 2021) with microwave blanching drying pretreatment.

Rehydration properties of dehydrated vegetables

The rehydration curves for dehydrated carrot, potato, and yam showing the absorption of moisture in kg moisture/kg dry matter per hour are presented in Figures 2 – 4. Moisture absorption was greatest at 60°C and least at 40°C for most blanching methods as well as the unblanched carrots. The moisture absorption of dehydrated potatoes was greater at 80°C for all pretreatment methods with the exception of unblanched potatoes. Ohmic blanching at one minute resulted in higher moisture absorption of dehydrated yams at 80°C. There was an increase in rehydration with an increase in rehydration temperature of yams pretreated by blanching for 1 minute in ohmic heat. Previous studies (Lopez-Quiroga et al., 2020; Rana et al., 2021) revealed greater moisture absorption at higher rehydration temperatures. The higher rate of moisture absorption at higher temperatures of the rehydration medium could be a result increased mass transfer (Xiao et al., 2017).

Moisture absorption in carrot and yam was greater at 80°C in the early stages of rehydration. As rehydration time increased, the moisture absorption gradually decreased. This suggests that the heat may have caused irreversible changes at 80°C. It is reported that above 60°C, starch may undergo some irreversible reactions like gelatinization and retrogradation (Chhe et al., 2018; Tako et al., 2014) in the presence of moisture. Thereby resulting in an early and lower saturation time and moisture content respectively.

The corresponding points of saturation in carrots at 80°C and 60°C rehydration temperature were closer in the ohmic blanched samples. Thus, ohmic heating may have caused minimal damage to the tissue of carrots. Similarly, at 1 minute of blanching in ohmic heat, rehydration was significantly higher in yams. Moreover, (Indiarto and Rezaharsamto, 2020) revealed that the generally low conductivity of food products enhances uniform heat distribution, causing less damage to the tissue.

Rehydration Kinetics of dehydrated vegetables

Peleg's kinetic model parameters are presented in Tables. The Peleg model was satisfactorily fitted with the experimental data with observed deviations in water-blanched potatoes and yams. The regression coefficients calculated from model fitting ranged from 0.7279 to 0.9840 for carrots, 0.5652 to 0.9875 for potatoes, and 0.4533 to 0.9965 for yam. The low regression coefficients observed in water-blanched yams and potatoes resulted from an irregular rehydration pattern with an observed loss of mass along with moisture absorption. The loss of mass was probably due to starch solubility in water. The granular structure of starch is usually lost as a result of gelatinization as starch molecules get dispersed in water (Chakraborty et al., 2022). Particles of yam and potato were observed to leach into the rehydrating medium (hot water). However, this was not apparent in all

other treatments. The unblanched food materials absorbed water minimally and attained an equilibrium moisture content between 0.86 kg/kg and 1.41 kg/kg with minimal leaching.

The Peleg's rate constant is related to the rate of water absorption in food samples. The relatively lower k_1 values correlate with higher initial water absorption rates (Okpala and Ekechi, 2014). The non-linear variation of k_1 with rehydration temperature observed in previous studies was discussed by (Miano and Augusto, 2018; Oli et al., 2014). Except for hot water-blanched carrots where there was a decreasing trend with increasing rehydration temperature, k_1 was greatest at 60°C with an increase from 40°C followed by a decrease at 80°C. Also, the Peleg's rate constant decreased significantly with an increase in the rehydration temperature of hot water-blanched potato. At 60°C, k_1 was greatest in unblanched, microwave-blanched, and ohmic-blanched potatoes. In yams, k_1 increased with increasing rehydration temperature in unblanched and microwave-blanched yams. The Peleg's rate constant was greatest at 40°C rehydration temperature in ohmic-blanched yams. Moreover, several researchers (Lopez-Quiroga *et al.*, 2019; Indiarto and Rezaharsamto, 2020; Rana *et al.*, 2021) have previously revealed and confirmed the temperature dependence of k_1. In the current study, it was found that k_1 is not only dependent on rehydration temperature but also on the blanching method used as a pretreatment which implies that k_1 is affected by the effect of microstructural damage on the rehydrating food sample.

Lower k_2 corresponds to higher water absorption capacity (Miano and Augusto, 2018). The variation in k_2 with temperature was not linear. The effect of temperature on water absorption capacity (k_2) is variable, depending on the nature of the material (Oli et al., 2014). The loss of soluble solids during soaking may affect the moisture absorption capacity of the food material (Oli et al., 2014).

The saturation moisture content of the food materials has not reached the moisture content of the fresh products indicating that dehydration is irreversible. This observation supports previous reports (Lopez-Quiroga *et al.*, 2019; Rana *et al.*, 2021). The predicted equilibrium moisture contents (X_{eq}) were lower than the moisture content at saturation (X_f). The rehydration capacities revealed that the dehydrated potato was able to rehydrate up to 299.55% of its mass when blanched in microwave heat. Ohmic blanching of potato for a period of 2 minutes rehydrated to 274.32% and 241.43% (80°C and 60°C rehydration temperature respectively). Microwave blanched yams had the highest rehydration capacity (200.06%) at 60°C rehydration temperature. Moisture absorption capacity (k_2) was high in water-blanched potato and yam, this was followed by a simultaneous mass loss and moisture absorption during soaking. The relatively high equilibrium moisture content (X_{eq}) could be a result of water emptying from the free capillary and intermicellar spaces of the tissue of the yams and potatoes thereby, creating a concentration gradient for more absorption as observed by Sayar, Turhan and Gunasekaran (Miano and Augusto, 2018; Oli et al., 2014). As a result, saturation moisture content was not confirmed as the experiment couldn't be continued due to material disintegration. Microwave and ohmic heat treatments showed little signs of leaching with yam samples remaining intact throughout the period of rehydration. It was previously concluded that ohmic blanching was unsuitable in carrots due to the structural destruction of material tissue (Xiao et al., 2017). However, our results from rehydration experiments show that despite progressive shrinking, the carrot cylinders recovered 424.02% and 411.64% of its mass.

CONCLUSION

The Peleg model was satisfactorily fitted with the experimental data with deviations observed in water-blanched potatoes and yams. Moisture absorption in water-blanched yam and potato proceeded with simultaneous mass losses due to porous capillaries caused by hot water-blanching. The rehydration capacities (RC) of dehydrated carrots were higher than those of potato and yam. Pretreatment of vegetables by blanching in ohmic heat had a satisfactory impact on the rehydration properties as observed in this study.

