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PREFACE

By the Grace and Blessings of Allah the Almighty, we would like to present, with great pleasure, the Volume 04 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 2022

Editorial Team

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Image Analysis as a Tool for Estimation of Red Peppers' Color

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ABSTRACT

Red pepper is an excellent source of natural red color. The estimation of color is of great importance, as it is being used for grading red peppers to suit various applications in the food industry. A novel method for quick and easy determination of the color of red peppers by analyzing the color images obtained from a flatbed scanner was developed and validated. ImageJ software was used for measuring the RGB values of the images and the RGB values thus obtained were converted into the industrially accepted American Spice Trade Association (ASTA) color values in red peppers by using an empirical formula. The results were compared with color values obtained through ASTA chemical analytical method. The developed image analysis method was used for the analysis of red peppers with color values ranging from 15 to 154 ASTA units. The image analysis method showed good agreement with the chemical method for color values ranging from 40 to 140 ASTA units. The new method is also fast and easy to adopt and is deemed useful in the field, of processing and storage facilities, where access to sophisticated instrumentation for color estimation and color stability studies is limited.

Keywords: Image Analysis: Flatbed Scanner; Red peppers; ASTA Colour Value; RGB Colour Model

INTRODUCTION

Red pepper is being used both as a flavoring and coloring agent to culinary preparations. The red pepper extracts containing carotenoids, after the removal of pungent compounds like capsaicin, are used in the food industry for imparting shades of red color to products. In the spice industry, the accurate estimation of color is of great importance, as it is used for grading red pepper to suit various applications. A standardized arbitrary unit developed by American Spice Trade Association (ASTA), namely ASTA color units is being used to express the color of red pepper in trade and industry. The ASTA method involves the solvent extraction of coloring compounds from the red pepper followed by the measurement of absorbance using a uv-visible spectrophotometer (ASTA 2004, AOAC 1980).

Non-destructive and chemical-free analytical methods such as image-based estimations are being increasingly preferred to traditional chemical methods for the quality analysis of food products by the industry. Among the image analysis softwares, ImageJ holds a unique position as its source code is openly available to perform necessary modifications or insert add-ons to make it suitable for the required image analysis. ImageJ is a java-based, readily available, open-source, platform-independent, and public domain software developed by the National Institute of Health (NIH), Bethesda, Maryland USA (Rasband 2013). Studies have been reported on the application of ImageJ for color analysis in food products: e.g. apples (Garrido-Novella et al. 2012) and tomatoes (Lana et al. 2006). However, studies establishing a direct correlation between image analysis and standard chemical methods are rather limited. Availability of a non-chemical, image-based estimation technique for color evaluation in red pepper suitable to be used in food processing areas would be useful to monitor and manage the quality and consistency of chilly-containing food products. This study was aimed at the development of a flatbed scanner-based estimation of the color value of red pepper and to validate against the available chemical method of color value estimation.

MATERIALS AND METHODS

Tools and Materials

Red pepper: Samples (40 Nos.) of red pepper (*Capsicum annum*) powder were collected from the local markets of Maharashtra and Gujarat in India. The samples were powdered and sieved through a US No. 40 standard sieve (4760 microns), sealed, and stored at room temperature till analysis. Certified reference red pepper powders for color value were obtained from ASTA (category number 5500012).

Chemicals and Reagents: Acetone (spectroscopic grade), sulphuric acid, NIST-traceable potassium dichromate, and cobalt (II) chloride hexahydrate were purchased from Merck India.

Chemical estimation of color

About 0.0125 g potassium dichromate and 1.35 g of cobalt (II) chloride hexahydrate were dissolved in 50 mL 5% (v/v) sulphuric acid. The absorbance (A_{std}) of the solution was measured at 420nm, and the Instrument factor (I_f) was calculated (ISO 7541, 2010) using the equation $I_f = 0.315 / A_{std}$

The extractable color of red pepper powder was analyzed by ASTA Method No. 20.1 (ASTA 2004, AOAC 1980). About 0.1g (W_s) of red pepper powder was added into a 100 mL amber-colored volumetric flask and made up to the volume with acetone. This was kept for 16 h in dark for extraction. A reagent blank was also run simultaneously. The extracts were filtered through Whatman No. 1 filter paper and the absorbance of the extract at 460 nm for the sample (A_s) and the blank (A_b) were determined using a UV-visible spectrophotometer (Shimadzu Model UV 1700, Kyoto, Japan). The color value was calculated as:

$$Colour\ Values\ (ASTA) = \frac{[(A_s - A_b) \times 16.4 \times I_f]}{W_s}$$

Where 16.4 is the constant to convert the absorbance into ASTA color units.

Image Analysis

Generating and Analyzing Images: A sample loading system with multiple sample wells was fabricated using a transparent plastic sheet of 7 mil (0.18 mm) thickness and circular rings of about 5 cm in diameter and 3 mm height, thus enabling multiple samples (up to 8) to be loaded at the same time. About 2 g each of the samples were loaded into the well and gently tapped down to form a

uniform surface against the plastic sheet. The setup was then scanned by placing it in a flatbed scanner (HP Scanjet model G2410) at a scanning resolution of 300 pixels per inch (ppi) and stored as images in JPEG format. The individual images were then loaded into ImageJ software and cropped into rectangular sections of uniform color (Fig. 1). The 'Measure RGB' plugin of ImageJ (Rasband, 2018) was used to analyze the images to obtain the R, G, and B values corresponding to the image. The performance of the scanner was verified by scanning a white paper of 80 gsm (g/m^2). The resultant image was analyzed in ImageJ and the R, G, B values were found to be within 250± 0.01.

Calculating Color Values: A general equation for the calculation of the color of red pepper from the RGB values obtained from a digital photograph of the powder can be stated as follows:

$$Color\ Value\ (Image\ Analysis) = rR + bB + gG$$

where R, G, and B are the red, green, and blue components obtained from the image analysis of the sample, and r, g, and b are coefficients that determine the contribution of red, green, and blue components to the final color of the red pepper powder. The values of these coefficients can be chosen so as to give the best correlation with the values obtained from standard methods. The coefficients were calculated by resolving 3 equations generated from the image analysis and chemical analysis of 3 samples with varying color values get the best fit with the empirical values as determined by the ASTA chemical method.

$$C_i = r_i R + b_i B + g_i G$$

where C is the color value obtained through ASTA chemical method, i = 1, 2, or 3

A plugin was written for ImageJ by incorporating the coefficients r, g, and b obtained through 5 sets of experiments (r = 1, g = -1.5, b = 0.4) which calculates the color of red pepper from the digital image (Fig. 1).

Method Validation

The precision of the method was validated in terms of repeatability and intermediate precision. Repeatability was studied by analyzing 5 replicates of each sample using both the chemical and image analysis methods. Intermediate precision of chemical method included the analysis of 3 replicates of each sample, analyzed by three analysts at three different times. Intermediate precision of the image analysis method was established by analyzing the same sample on two different scanners (HP Scan jet model G2410 and Canon LiDe 110) on different days. The ruggedness of the image analysis method was established by analysis of the same sample at scan resolutions of 150, 200, 300, 600, 1200, and 4800 ppi. The efficacy of the method was confirmed by analyzing 40 red pepper powder samples with varying color intensities simultaneously using both image analysis and chemical methods.