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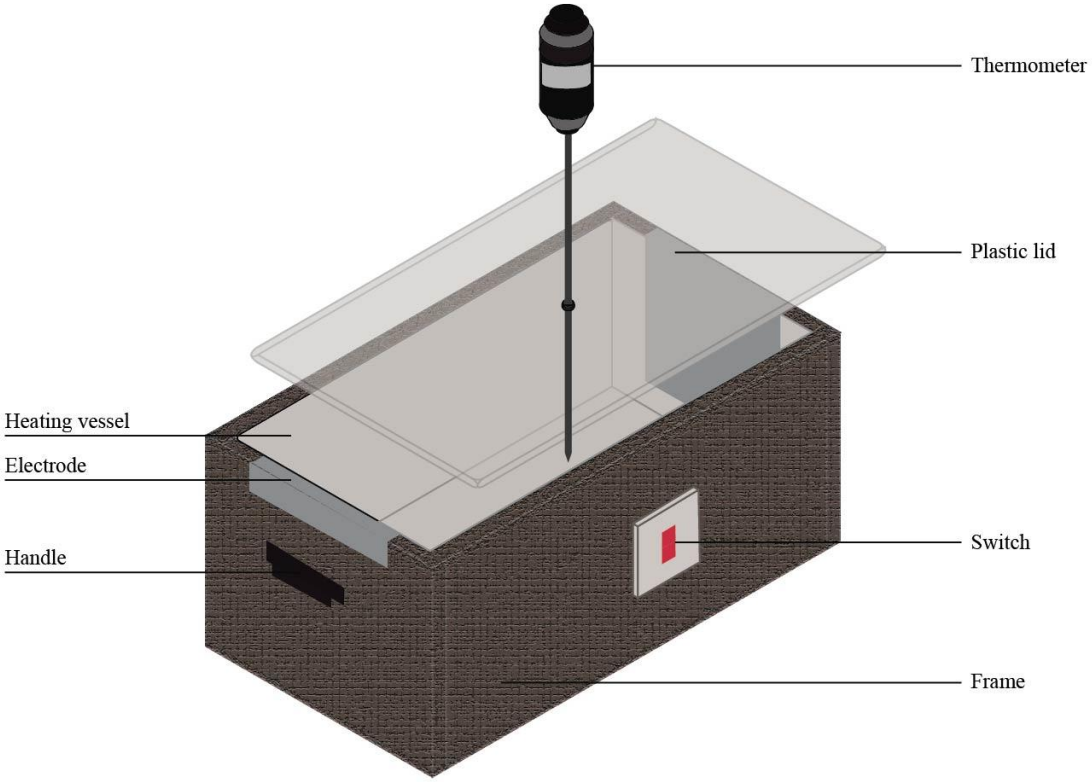
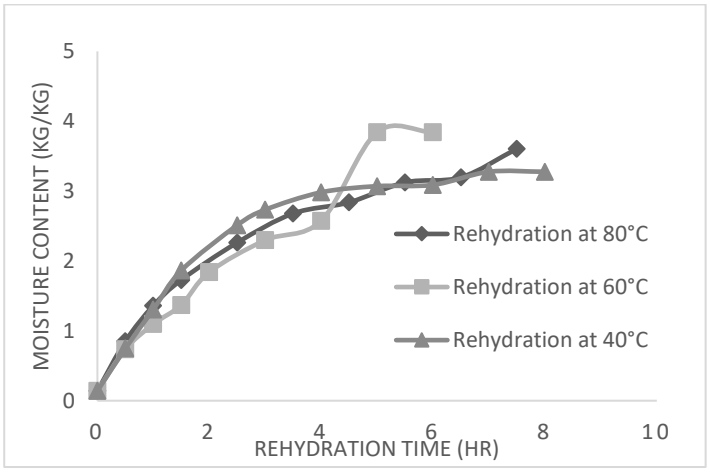


Figure 1. 3D Model of Bench-top Ohmic heating device



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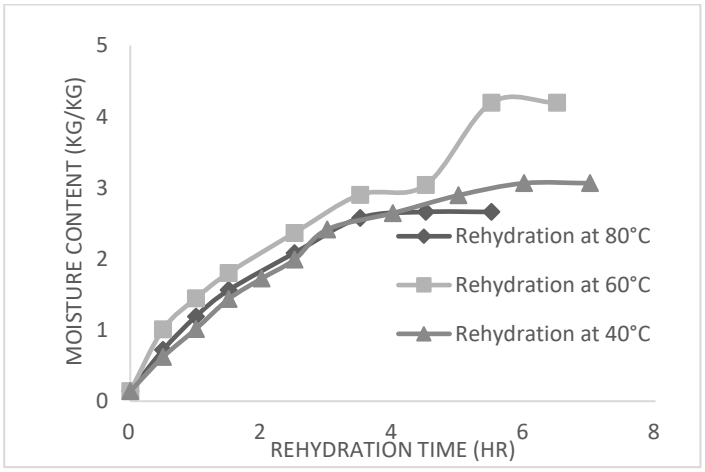

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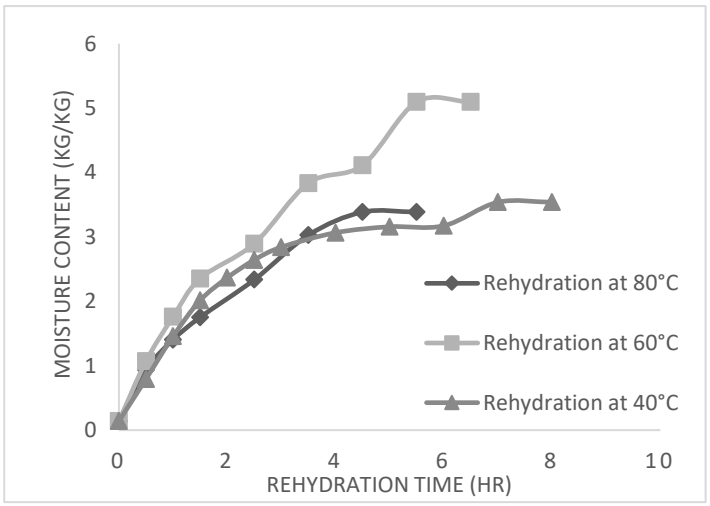

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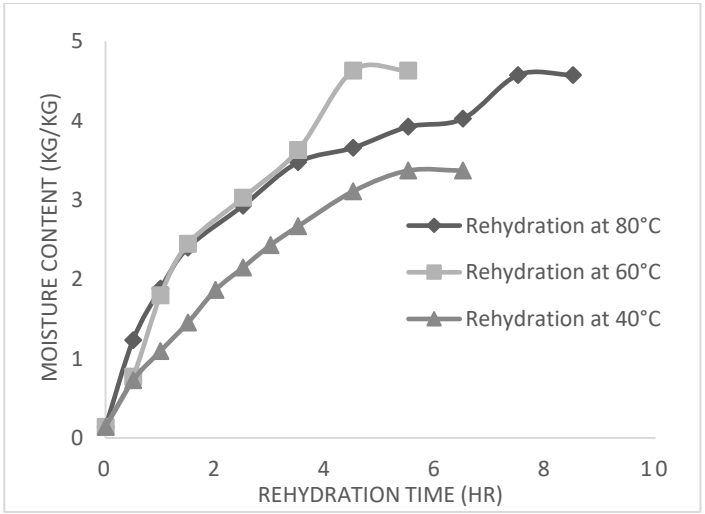

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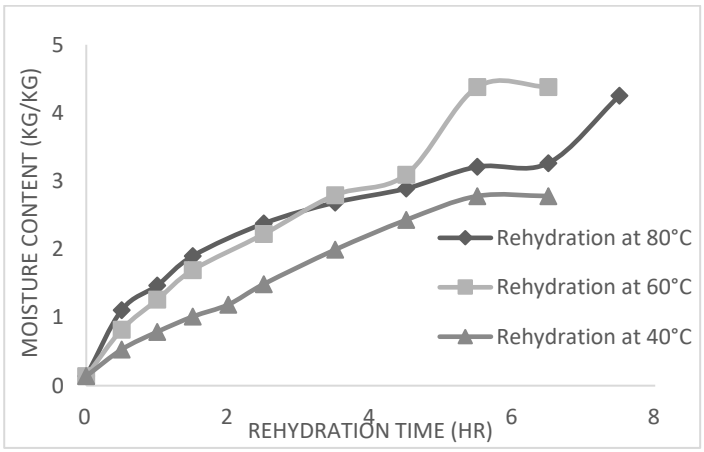
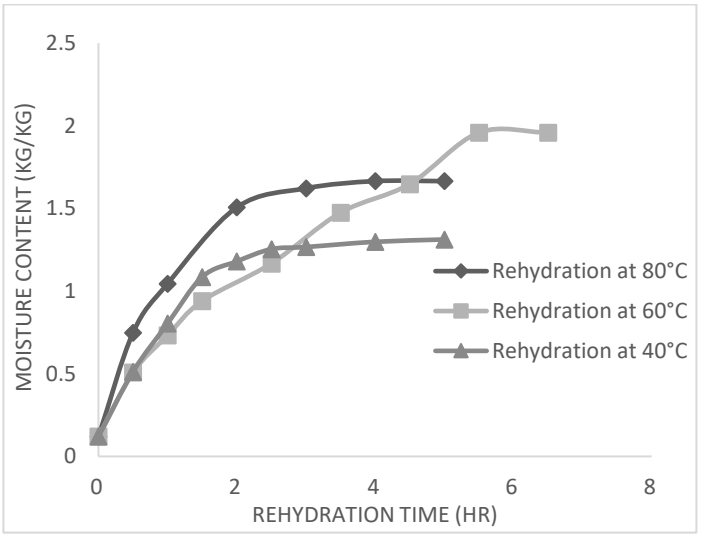


(e)

Figure 2. Rehydration curves of dehydrated carrots (a) unpretreated (b) water blanched (c) Microwave blanched (d) 1 minute blanching in ohmic heat and (e) 2 minutes blanching in ohmic heat.



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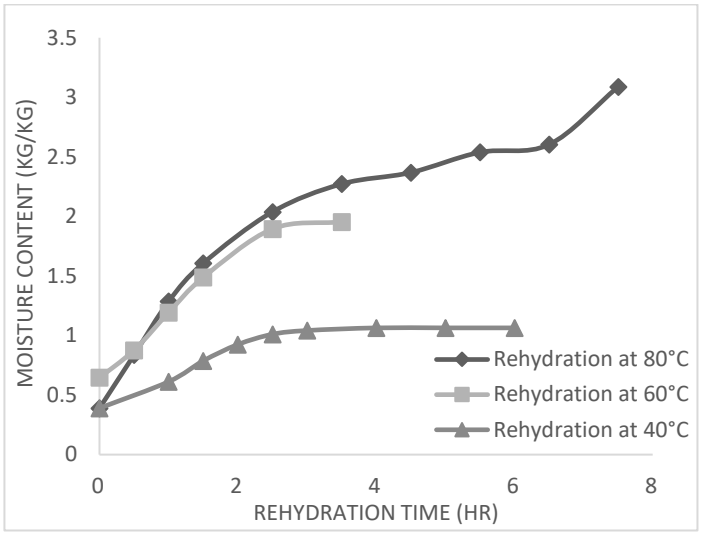

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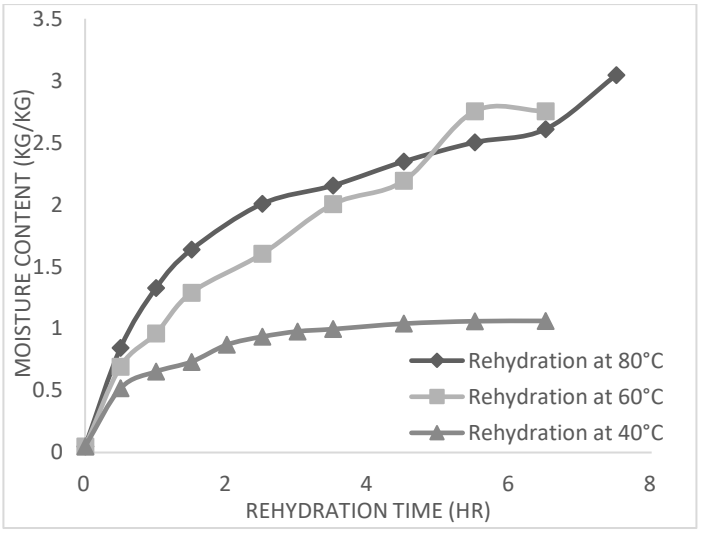