Statistical Analysis

Results were reported as mean ± standard deviation of 5 trials. The image analysis and ASTA methods were compared by regression analysis (Linnet 1993) and Bland-Altman plot (Altman and Bland 1983; Dewitte et al. 2002). Statistical analysis was performed using Microsoft Excel 2007

RESULTS AND DISCUSSION

Method Development

The RGB color model was chosen for image analysis. This is an additive color model in which three primary colors, viz. red (R), blue (B), and green (G), are added together to form a broad array of colors (Burger and Burge 2008). In this model, the (R, G, B) values of black color are (0, 0, 0) and those of white color are (255, 255, 255). Other colors are represented as combinations R, G, and B, varying from 0 – 255. It was established initially that varying the scan resolution from 150 – 4800 ppi did not

significantly affect the results; hence an optimum scan resolution of 300 ppi was chosen based on acceptable visual image quality and scan time. Ten red pepper powders of widely varying color values (30 – 100 ASTA units), including 2 certified reference materials supplied by ASTA, were selected for optimization of the method. Images of these samples were generated and the 'Measure RGB' plugin of ImageJ (Rasband 2004) was used to obtain the R, G, and B values from the images. The values of the coefficients r, g, and b were fixed so as to obtain the best correlation with the reference values obtained from the chemical analysis of these samples.

Color values of red peppers

The ASTA method, as an internationally accepted method being used in trade and industry for the evaluation of color in red pepper, was taken as the gold standard to develop the image analysis method. The efficacy of the method was verified with 40 samples ranging from 15.7 to 153.7 ASTA units. The results for each sample obtained by image analysis and chemical methods were compared. It was observed that the results of the samples studied could be divided into three ranges based on the level of correlation of the image analysis results with ASTA results - Range 1: results < 40 ASTA units, Range 2: results from 40 – 140 ASTA units and Range 3: results > 140 ASTA units. It was observed that in Range 1, the results from image analysis deviated widely from the results from chemical analysis. Range 2 showed a good correlation between the image analysis and chemical analysis results, to the extent of ≤ 10%. In Range 3, the degree of correlation between methods decreased but was still seen to be within ≤ 25%. Hence it was concluded that the method has a linear range within 40-140 ASTA units. The repeatability and intermediate precision obtained for the two methods in the three ranges are shown in Table 1.

To evaluate the correlation between the two methods in Range 2, two statistical methods were used. The regression analysis using a scatter plot of image analysis results versus chemical analysis results is given in Fig. 2. A Bland-Altman plot was constructed by plotting the difference vs. mean of the results obtained from the two methods, as given in Fig. 3. The regression analysis showed that the data obtained from the two methods showed a good correlation between the methods, with a correlation coefficient of 0.9895 ($P < 0.01$), slope 0.9405 ($P < 0.01$), and intercept 5.1895 ($P = 0.06$). From the Bland-Altman plot, the limits of agreement were calculated at 95% confidence limits as $d + 2s = 7.95$ and $d - 2s = -8.86$, where d is the mean difference between the methods and s is the standard deviation of the differences. Since this difference is not significantly important in measuring the color values of red peppers, it is inferred that the two methods can be used interchangeably within the range of 40 – 140 ASTA units. In the case of repeatability, the average %CV was found to be the lowest in Range 2 in both methods. In this range, the %CV for the chemical method was 0.6 and for the image analysis method was 2.0. In other ranges, the values were slightly higher. Intermediate precision (%CV) for Range 2 for chemical method and image analysis method were 1.3 and 2.0 respectively.

CONCLUSION

A novel method was developed by which the color of red peppers can be quantitatively estimated using a flatbed scanner and image analysis software. The image analysis method showed good agreement with the standard method for color values ranging from 40 to 140 ASTA units and was shown to be statistically comparable to the accepted method. Optimization of the

coefficients for different scanning instruments can be easily done by comparing the results with the ASTA color values obtained from the chemical method. The process can be adopted inexpensively by small-scale spice processers lacking instrumentation for chemical estimation of color, for a quick estimate of the color of sample lots and to ensure color consistency of sample lots. It can also be used for studies on the stability of color on storage, where the absolute correlation with the chemical method is not necessary. The image analysis method is also much faster and easier to perform than the corresponding chemical methods.

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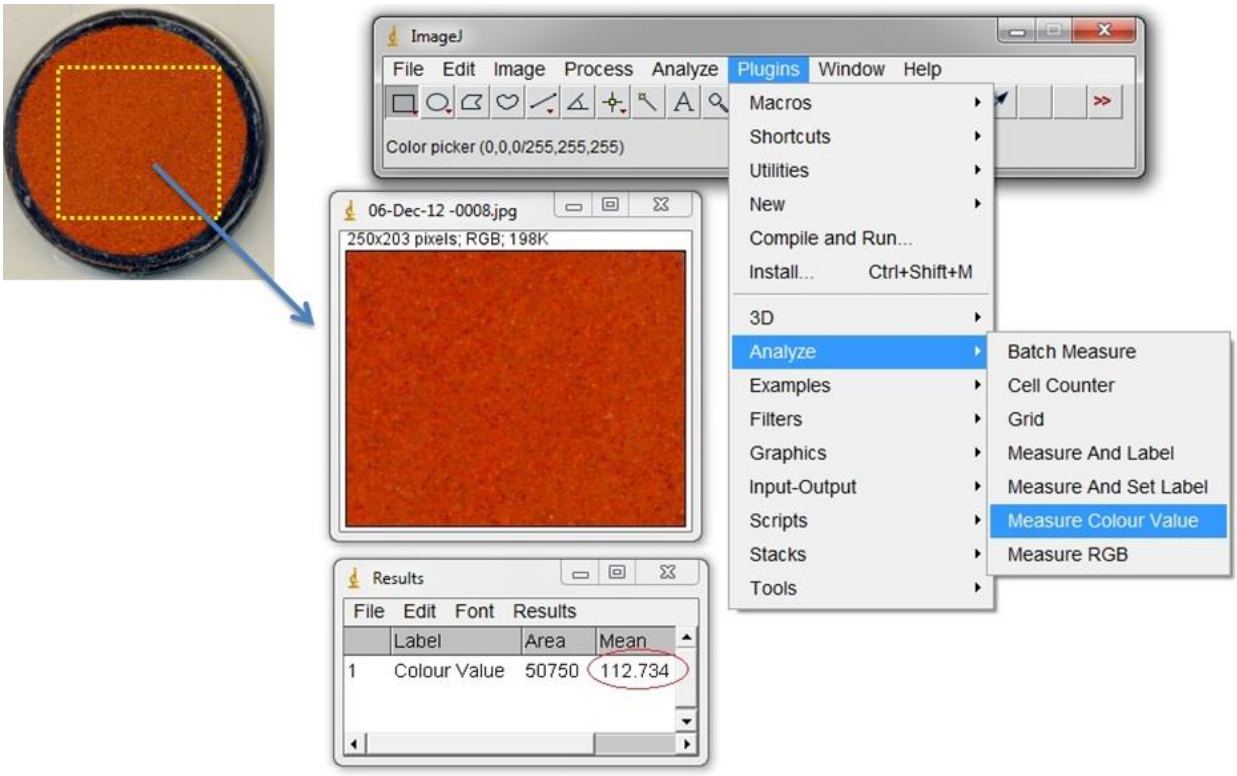