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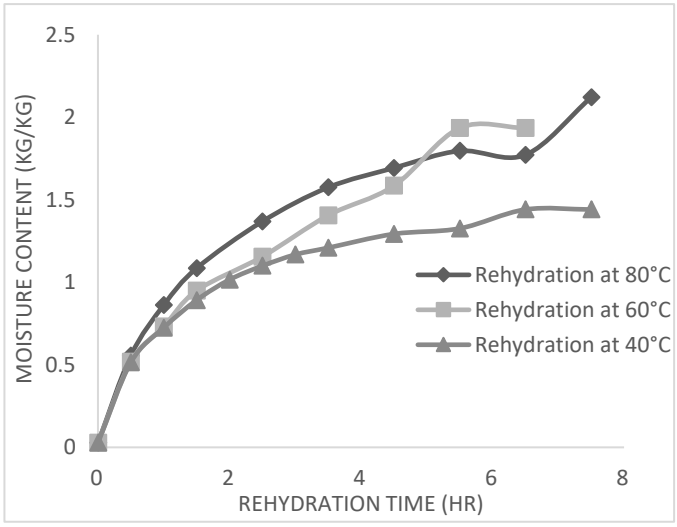

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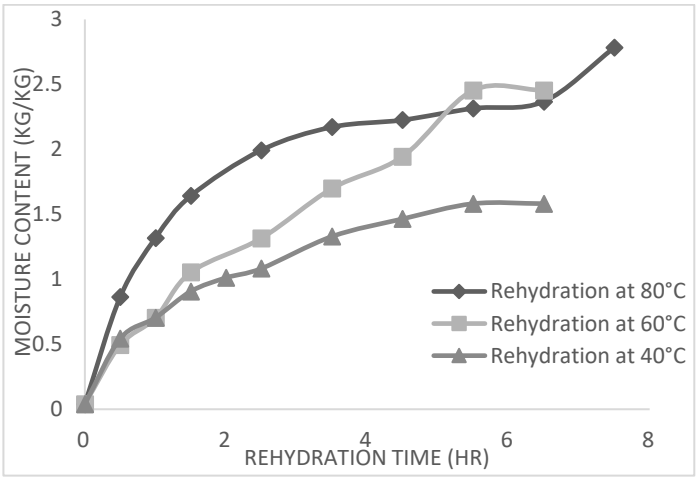
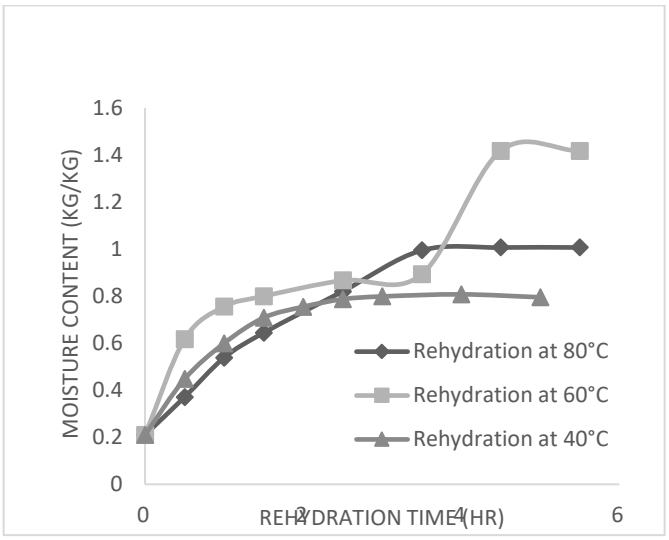
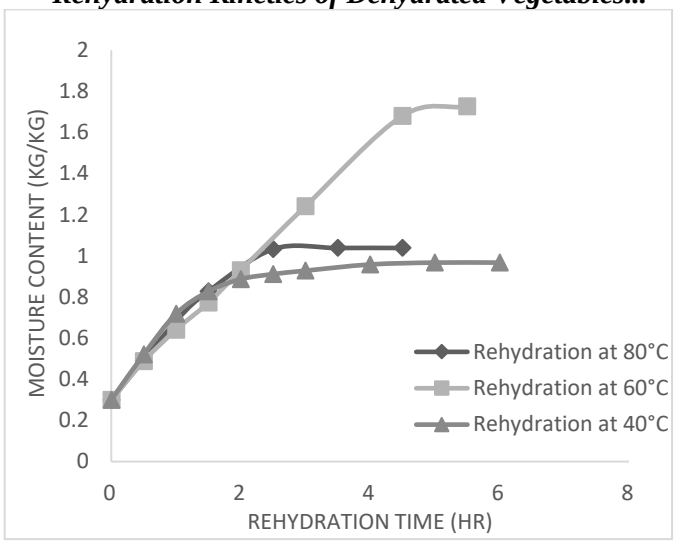


(e)

Figure 3. Rehydration curves of dehydrated potato (a) unpretreated (b) water blanched (c) Microwave blanched (d) 1 minute blanching in ohmic heat and (e) 2 minutes blanching in ohmic heat.



(a)



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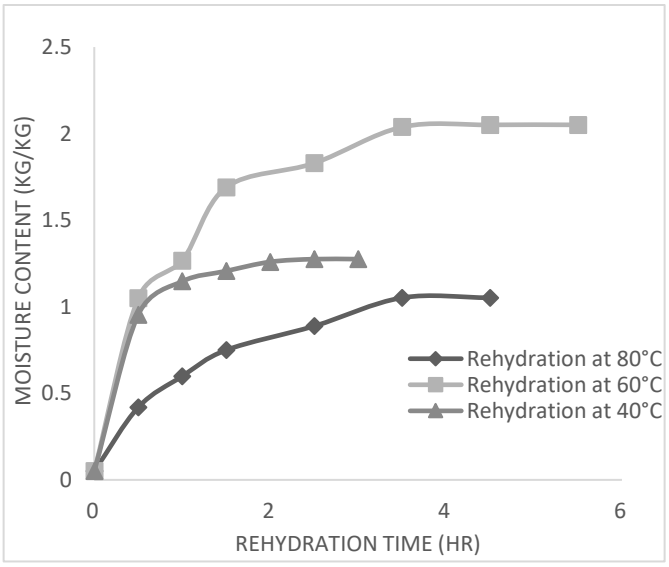

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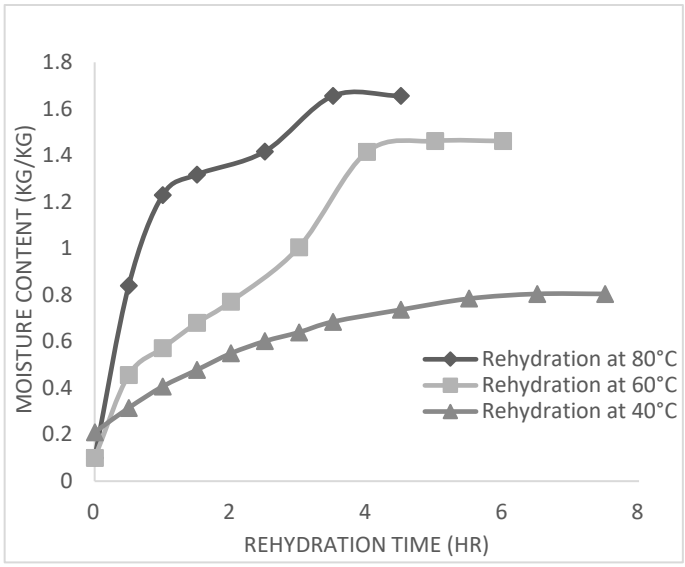

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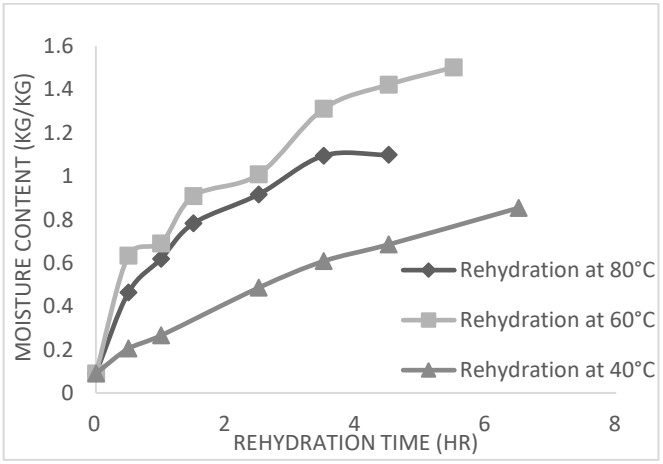

(e)

Figure 4. Rehydration curves of dehydrated yam (a) unpretreated (b) water blanched (c) Microwave blanched (d) 1 minute blanching in ohmic heat and (e) 2 minutes blanching in ohmic heat.

Table 1. Moisture content of fresh and dried food materials

	Initial Moisture Content (kg/kg)			Final Moisture Content (kg/kg)		
	Carrot	**Potato**	**Yam**	**Carrot**	**Potato**	**Yam**
CON				0.160±0.028	0.123±0.025	0.216±0.020
WB				0.145±0.007	0.385±0.005	0.390±0.005
MWB	8.320±0.554	4.016±0.217	1.900±0.100	0.023±0.002	0.056±0.005	0.050±0.005
OH1.0				0.024±0.005	0.025±0.005	0.100±0.050
OH2.0				0.027±0.001	0.046±0.007	0.090±0.010

Key:
CON: Control
WB: Water blanched
OH1.0: Ohmic blanched for 1 minute.
OH2.0: Ohmic blanched for 2 minutes.

Table 2. Rehydration Kinetics of Dehydrated Carrots

Pre-treatment Method	Pre-treatment time (mins.)	Rehydration Temperature (°C)	Carrot						Potato						Yam					
			R^2	k_1	k_2	X_e	X_f	RC (%)	R^2	k_1	k_2	X_e	X_f	RC (%)	R^2	k_1	k_2	X_e	X_f	RC (%)
Unblanched	0	40	0.97	0.64	0.26	3.93	3.27	313.64	0.95	0.46	0.73	1.47	1.31	119.19	0.97	0.67	1.53	0.86	0.79	58.5
	0	60	0.72	1.00	0.13	7.59	3.84	370.28	0.85	0.91	0.43	2.43	1.95	183.82	0.76	1.00	0.77	1.49	1.41	120.72
	0	80	0.98	0.75	0.23	4.33	3.6	346.67	0.97	0.29	0.58	1.83	1.66	154.5	0.85	1.58	0.95	1.26	1	79.65
Water blanching	2	40	0.96	0.94	0.22	4.63	3.06	292.5	0.87	1.38	1.21	1.21	1.06	67.4	0.97	0.78	1.35	1.04	0.96	66.73
	2	60	0.86	0.72	0.17	5.87	4.19	405.47	0.56	1.11	0.30	3.67	2.63	224.45	0.45	0.40	0.40	2.76	1.72	142.62
	2	80	0.98	0.80	0.28	3.59	2.66	252.05	0.90	0.33	0.64	1.95	3.08	269.8	0.84	1.46	1.39	1.01	1.03	73.84
Microwave blanching	2	40	0.97	0.60	0.25	4.14	3.53	339.87	0.98	0.52	0.90	1.15	1.06	101.19	0.99	0.08	0.79	1.31	1.27	122.5
	2	60	0.93	0.58	0.13	7.55	5.09	495.51	0.86	0.58	0.30	3.32	2.75	270.37	0.98	0.20	0.46	2.61	2.05	200.06
	2	80	0.96	0.69	0.21	4.72	3.38	324.53	0.95	0.39	0.32	3.15	3.04	299.55	0.94	0.63	0.87	1.59	1.05	100.19
Ohmic heating	1	40	0.97	0.88	0.18	5.43	3.37	323.14	0.97	0.62	0.64	1.58	1.44	141.4	0.89	2.90	1.28	0.87	0.8	70.46
	1	60	0.80	0.64	0.11	8.5	4.63	449.24	0.89	0.74	0.44	2.28	1.93	190.76	0.79	1.18	0.56	1.88	1.46	136.17
	1	80	0.98	0.52	0.19	5.29	4.57	443.71	0.95	0.58	0.45	2.24	2.12	209.38	0.97	0.24	0.60	1.76	1.65	155.54
	2	40	0.87	1.46	0.16	6.08	2.78	264.04	0.94	0.70	0.55	1.83	1.58	154.21	0.69	2.99	0.98	1.02	0.85	76.37
	2	60	0.79	0.86	0.13	7.65	4.38	424.04	0.78	0.83	0.31	3.22	2.45	241.43	0.92	0.66	0.63	1.59	1.5	141.19
	2	80	0.90	0.68	0.2255	4.57	4.25	411.64	0.96	0.33	0.3595	2.82	2.78	274.32	0.941	0.6644	0.86	1.16	1.09	100.81

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Halal Critical Point of Beneng Taro Products Identification Produced by Micro Enterprise in Serang City, Banten

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ABSTRACT

Food products that have a halal certificate must fulfill halal standards. The halal assurance system is created and implemented in order to ensure the halal production process. The concept of Halal Assurance System (HAS) 23000 is used as a reference to implement halal product processing. The problem is that there are many ingredients, the main raw materials and additional ingredients—that are not clear about their origin and halal assurance. This study aims to identify the Halal Critical Point (HCP) of derivative of beneng taro produced by microenterprises. This study uses a qualitative method. Sources of research data were taken from observations and interviews with owners of businesses, as well as observations at the research location. Halal critical materials are beneng taro flour, cassava flour, vegetable oil, and dry coriander. Halal critical processes are production, washing facilities, and transportation. The critical control points in production include soaking, washing, and frying.