Figure 1. Image of red pepper powder used for analysis (300 ppi, HP Scanjet model G2410) and the ImageJ plugin used for estimation of color value

Table 1. Repeatability and Intermediate Precision data for Chemical and Image Analysis methods for estimation of color in red peppers

		Chemical Analysis		Image Analysis	
	ASTA Values	**Repeatability (%CV)**	**Intermediate Precision (%CV)**	**Repeatability (%CV)**	**Intermediate Precision (%CV)**
Range 1	< 40	1.5	3.4	3.3	5.7
Range 2	40 – 140	0.6	2.0	1.3	2.0
Range 3	>140	1.1	2.5	1.4	1.7

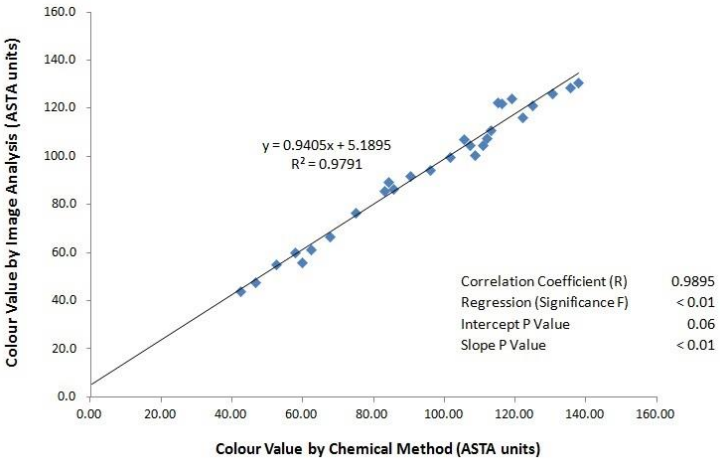

Figure 2. Regression analysis of results of colour values of red peppers from image analysis and chemical analysis methods in the range 40 – 140 ASTA units (mean of five trials)

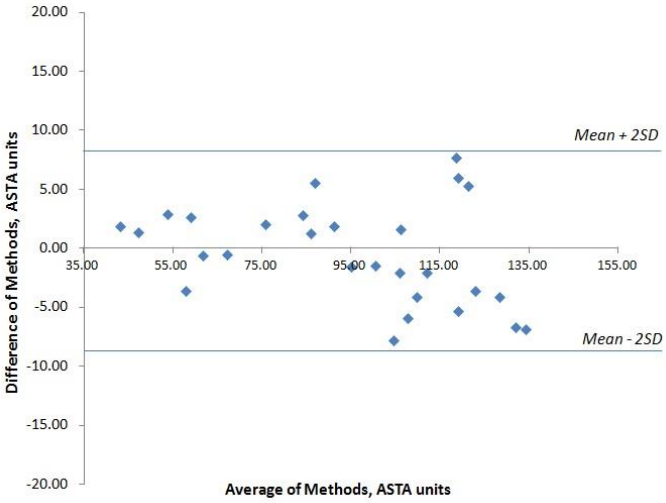

Figure 3. Bland-Altman plot for results of colour values of red peppers from image analysis and chemical analysis methods in the range 40 – 140 ASTA units (X-axis = $[C_{image}+C_{chem}] / 2$, Y-axis = $[C_{image} - C_{chem}]$, where C_{image} is the colour value obtained by image analysis and C_{chem} is the colour value obtained by chemical analysis.

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Nutritional and Anti-Nutritional Composition of Fermented/Pickled Garden Egg (*Solanum aethiopicum* L.)

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ABSTRACT

Garden egg (*Solanum aethiopicum* L.) is highly perishable. Shelf life of the crop can be extended via pickling. Therefore, this study focused to observe the pickled garden egg, in order to make it available in and out of season. Three samples were prepared, namely unpickled (control) garden egg (UPRGE); pickled garden egg in brine with sugar (PGESU); and pickled garden egg with salt (PGESA). Samples were pickled for seven days and evaluated for the nutritional, phytochemicals and sensory qualities using standard methods. On dry weight basis, moisture contents ranged from (1.11 to 1.12 %), protein ranged from (13.8 to 19.5 %), ash ranged from (12.7 to 18.0 %), fiber ranged from (8.5 to 15.8 %), fat ranged from (2.7 to 5.4 %) and the available carbohydrates ranged from (45.1 to 54.3 %). There was significance difference ($p<0.05$) in protein, ash, crude fiber, and carbohydrates. The mineral compositions were as follows: Potassium (183.0 to 183.7 mg/g), magnesium ranged from (162.3 to 194.5 mg/g), calcium ranged from (105.6 to 207.3 mg/g), copper ranged from (67.9 to 747.1 mg/g) and sodium ranged from (315.4 to 346.2 mg/g). Vitamin C ranged from (3.25 to 3.37 mg/g), saponin ranged from (10.74 to11.58 mg/g) and tannin (1.93 to 2.73 mg/g). Unpickled garden egg was scored higher in all of the sensory attributes. Conclusively, although pickling improves the nutritional composition of garden egg, and reduces the anti-nutritional content, the raw samples were preferred.

Keywords: Pickling; garden egg; phytochemical; proximate; minerals

INTRODUCTION

Garden egg (*Solanum aethiopicum* L.) also called eggplant belongs to the family *Solanaceae* which has over 1000 species worldwide. According to Bergley (2009), there are about 25 species including domesticated and wild types, with the leaves, and fruits used as vegetables or in traditional medicine in Nigeria. Garden egg is a popular traditional vegetable in tropical Africa, called "afufa" in Igbo, "dauta" in Hausa and "Igba" in Yoruba (Anosika *et al.*, 2012). It is one of the healthiest foods one can consume (Bergley, 2009), composed of approximately 92% water, 6% carbohydrates, 1% protein, good source of dietary fiber and negligible fat. Furthermore, it is rich in minerals such as potassium, manganese, copper, magnesium, phosphorus and vitamins such as folate, niacin, thiamine (vitamin B1) and vitamin K.

Garden egg contains phytonutrient such as Nasunin and Chlorogenic acid (Noda *et al.*, 2000). Nasunin is found in the skin of the fruit, a potent antioxidant and free radical scavenger. Furthermore, nasunin has been revealed to prevent destruction of cell membrane that can promote cancer and lessening free radical damage in joints (Anosika *et al*., 2012). Due to the nutritional contents of garden egg, consumption has been documented to have a lot of health benefits, including weight loss, lowering of cholesterol, lowering of blood sugar, improvement in vision and increases vitamin K level (Eze and Kanu, 2014).

The phytochemicals in garden egg such as oxalates, tannin, saponin and alkaloids are considered as anti-nutrients since it has been documented to chelate with minerals (Eze and Kanu, 2014). However, pickling/fermentation process has been shown to reduce the anti-nutritional content of crops (Saranya and Ranjani, 2017).