Keywords: Halal Critical Point, Beneng Taro, Crackers, Chips

INTRODUCTION

Indonesia as a country with the largest Muslim population in the world, apart from in addition, Indonesia is also a very potential Muslim consumer market. The government has a great responsibility to protect the public overall, especially consumers for the halal of the products in circulation and marketed. The words "halal" and "haram" are terms of the Qur'an and this used in various places with different concepts, and some related to food and drink. The basis used for shows the necessity of consuming food and drink, plants and animals/animals that have been lawful and thayyib (good) are listed in the Qur'an and Hadith (Ali 2016). In today's food industry, foodstuffs are processed through various new processing techniques and methods by utilizing the development of science and technology, so that it becomes a product that ready to be thrown for consumption by people around the world.

According to (Faidah 2017) one form of protection and guarantee is to consumers that the products consumed have been researched and declared. Halal, namely having a halal certification so that it gives a sense of calm and comfort peace of mind for consumers. In terms of producers according to (Atma et al. 2018) halal products make products have prestige and trust from consumers so that it becomes one

of the product requirements in order to penetrate the global market, either local and international arena.

The combination of HACCP and Halal ensures that the food product and food contact products are safe and halal. Determination of Halal Critical Point can be done by referring to Hazard Analytical Critical Control Point steps. According to Riaz and Chaudry (2019), the Halal principle refers to HACCP system. Halal-HACCP principles consist of 7 principles, namely 1). Identification and analysis of all potential haram materials. Haram materials are various materials that are prohibited in Islamic for consumption. Haram substance analysis was carried out prior to hazard analysis. 2). Determination of HCP and CCP. HCP is determined before CCP decision tree. 3). Determination of critical and forbidden limits. The critical limit for hazard is measured with the presence or absence of illicit substances. 4). Determination of control measures. 5). Determination of corrective. 6). Determination of validation and verification procedures. Validation and verification can carry out by checking the material halal certificate and testing production processes and products.

In accordance with mandatory of process product halal and every food product must meet halal certification, so study of HCP and HCCP product need to be done in the small and micro enterprise. CCPs need to be quantifiable to achieve measurable limits and monitoring. It is a challenging task to identify CCPs for products especially those products which fall into minimal processed category (Kohilavani et al. 2013). The objectives of this research are to identify HCP and HCCP product derivatives of Talas Beneng include chips and crackers. This product is produced by some micro enterprises in Serang City, Banten Province.

MATERIALS AND METHODS

HCCP can be defined as a point, stage or procedure in halal food production so that cross-contamination of illicit materials can be prevented or removed (BSN 2016). Identification of the critical point of product halalness can be done by using a tree.

The CCPs are generally identified by using codex CCP decision tree. A Decision Tree is a predictive assessment, by mapping from observations about an item (Process or Materials) to infer conclusions about its status. This also known as "Classification Tree" or "Reduction Tree", the technique is used primarily as a decision support tool that uses a tree-like graph or model of decisions and its possible consequences, including chance event outcomes, resource costs, and utilities. Process step CCP decision tree by Horchner et al. (2006) were adopt in this study. Determination of the CCP based on CCP decision tree followed this question:

1. Do control measure(s) exist?
2. Is the step specifically designed to eliminate or reduce the likely occurrence of a hazard to an acceptable level?
3. Could contamination with identified hazards(s) occur in excess of acceptable level(s) or could these increases to acceptable levels?
4. Will a subsequent step eliminate identified hazards or reduce likely occurrence to acceptable levels?

Meanwhile, HCCPs are able to identify by using the HCCP decision tree for the ingredients and process control. Halal Critical Control Point (HCCP) decisions and some questions (Kohilavani et al. 2013):

1. Do all raw materials have halal certification?
2. Is there any possibility for cross contamination of haram substance?
3. Are the noncertified products are being used in the process?
4. Does the material contain any haram substances?
5. Is the specific production line and storage area for certified and non-

certified process and ingredients clearly identified?
6. Could the sanitation procedure able to eliminate the fat, smell, color and taste (dibagh)?
7. Is there any potential cross contamination of haram substances?

RESULTS AND DISCUSSION

General Description

The product description contains information about the product. Beneng taro chip and beneng taro cracker is produced by some of micro enterprises in Serang City, Banten Province. These products were produced as a form of diversification activity from Banten's local food materials. These products were produces by many micro or small enterprises in Banten Province, but this research only analyze one enterprise that located in Serang City, Banten Province. This micro enterprise was managed by Kelompok Wanita Tani (woman farmers group) of Tanjung Kulon, Talaga Warna, Pabuaran, Serang City, Banten Province. In 2022, this enterprise produces 2 types of food product namely chips beneng taro and cracker beneng taro. The chemical composition of product can be seen in the Table 1.

Crackers are a type of dry food made from high levels of beneng taro starch meanwhile chips are a kind of snack in the form of thin slices of tubers that are fried in vegetable oil. The packaging of these products are plastics and netto 100 grams.

Processing of Products

Chips Beneng Taro

Taro chips was processed by stripping raw materials and followed by washing and soaking in the citric acid solution/salt solution for 24 hours. The process continued with drain the material using a traditional machine and continued by draining and frying. The chips then added with flavoring and seasoning materials.

Peel the skin of the taro beneng and then wash it with water until clean, thinly slice the taro beneng with a sharp knife or use a cutting tool. Taro beneng that has been cut into thin is then soaked in 10% salt solution/acidic acid for 24 hours to remove the oxalate content. The taro that has been soaked is then washed thoroughly until the rinse water is clear. The rinsed taro is then drained and fried until cooked and crispy.