The perishability of garden egg is similar to that of cucumber (*Cucumis sativus L.*) which is about 10 to 14 days after harvest at optimum temperature of 10 to 12 ^{0}C and relative humidity of more than 80% (Snowdon, 1990). In order to improve the perishability of garden egg, similar forms of preservation applied to cucumbers can also be applied to make them available in and out of season (Snowdon, 1990).

Preservation techniques used for cucumbers include pickling, which also increases nutritional value of the foods (Lee and Kang, 2004). Cucumbers are often preserved by making them into pickles. Pickling is one of the oldest known methods of food preservation technique used in foods especially vegetables (Okafor, 2007). A wide range of vegetables and fruits can be pickled including onions, tomatoes, carrots, mangoes and cucumbers (Okafor, 2007) and the process involves anaerobic fermentation in acidic environment usually vinegar (Egbe *et al*., 2017). Other materials such as sugar, salt, spices, red pepper flakes, cinnamon sticks, mustard seed and bay leaves could be added into the brine solution (Kolbe and Kramer, 2007). Furthermore, this method of preservation could be applied to indigenous crop such as garden egg. In Nigeria, this fruit is usually consumed raw or cooked in sauce but never pickled. Therefore, the focus of this study was to pickle garden egg with the goal of making it available in and out of season.

MATERIALS AND METHODS

Materials

About 2 kg of mature but unripe fresh garden eggs (*Solanum aethiopicum* L.) variety was purchased from Beere market in Ibadan, Oyo State and was transported to Food Science and Technology Laboratory. Apple cider vinegar, sugar, salt and pickling jars with lids were also procured.

Methods

Preparation of samples

The gardens eggs were sorted to remove dirt, unwanted particles and damaged fruits. The stalks were removed with stainless knife and washed in portable water and divided into three sample groups of 0.6 kg each.

Group A: Unpickled garden egg (UPRGE)
Group B: Pickled Garden egg with sugar (PGESU)
Group C: Pickled Garden egg with salt (PGESA)

Preparation of brine

Brine solution with sugar was prepared according to (Kolbe and Kramer, 2007). Briefly, 1 L of distilled water, 1 L of apple cider vinegar, 16 g of salt and 38.9 g of sugar was heated to 100 ^{0}C for 5 min so as to allow the solution to dissolve properly and then it was cooled to room temperature (32 ± 2 ^{0}C). Brine solution without added sugar was also prepared as previously mentioned.

Pickling of Garden eggs

The garden eggs to be pickled were placed in a thoroughly cleaned jar along with a tight lid. Approximately 0.6 kg of garden eggs were placed in four jars and filled with 500 mL of brine with sugar, and left undisturbed at room temperature ($32 \pm 2\ ^0C$) to ferment for 7 days (Egbe *et al.,* 2017). Similar process was followed for the garden eggs pickled with brine without added sugar. Portions of samples were taken immediately following pickling for sensory analysis and determination of moisture content. The rest of the samples were sliced, oven dried in a cabinet dryer (Model F300, Chris Alex Engineering, Ibadan, Nig.) at 105 ^0C for 35 minutes, milled and stored in a Ziploc bag at room temperature for other analyses.

Determination of pH

The pH of the brine was determined by potentiometric method (AOAC, 2005) using Hanna instrument (HI 2209 pH/ORP Meter, USA). pH of brine was monitored every 24 h for 7 days during fermentation.

Proximate Composition

Proximate composition of the fruits including moisture, protein, lipid, crude fiber, ash contents were determined using the official method of the Association of official Analytical chemist AOAC (2005). All chemical analyses were performed in triplicates. Carbohydrate was determined by difference, 100 % – (protein +ash + fat + fiber contents). Energy in Kcal was calculated for vegetables according to Asibey-Berko and Taiye (1999), (2.44 Kcal, 2.57 Kcal and 8.37 Kcal), for protein, carbohydrate and fat respectively. Mineral content was determined according to (AOAC, 2005). The elements, calcium (Ca), magnesium (Mg) and copper (Cu) were determined using Atomic Absorption Spectrometer (PG 990, United Kingdom) at wavelength of 422.7 nm, 285.2 nm and (324.8 nm) respectively, and sodium (Na) and potassium (K) were determined by flame photometry method using a flame photometer (Jenway PFP7, United Kingdom) at wavelength of 589.0nm and 766.4nm respectively.

Determination of Phytochemicals

Vitamin C (Ascorbic acid) was determined according to AOAC (2005) method. Saponin was quantitated according to (Price *et al.,* 2006) and tannin was estimated according to the procedure of (Makkar *et al*., 1993).

Sensory Analysis

Sensory evaluation of the (control) un-pickled, pickled with sugar and pickled without sugar samples were carried out immediately following the pickling process by un-trained panel of 15 individuals. The Garden egg samples were coded and presented randomly to the panelists. Although the panelists were untrained prior to testing, they were familiar with the fruit and instructed to score the samples on a 5-point Hedonic scale, where 1 = dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely (Meilgaard *et al*., 1991), based on the attributes such as colour, aroma, taste, crunchiness and overall acceptability. Water and crackers were provided to cleanse palate in between sampling.

Statistical Analysis

Data from proximate, phytochemicals, minerals and sensory analysis were analyzed using descriptive statistics, Analysis of variance (ANOVA) with a post-hoc Duncan New Multiple Range Test in IBM SPSS Statistics 23.0 version at ($p<0.05$) significant level.

RESULTS AND DISCUSSION

Acidity

The pH values of brine without sugar were lower than the sample with sugar throughout the pickling period. Values for brine without sugar ranged from (1.95±0.01 to 2.81±0.03), compared to brine with sugar (2.45±0.06 to 3.33±0.01), possibly due to osmotic dehydration. According to Yadav and Singh (2014), osmotic dehydration increase uptake in sugars and removes acid in fruits and vegetables during pickling. All the pH values increased during the period of pickling as presented in Figure 1. High acid range food confers more antimicrobial effects on contaminating organisms, by inhibiting microbial activity as reported by Medina *et al* (2015). The pH values obtain from pickled garden egg in this study are lower than 4.0. Vinegar is so useful at preserving food, because it is an acid that is safe to consume and it inhibits the growth of pathogenic and spoilage microorganisms (Entani *et al.*, 1998).

Nutrient content of pickled garden egg

The nutritional compositions of pickled and unpickled garden eggs on dry weight basis are presented in Table 1. The moisture content of the garden egg samples ranged from (1.11 to 1.12 %). Protein content ranged from (13.8 to 19.5 %). Crude fiber content ranged from (8.5 to 15.8 %). Fermentation or pickling of foods increases the availability of nutrients, making digestible and indigestible constituents readily utilizable (Evans *et al.*, 2013). Therefore, pickled garden egg had higher fiber content compared to unpickled garden egg. Fat content of garden egg samples in this study ranged from (2.17 to 5.4 %) and ash content ranged from (12.7 to 18.0 %). The unpickled sample had the lowest values in protein and fibre, and statistically ($p<0.05$) different compared to the pickled samples. Protein content of (13.8 %) is within range of (12.43 %) reported by Ifon and Bassir (1980). The protein content of pickled garden egg sample was higher than the unpickled sample, because fermentation increases the nutrients, providing nutritious and palatable foods (Okorie and Okaka, 2017). According to Pearson (1976), vegetables that provide more than 12% of its calorie value from protein are good source of protein. Fat content of (1.65 %) reported by Agoreyo *et al* (2012) for S*olanum melongena* specie is within range observed in the pickled samples. Furthermore, it has been documented that most fruits and vegetables are low in crude fat content (Aliyu, 2006). Also, garden egg has no cholesterol and is virtually low in fat. In view of the low-fat content recorded in this study, garden egg can be consumed by a wide range of people including adults, elderly and children and can also serve as a weight restricted diet (Eze and Kanu, 2014). The unpickled sample had the least fiber content and significantly different ($p\leq0.05$) to the pickled samples. Garden eggs are valuable source of dietary fiber. The crude fiber content obtained in this study was within range of (3.90 – 6.22 %) reported by Auta and Ali (2011) for S*olanum incanum*. Also, high fiber content in the garden egg helps in reducing cholesterol level in the human body, protecting the heart in the process (Eze and Kanu, 2014). Unpickled garden egg had the highest ash content of (18.0 %), the pickled sample with sugar had (12.7 %), and was observed to have the least value. This could be as a result of leaching of soluble inorganic salt from the fruit during pickling (Aluge *et al.*, 2016). The ash content obtained in this study was within range (15 %) reported by Eze and Kanu (2014) but contradicts the value (1.96 %) obtained by Agoreyo *et al* (2012). There was significant difference between the samples at ($p\leq0.05$). The overall carbohydrates content ranged from (45.1 to 54.3 %). Garden egg pickled in salt had the lowest carbohydrates content, while the unpickled garden egg had (54.3 %).

Carbohydrate reduction in the pickled samples could be the result of microbial activities/growth involved in pickling (Akinola and Osundahunsi, 2017). Available carbohydrates content was comparable to (51.74 %) reported by Auta and Ali (2011) but contradicts (6.01 %) reported by Aliyu (2006).

Mineral composition of fermented and unfermented garden egg samples

The results of the mineral contents are presented in Table 2. Calcium content ranged from ($105.6^{c} \pm 0.02$ to $207.3^{a} \pm 0.01$ mg/g). The pickled sample had the highest calcium content of (207.263 mg/g). Sodium content ranged from ($315.4^{c} \pm 0.01$ to $346.2^{a} \pm 0.01$ mg/g). Magnesium ranged from ($162.3^{c} \pm 0.02$ to $194.5^{a} \pm 0.01$ mg/g). It was observed that magnesium content of the pickled samples was lower than the raw sample, possibly because magnesium availability is essential for microbial cell growth (Walker, 1994). Copper ranged from ($67.9^{c} \pm 0.01$ to $747.1^{a} \pm 0.01$ mg/g). The calcium content obtained in this study for unpickled garden egg was (105.6 mg/g) which contradicts (15.29 mg/g) obtained by Auta and Ali (2011). Potassium content of (183.0 to 183.7 mg/g) is within the range of *Solanum incanum* (216.89 mg/g) reported by Auta and Ali (2011). Pickled sample with salt (PGESA) had the highest sodium content and significantly different. The sodium content contradicts (149.34 mg/g) reported by Auta and Ali (2011). However, Yoshimura *et al* (1991) has documented that *Solanum macrocarpon* has high level of sodium. Further increase in mineral content could be as a result of the direct addition of vinegar, as it has been documented that vinegar has at least 20 minerals including copper, magnesium, sodium, potassium (Paneque *et al.,* 2016; Fu *et al.,* 2013).

Selected phytochemical and Anti-nutritional content of garden egg

The vitamin C content of (*Solanum aethiopicum* L.) garden egg in this study ranged from (3.3 ± 0.08 to 3.4 ± 0.2 mg/100g) (Table 3). The vitamin C content of the unpickled sample is slightly higher than the pickled, but not statistically different from each other ($p<0.05$). Vitamin C values are within the range of (2.40mg/100g) reported by Offor and Igwe (2015), but lower than (6.27 mg/100g) reported by Auta and Ali (2011). Vitamin C content was found to be reduced in pickled guava fruit (Ramli and Saadon, 2021) as also observed in this study. The tannin content of garden egg ranged from ($1.93^{c} \pm 0.67$ to $2.73^{a} \pm 0.85$ mg/g) (Table 3). The samples were statistically different from each other at ($p<0.05$). The unpickled sample had the highest tannin content of (2.7 mg/g). The tannin content obtained compares favorably with (2.45 mg/g) tannin reported by Auta and Ali (2011). The bitter property of S*olanum macrocarpon* is due to the presence of tannin according to Ekop *et al (*2005). Similar trend was observed with the saponin content of garden egg as the unpickled raw sample had the highest saponin content of ($11.58^{a} \pm 0.1$ mg/g). The saponin content observed is lower than (14.40 mg/g) reported by (Auta, 2008). Numerous processing such as pickling/fermenting and cooking methods have been shown to possibly reduce the amount of these antinutrients and hence their adverse effects (Swain *et al.,* 2014).

Sensory Evaluation

Figure 2 shows the results of the sensory analysis of the samples. Colour ranged from (2.7 to 3.5); aroma ranged from (2.6 to 3.7); taste ranged from (2.3 to 3.7); crunchiness ranged from (2.8 to 3.7) and general acceptability ranged from (2.5 to 3.7). There were significant differences ($p<0.05$) between samples UPRGE, PGESU

and PGESA using Duncan's Multiple Range Test. It was observed in this study that panelists consistently scored unpickled garden egg higher in all the sensory attributes analyzed, probably because it is more familiar to them than pickled garden egg. Between the pickled samples, the garden egg pickled with sugar received the second highest score. Apple vinegar may have negatively impacted these sensory qualities. Furthermore, pickling of fruits and vegetables is not common in this society and consumption of pickled foods may require acquired taste. Further, spices may also be added for improved palatability. Pickled garden egg (*Solanum aethiopicum L.*) in brine with or without sugar is presented in Figure 3.

A Spearman *rho* correlation coefficient was calculated for the relationship between the various treatments and sensory attributes (color, aroma, taste, crunchiness, and overall acceptability) of pickled garden eggs. Correlation between color (*rho* (45) = -.346*, *p* .020); aroma (*rho (*45) = -.495**, *p* .001); taste (*rho* (45) =-.368*, *p* .013); crunchiness (*rho* (45) =-.294*, *p* .050); overall acceptability (*rho* (45) =-.457*, *p* .002) and method of treatment. All the sensory attributes analyzed were observed to have strong and significant relationship with overall acceptability of the garden eggs (Table 4). Correlation between method of treatment and nutritional composition (protein, fibre, carbohydrate and saponin) were also strong and significant (data not included).