Cracker beneng taro

Cracker beneng taro was processed by through several stages, including: cleaning the skin, cutting the taro into thin strips, soaking in salt solution for 24 hours, rinsing, draining, mixing flour, kneading, mixing dough and beneng taro, printing, steaming, drying, and frying.

Peel the skin of the taro beneng and then wash until clean, thinly slice the taro beneng with a sharp knife or use a cutting tools so that the result is same. Taro that has been cut is then soaked in 10% salt solution for 24 hours to remove the oxalate content. The taro that has been soaked is then washed thoroughly until the rinse water is clear. The rinsed taro is then drained and crushed. The cracker dough is made by grinding the spices and mixing them with tapioca flour, fine taro beneng and water, then the dough is shaped until smooth. The cracker dough is laid out in a container and steamed until cooked. The cooked dough is then dried in the sun to dry and fried.

Critical Point on Raw Materials

Critical activities effect on halal status of a product. Critical activities may include selection of new materials, purchase of materials, inspection of incoming materials, product formulation, production, washing of production facilities and auxiliary equipment, storage and handling of materials and products, transportation, display, visitor management, menu determination, slaughtering, customizing with the company's business processes (manufacturing industry, RPH, restaurant/catering/kitchen) (LPPOM MUI 2008).

In the material selection, all materials are categoriz into 3 types namely vegetable, animal and microbial materials. The material selection procedure must guarantee that every ingredient used is approved by the Halal Certification Body.

HCP identification of each ingredient is based on a decision tree. The halal decision tree is used as a reference to replace unclear halal material with clearly halal materials. According to material categories, beneng taro's crackers and chips mainly use vegetable materials so determination of HCP materials only based on decision tree of vegetable materials. Other material such as water and organic material categorized based on positive list halal materials (LPPOM MUI 2013; Kemenag 2021).

Mapping Decision Tree of Vegetable materials

Vegetable materials are obtained from plants. Decision trees from vegetable materials can be seen in Figure 1.

The identification of the halal status of vegetable material is based on the decision tree in figure 1. The HCP of all materials to produce chips and crackers of beneng taro must be identified through the decision tree. The identification of halal status of cracker and chips materials is shown in Table 2.

Flour can be added as a coating material. Coating materials other than halal materials such as gum are also from syubhat (dubious) materials such as gelatin. L-Cysteine is an improving agent to improve the properties, and quality of the flour (Apriyantono, 2007). L-cysteine could be made from human hair and animal hair. If L-cystein is made from human hair, it is including non halal material. L-Cysteine from animal hair must be ascertained from halal animals (Sucipto et al. 2022).

Coriander is harvested from plant. But Coriander used in the chips and cracker was dry coriander. Although, it does not contain additives material, coriander meet physical drying process. Drying facilities should meet the Islamic law that is free from haram substances contamination.

Cooking oil is from vegetable oil or animal oil, which in the processing involves pale material in the form of bleaching earth or activated charcoal and the addition of antioxidants. It makes halal certified should be required in oil production process (HAS 23201: Persyaratan Bahan Pangan Halal, 2012). This business use packaged oils that have halal labels. However, there are still some moments, which still use uncertain bulk oil from market. According to Sugito et al. (2018), some producers mix vegetable cooking oil with animal oil to produce more savory food taste. Mohammed et al. (2012) state that produces adsorbents in palm oil can use pig bones. The enterprise should be aware of this issue and change vegetable oil material with packed oil.

Mapping Decision Tree of other materials

Salt, acetic acid and water are additive materials in making chips adnd crackers products. Decision tree of salt, water, acetic acid can be determined based on the Figure 2.

Based on Figure 2, halal status of materials can be categorized. Table 3 show materials category from product.

Salt is a mineral that is categorized as uncritical because it has not undergone any processing. Salt includes in mineral that has no HCP. Water is also materials uncritical or positive list. Acetic acid and packaging material (plastics/ polyethylene) categorize as synthetics that is positive list.

Purchasing Materials

In the manual halal self-declare, purchasing activities must have supporting data such as the name of the materials, brand, company name and halal certification materials, date purchasing, and total purchasing. According to HAS 23000, purchasing data include the name of the material, code, company name, factory location, and halal logo. Suppose there is a

material that has not been certified as halal. In that case, it must be replaced with halal-certified material or have a halal production process statement from the producer that needs to be verified by the halal inspector (Sucipto et al. 2022). But, in the case of self-declare certification, it is recommended that the materials used must have a halal certificate.

Inspection of Incoming Materials.

In the case of micro enterprise, materials were purchased based on customer order. The owner buys some materials by themselves. Suppose purchasing was carry out in lot number, inspection was done when materials come in owner house production. The inspection included physical condition (freshness, colour, odor), brand named, expired date, and amount. Administration record was carried out after inspection with listed information of purchasing date, amount, type product, and brand.

Critical Point on Process

Production

Production is carried out at facilities that meet the criteria, halal, and free from haram substances and najis (ritually unclean) materials. This requirement applies to self-owned production facilities and other parties' facilities if subcontracting production (Sucipto et al. 2022). Production in this micro enterprise is carried out after receiving a customer order. Production activities are in kitchen. Production was done in the house. Before carrying out production, employees must wear closed clothing and gloves when processing materials. This must be done because hygiene and sanitation are also prerequisites in the halal production process program

Production of chips are peeling, washing, cutting, soaking, draining and frying. Production of cracker are peeling skin, cutting, soaking rinsing, draining, mixing, kneading, mixing dough, printing, steaming, drying, and frying. Halal critical point of product lays on selection materials. To prevent haram substances and Najis (ritually unclean) materials contamination, all facilities must be cleaned before it is used. This is possible for micro entrepreneurs.

Washing of Production Facilities and Equipment.

Washing facilities in this enterprise are carried out before and after production using flowing water. A washing uses liquid dishwashing soap for all types of tools. The pans, container, and knife are cleaned before using every production. The dishwashing soap has a halal certificate so that it can guarantee product halalness. Washing using flowing water is better than soaking (Azari in Sucipto et al. 2022). Washing using running water removes all dissolved impurities without recontamination of the equipment (Sucipto et al. 2022. *Storage and Handling of Materials and Products*

There is no product storage in this industry. Products that have been produced are packed in plastic packaging/pouches and delivered directly to the consumers. Materials that come directly processed into products. For packaged materials, materials are stored in a closed condition.