CONCLUSION

This research has shown that the shelf life of garden egg (*Solanum aethiopicum* L.) can be extended through pickling. Pickling improves the nutritional composition of garden egg such as protein, fat and some minerals and also reduces the anti-nutritional content of the fruit. Panelist preferred the raw unpickled garden egg probably because they are not familiar with the pickled fruit. However, the pickled garden egg samples with sugar (PGESU) had the highest score than the pickled without sugar sample. For further studies, spices could be added to the pickling process so as to improve the taste and consumer sensitization of pickled garden egg is also recommended.

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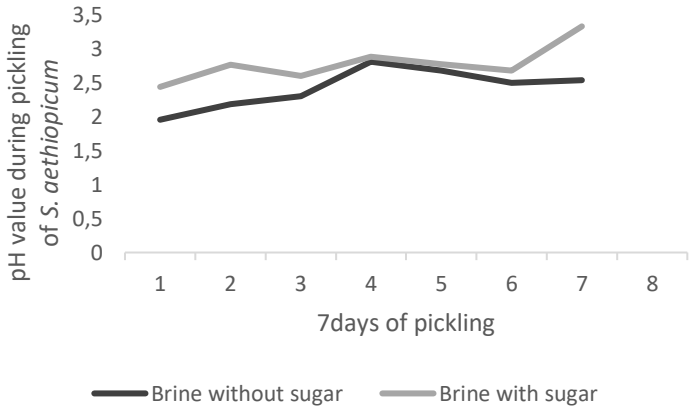

Figure 1. pH values of brine during the 7 days of pickling garden eggs

Figure 2. Sensory attributes of garden egg samples. UPRGE = Un- pickled garden egg; PGESU = Pickled with sugar; and PGESA = Pickled with salt garden eggs

Figure 3. Garden egg (*Solanum aethiopicum* L.) pickled in brine with salt or sugar.

Table 1. Proximate composition of unpickled and pickled garden egg on dry weight basis

	Proximate composition (%) *						
Sample	**Moisture**	**Protein**	**Fibre**	**Fat**	**Ash**	**CHO**	**Energy Kcal**
UPRGE	1.11±0.00[a]	13.8±2.10[b]	8.5±2.3[b]	5.4±2.8[a]	18.0±1.7[a]	54.3±0.7[a]	272.72
PGESU	1.11±0.00[a]	17.3±3.4[ab]	14.8±1.4[a]	2.7±2.4[a]	12.7±1.3[b]	52.6±1.9[a]	252.59
PGESA	1.12±0.01[a]	19.5±1.7[a]	15.8±1.1[a]	2.7±1.3[a]	17.1±3.1[a]	45.1±3.8[b]	231.19

*Values in means of triplicate determination ±SD. Mean with the same superscript in the same column are not significantly different ($p<0.05$). UPRGE = Un-pickled raw Garden egg; PGESU = Pickled Garden egg with sugar; PGESA = Pickled Garden egg with salt.

Table 2. Mineral content (mg/g) of unpickled and pickled garden egg samples

Sample	**Calcium**	**Potassium**	**Sodium**	**Magnesium**	**Copper**
UPRGE	105.6±0.02[c]	ND	315.4± 0.01[c]	194.5± 0.01[a]	67.9± 0.01[c]
PGESU	207.3±0.01[a]	183.0±0.01 [a]	332.2±0.01[b]	186.4±0.01[b]	747.1±0.01[a]
PGESA	153.9±0.01[b]	183.7±0.01 [a]	346.2±0.01[a]	162.3±0.02[c]	511.6±0.01[b]

*Values in means of duplicate determination ±SD. Mean with the same superscript in the same column are not significantly different ($p<0.05$). UPRGE = Not pickled raw Garden egg; PGESU = Pickled Garden egg with sugar; PGESA = Pickled Garden egg with salt; ND=Not determined.

Table 3. Phytochemical Content (mg/g) of garden egg

Sample	**Vitamin C**	**Tannin**	**Saponin**
UPRGE	3.37 ±0.21[a]	2.73 ±0.01[a]	11.58 ± 0.01[a]
PGESU	3.22 ±0.08[a]	1.93 ± 0.01[c]	11.06 ±0.01[b]
PGESA	3.27 ±0.41[a]	2.34 ±0.01[b]	10.75 ± 0.01[c]

*Values in means of triplicate determination ±SD. Mean with the same superscript in the same row are not significantly different ($p<0.05$). UPRGE = Unpickled raw Garden egg; PGESU = Pickled Garden egg with sugar; PGESA = Pickled Garden egg with salt.

Table 4. Spearman Correlation between method of treatment and sensory of pickled garden egg

	Colour	Aroma	Taste	Crunchiness	Overall Acceptability
Treatment	ρ(45)=-.346* p<0.020	ρ(45)=-.495** p<0.001	ρ(45)=-.368* p<0.013	ρ(45)=-.294* p<0.050	ρ(45)=-.457* p<0.002
Colour		ρ(45)=.514** p<0.000	ρ(45)=.570** p<0.000	ρ(45)=.434** p<0.003	ρ(45)=.590** p<0.000
Aroma			ρ(45)=.562** p<0.000	ρ(45)=.582** p<0.000	ρ(45)=.595** p<0.000
Taste				ρ(45)=.635** p<0.000	ρ(45)=.682** p<0.000
Crunchiness					ρ(45)=.695** p<0.000

*Correlation is significant at the 0.05 level (2-tailed); ** Correlation at the 0.01 level (2-tailed)

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Pasting Properties of MOCAF (Modified Cassava Flour) Using Rapid Visco Analyzer with Variations of pH Solution

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ABSTRACT

Starch is a glucose polysaccharide which includes amylose and amylopectin. Natural starch in its utilization has several weaknesses so that modified starch begins to develop, one of which is MOCAF. MOCAF is a modified cassava flour by fermentation using lactic acid bacteria (BAL). The weakness of natural starch is become thicky when the food processing at high temperature and acidic conditions. MOCAF is expected to be able to provide solutions for food processing at high temperatures and acidic conditions. This research was conducted to determine the gelatinization profile of MOCAF during processing at several pH levels. Physical properties of the MOCAF firstly were analyzed including water, starch, amylose, and amylopectin content. After that, the MOCAF gelatinization profile was measured using a Rapid Visco Analyzer (RVA), with observation parameters namely peak viscosity (PV), minimum viscosity (MV), breakdown (BD), final viscosity (FV), setback (SB), pasting temperature (PT), and peak time (Ptime). The results showed that the acidic conditions affected the gelatinization profile of the starch. In the sample which was mixed with citrate buffer solution pH 3 showed the highest peak viscosity (4206.4 cP) and breakdown (3047.6 cP), while the lowest minimum viscosity (1158.6 cP), final viscosity (1604.0 cP), setback (445.2 cP), pasting temperature (74.28°C), and peak time (4.31 minutes). Thus, the low-pH processed MOCAF has the lowest viscosity stability and retrogradability.

Keywords: Acid, modified starch, pasting properties

INTRODUCTION

Starch in everyday life is widely used not only on a household scale but also on an industrial scale. Starch has an important role not only in the food industry but also in non-food industries such as the paper, glue, textile, and so on (Koswara, 2009). In the world of trade, starch is known to be of two types, namely natural starch and modified starch. One example of natural starch that is widely used, especially in the food industry, is cassava starch or commonly known as tapioca. The function of tapioca in the food industry is diverse, namely as a thickening agent, filler, and binder such as in the production process of baby food, ice cream, meat processing, sauces, and others including the pharmaceutical industry (Putri, 2015).

The use of natural starch such as tapioca raises several weakness, namely: when natural starch is cooked natural starch takes a long time so it requires high energy, has sticky characteristics, and is not resistant to acid treatment. This causes starch to have limitations in its application. According to (Koswara, 2009), the industry requires starch to have stable characteristics both to high and low temperature treatments, good resistance to mechanical treatment, and its thickening power to withstand acidic conditions and high temperatures. Therefore, several studies have emerged that have developed modified starch to improve the characteristics of natural starch.

MOCAF (Modified Cassava Flour) is a form of starch product development by modifying cassava cells by fermentation using lactid acid bacteria (BAL). MOCAF has different characteristics from ordinary cassava flour, including: increased viscosity, gelation ability, rehydration power, WHC (water holding capacity), and ease of dissolving. Today, there are various kinds of products that can be produced with MOCAF both as raw materials and as substitute materials such as in noodle products, bakery, cookies, and semi-wet food.

The use of MOCAF is expected not only as a substitute material but also as a filler, one example of which can be applied to sauce products. According to (Koswara, 2009), the sauce-making process is carried out under acidic conditions (pH 3.8 - 4.4) and quite high temperatures (80-100°C). These conditions will affect the characteristics of gelatinization (gelatinization profile) which can be known from the amylograph curve. The content of amylose and amylopectin has an important role in determining the gelatinization profile of starch. Amylose and amylopectin levels in sorghum flour affect the peak viscosity and gelatinization temperature of the starch (Budijanto and Yuliyanti, 2012).

Gelatinization of starch is a condition where the granules experience swelling and at a certain point are unable to return to their original condition. The gelatinization temperature is the temperature at which the starch granules break. According to BeMiller (2007), the gelatinization temperature of starch is influenced by the type of starch, the method of measurement, the ratio between starch and water, pH, the presence or absence of swelling by salt, the concentration of salt, and the concentration of solutes such as sucrose. The characteristics of starch gelatinization are divided into various types, tapioca has the characteristics of type A gelatinization, which has a high peak viscosity value and is followed by rapid dilution during the heating process (Syamsir et al., 2012). This is because the hydrogen bonds linking amylose and amylopectin in natural starch are prone to breaking during the starch gelatinization process.

Thus, tapioca is considered to have poor resistance when used in high temperature processing and under acidic conditions. Therefore, this study was conducted conducted to determine the gelatinization profile of MOCAF during processing at several pH levels.

MATERIALS AND METHODS

Tools and Materials

The tools used in this research are analytical equipment such as Techmaster's Rapid Visco Analyzer (RVA), Horiba's pH meter, Precisa analytical balance, Troac brand of thermometer, pyrex brand of glassware, pipettes and pyrex stirrer. The material used in this research is MOCAF obtained from Mr. Te Jember, citrate buffer solution, and aquades.

Methods

The first MOCAF used was measured for its chemical content including water content, starch content, amylose content and

amylopectin content. This research was conducted to observe the gelatinization profile of MOCAF starch using a Rapid Visco Analyzer using acid solvents with different acidity levels. The solvent used was citrate buffer solution with the following variations in pH: 3, 4, 5, 6, and 7. The measurement of gelatinization profile in each sample was carried out three times. This research consists of several stages of activities, namely: preparation of tools and materials; analysis of moisture, starch, amylose and amylopectin content; preparation of citrate buffer solution; starch gelatinization profile measurement; and data analysis.

Preparation of Citrate Buffer Solution

The citrate buffer solution was prepared using 0.1 M citric acid solution and 0.1 M Na-citrate solution as shown in Figure 1. The first step was that x mL of citric acid solution was added with y mL of Na-citrate. After that, the dilution was carried out to a predetermined pH of 100 mL. Then, the pH of each solution was measured (Sudarmadji dan Haryono, 1997).

Chemical Content Analysis

The sample to be used was measured water content using the gravimetric method, starch content using the Nelson-Somogyi method, and analysis of amylose content using the spectrophotometric method.

Gelatinization Profile Measurement

Gelatinization profile measurements were carried out using a Rapid Visco Analyzer (RVA) using the AACC 61-02.01 method. 3 grams of MOCAF dissolved in 25 mL of solvent (aquades or buffer solution), 12% water content (Imanningsih, 2012), and a stirring speed of 160 rpm (An, 2005) which is shown in Figure 2. The solvent used is divided into two, namely the control solvent and the citrate buffer solution as follows:

K: control solvent (aquades)
A: citrate buffer solution pH 7
B: citrate buffer solution pH 6
C: citrate buffer solution pH 5
D: citrate buffer solution pH 4
E: citrate buffer solution pH 3

The RVA temperature measurement was in the range of 50-95°C for 13 minutes with a gradual increase in temperature as shown in Figure 3. The method used to measure the gelatinization profile of MOCAF on RVA is by using the flour method. Each method has different temperature, time, and rotational speed settings depending on the type of sample to be tested. Details of the settings used to measure the gelatinization profile of MOCAF using the flour method are shown in Figure 4.

The RVA heating and cooling viscometer provides information on the pasting properties, including: peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), breakdown (BD), setback (SB), pasting temperature (PT), and peak time (Ptime) (Shafie et al., 2016).

Data Analysis

The data obtained will be analyzed using a descriptive method by calculating the average data and standard deviation. Presentation of data in the form of tables and figures to facilitate the discussion of the data.

RESULTS AND DISCUSSION

Chemical Content of MOCAF

The chemical content of MOCAF including moisture content, starch content, as well as amylose and amylopectin content are shown in Table 1. Information on this chemical content is needed to determine its effect on the gelatinization profile of the tested MOCAF.

In Table 1 it is known that the water content of MOCAF is 12.15% which is still in accordance with Indonesian National Standar (SNI No. 7622 of 2011) concerning

MOCAF with a maximum limit of 13% water content. MOCAF starch content of 83.63% lower than tapioca starch content of 85.92% (Putri, 2015). Tapioca starch content was higher than MOCAF because tapioca starch content almost covered all dry matter, while MOCAF components other than starch were still in significant amounts. Starches are composed mainly of two polysaccharides, amylose and amylopectin, that organized in complex structure called granules (Castanha et al., 2021).

The amylose and amylopectin levels on the tested MOCAF were 16.22% and 83.78%, respectively. Amylose and amylopectin levels in flour will affect the process of starch gelatinization. Measurement of amylose content was carried out using a spectrophotometric method with the principle of staining using iodine. Amylose is a polymer part with -(1,4) bonds of glucose units in each chain there are 500-2000 D-glucose bonds, forming straight chains which are generally said to be linear from starch. The characteristic of amylose in solution is that it has a tendency to form very long and flexible coils that always move in circles. The structure that underlies the interaction of iodamylose forms a blue color (An, 2005).