Transportation

Transport procedures must be observed to ensure halal products' contamination by haram (unlawful) or najis (unclean Islamic ritually) materials during transportation (LPPOM MUI 2013). Transporting materials from suppliers must be clean from haram or najis substances and not transport haram material, animals, or humans. The halal supply chain in production is essential to halal product assurance (Tieman, 2011). In the case of micro enterprise, packing was done manually by employees. Transportation used motorcycle to the customer by owner/employee.

Halal Critical Control Point

CCPs are the control points at which food safety hazards can be eliminated, prevented and reduced to safety level (Kohilavani et al. 2013). Similarly, HCCPs are the steps where haram substances are identified and eliminated from the process steps. Determining the CCPs and HCCPs of both products was shown in Table 4 and Table 5. HCCPs are able to identify by using the HCCP decision tree.

CONCLUSION

Based on the analysis, it known that chips and crackers of has halal critical point in the selection materials. Halal critical materials are beneng flour, cassava flour, vegetable oil, and dry corriander. Halal critical process are production, washing and transportation. The critical point in the production includes soaking, washing, frying. Beneng taro chips and crackers don't have halal critical control point, but its only critical point.

SUGGESTIONS

Suggestion for further research are implementation of processing of halal product and quality control in the process.

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Table 1. Chemical composition of product

Chemical composition (%)	Chip beneng taro	Cracker beneng taro
Moisture content	7.10	2.58
Ash content	3.40	3.36
Fat content	24.77	32.29
Protein content	3.45	1.61
Carbohydrate	61.28	60.16

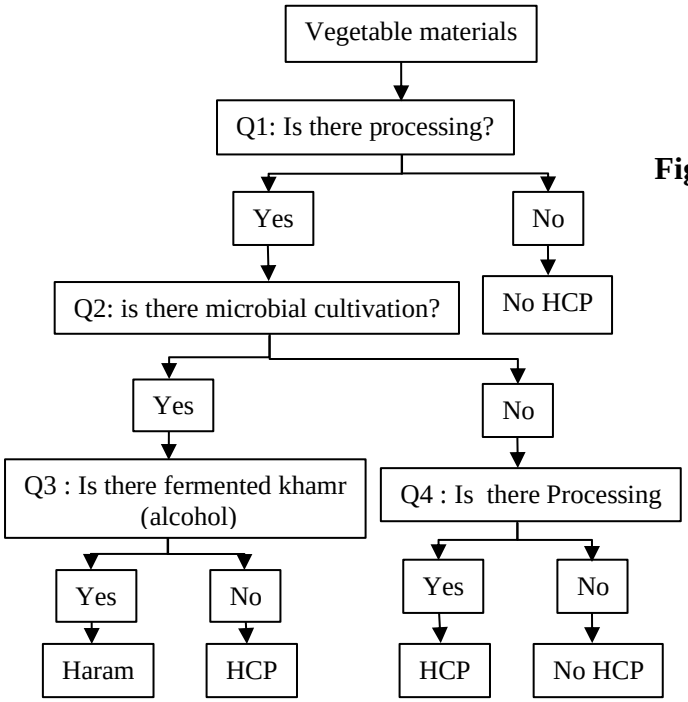

Figure 1. Decision tree for identification of Halal Control Points for vegetable materials (LPPOM, 2013)

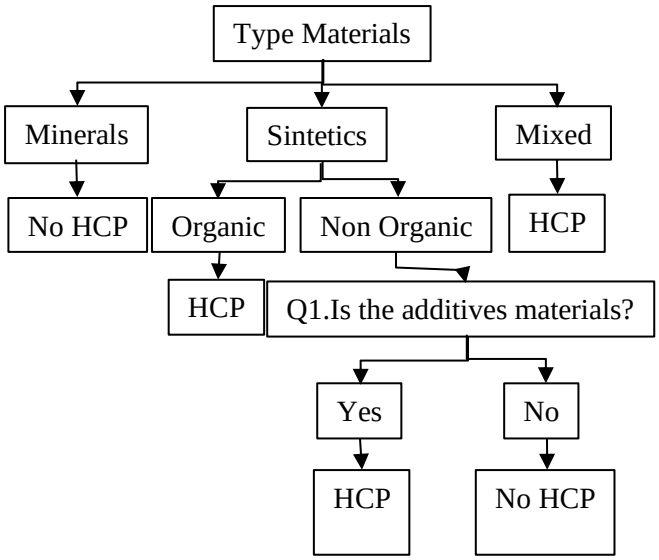

Figure 2. Decision tree for identification of Halal Control Points for other materials

Table 2. Materials identification based on mapping decision tree

Product	Materials	Question				Status HCP	Potential risk
		Q1	Q2	Q3	Q4		
Chips	Beneng Taro	No	-	-	-	No HCP	-
	Vegetable oil	Yes	No	-	Yes	HCP 1	Additives materials, process contamination bleaching agent
Cracker	Onion	No	-	-	-	No HCP	-
	Coriander	Yes	No	-	Yes	HCP 2	Additives materials, process contamination
	Vegetable oil	Yes	No	-	Yes	HCP 3	Additives materials, process contamination bleaching agent.
	Beneng flour	Yes	No	-	Yes	HCP 4	Additives materials, process contamination
	Cassava flour	Yes	No	-	Yes	HCP5	Additives materials, process contamination

Table 3. Materials category for product

Product	Materials	Positive list[a,b]	Category	Status HCP
Chips	Water	Yes	-	No HCP
	Salt	Yes	Minerals	No HCP
	Acetic Acid	Yes	Synthetics	No HCP
	Packaging materials (plastics)	Yes	Synthetics	No HCP
Crackers	Water	Yes	-	No HCP
	Salt	Yes	Minerals	No HCP
	Packaging materials (plastics)	Yes	Synthetics	No HCP

Sources: [a]LPPOM MUI (2013)
[b]Kemenag (2021)

Table 4. Determination CCP

Product	Question				CCP/ CP
	Q1	Q2	Q3	Q4	
Taro beneng Cracker	No	Yes	No	Yes	Soaking Washing Frying
Taro beneng chips	No	Yes	No	Yes	Soaking Washing Frying

Sources: direct observation in small enterprise

Table 5. Determination HCCP

Product	Questions							Status HCCP /HCP
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	
Taro beneng Cracker	No	-	No	No	No	Yes	No	HCP
Taro beneng chips	No	-	No	No	No	Yes	No	HCP

Sources: direct observation in small enterprise

SCOPE, POLICY, AND AUTHORS GUIDELINES FOR FOOD SCIENTECH JOURNAL

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