Amylopectin is a branched-chain polymer with α-(1,4)-glycosidic bonds and α-(1,6)-glycosidic bonds at the branching sites. Each branch consists of 25-30 D-glucose units (An, 2005). According to (Koswara, 2009), amylopectin usually contains 1000 or more units of glucose molecules for each chain. The molecular weight of glucose amylopectin for each chain varies depending on the source. Amylopectin in tubers contains a small amount of phosphate ester bonded to the 6th carbon atom of the glucose ring (Putri, 2015). The structure of starch including amylose and amylopectin is shown in Figure 5.

Gelatinization Profile of MOCAF

Gelatinization process occurs when propitious conditions are present, as sufficient amount of water and temperature higher than the gelatinization temperature of the given starch. In those conditions, the intermolecular bonds that maintain the crystalline structure of the granules are weakened, and the water in the vicinity can penetrate the formerly rigid granular structure (Castanha et al., 2021). At this conditions, the starch granule swells and loses its birefringence (Xie et al., 2006). MOCAF gelatinization profile measured using RVA with various solvents under different acidity conditions is shown in Table 2 which includes: peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), breakdown (BD), setback (SB), pasting temperature (PT), and peak time (Ptime). According to (Imanningsih, 2012), in measurements using RVA there are four phases that can be identified on the resulting amylographic curve.

In the first phase, the temperature is lower than the gelatinization temperature of starch so that the measured viscosity value is low. In the second phase, there was an increase in viscosity along with increasing temperature until it reached the gelatinization temperature of starch. The increase in viscosity indicates that the starch granules are swelling. The peak viscosity of the product is called the peak viscosity (PV). The third phase, occurs when there is an increase in temperature and stirring, the starch granules will break and amylose will come out of the granules which results in a decrease in viscosity. The lowest viscosity of this phase is known as minimum viscosity (MV). The last phase, is a decrease in temperature causing a re-association between starch molecules (setback) so that the gel is formed again and the viscosity increases again so that it reaches the final viscosity which is usually called final viscosity (FV).

Measurement of the gelatinization profile using RVA will produce an amylographic curve that shows several parameters of viscosity values in the sample during the heating and stirring process as shown in Figure 6. In addition, the amylographic curve generated from measurements using RVA has unique characteristics. In the RVA amylography curve, the x-axis represents time (minutes) and the y-axis indicates viscosity (cP). In principle, during the measurement the liquid is heated and stirred. Viscosity value is measured from the resistance of the liquid to the rotating blades (Putri, 2015).

In Table 2 it can be seen that pH can affect the value of starch viscosity during the heating and cooling process. The lower the pH, the higher the peak viscosity, and the lower the minimum viscosity, breakdown, final viscosity, setback, peak time, and pasting temperature. This is due to acid hydrolysis which can break the glycosidic bonds of starch.

The pasting curve of the starh solutions mixture at pH 7 and 3 show that starch solutions had a lower peak viscosity, final viscosity, setback viscosity, pasting time, peak time, and a higher breakdown viscosity in acidic conditions than in a neutral environment (Zhang et al., 2017). Given that acid hydrolysis destroys glycocidic binds in molecule (Choi et al., 2016), the complete pasting of starch molecules in acidic conditions requires less energy anf therefore it is gelatinized faster and at lower temperatures (Zhang et al., 2017).

MOCAF amylograph curves resulting from measurements using RVA with two types of solvents, namely distilled water and buffer solutions with different pH are shown in Figure 7.

Peak Viscosity (PV)

Peak viscosity was the highest viscosity achieved during heating at 95°C. The variation of peak viscosity is often associated with the swelling power of starch and the rate disruption of the starch granule (Shafie et al., 2016). MOCAF samples mixed with citrate buffer solution pH 3 had the highest peak viscosity value, and MOCAF samples mixed with citrate buffer solution pH 7 had the lowest peak viscosity values. The results of previous studies showed that the peak viscosity of arrowroot starch modified with 10% propylene oxide was higher at pH 3.5 than at pH 6.5 (Rahaju Maulani et al., 2013). In addition, amylopectin levels are responsible for the granule development process (Putri, 2015). MOCAF has a lower amylose content than cassava flour, this is because during the fermentation process it opens the amylopectin structure of starch resulting in an increase in amylose content which plays a role in the formation of a stable gel (Loebis and Meutia, 2012).

Minimum Viscosity (MV)

Minimum viscosity or hold viscosity was the lowest viscosity achieved during heating at 95°C. MOCAF samples mixed with citrate buffer solution pH 3 had the highest minimum viscosity value. It was reflecting different susceptibility of starch breakdown upon shearing and heating (Shafie et al., 2016). Starch granule became susceptible to shear disintegration when swelles, especially in starches with lower amylose content (Kaur et al., 2007).

Final Viscosity (FV)

Final viscosity measured the ability of the starch to form viscous paste after cooking and cooling. Final viscosity was the paste viscosity upon cooling at 50°C. At this stage, the starch granules experienced restructuring of starch molecules and retrograded (Shafie et al., 2016). The highest final viscosity value is in the sample MOCAF pH 7. This is because if the starch suspension at a certain

pH and heated at a certain temperature will cause the starch to be hydrolyzed into dextrin so that starch with low viscosity is produced. (Koswara, 2009).

Breakdown (BD)

Breakdown shows viscosity stability against the heating process (Faridah D.N, Fardiaz D, Andarwulan N, 2014). The highest breakdown value is the pH 3 MOCAF sample, it shows that under acidic conditions the viscosity stability is not good when compared to the pH 7 MOCAF sample which has the lowest breakdown value. This shows that the viscosity of starch is stable at neutral pH compared to acidic conditions. Breakdown viscosity defined as the difference between peak viscosity and minimum viscosity, while stability ratio was the ratio of viscosity at the onset of cooling to peak viscosity before cooling (Shafie et al., 2016). The stability explained the hydration, starch swelling power and shear resistance of starch paste during heating.

Setback (SB)

The highest setback value was in the MOCAF pH 3 sample, while the highest setback was in the control MOCAF sample. Setback viscosity indicated starch retrogradation tendency after gelatinization and cooling at 50°C. The viscosity changes while cooling were mainly due to amylose molecular re-association, and low setback viscosity indicated a low rate of starch retrogradation (Shafie et al., 2016). The lowest setback viscosity could be a good thickener and stabilizer for food processing industries (Corke, 1997).

Peak Time (Ptime) and Pasting Temperature (PT)

Peak time is the time required to reach peak viscosity, while pasting temperature is the temperature required for the sample to undergo the gelatinization process (Putri, 2015). The value of the peak time and pasting temperature of the sample decreased with the lowering of the pH. This shows that under acidic conditions, starch will be gelatinized faster and requires lower energy.

CONCLUSION

From the results of the recent study, it can be concluded that the gelatinization profile of MOCAF is influenced by acid treatment. From the measurement results using RVA, an increase in the value of peak viscosity and breakdown, as well as a decrease in the value of minimum viscosity, final viscosity, setback, time to peak and pasting temperature occur along with the lower pH (acid conditions).

The peak viscosity value indicates the ability of starch granules to expand, the breakdown viscosity indicates the viscosity stability during the heating process, and the setback viscosity indicates the level of retrogradation. Thus, the MOCAF processed at acidic conditions has the lowest viscosity stability and retrogradability.

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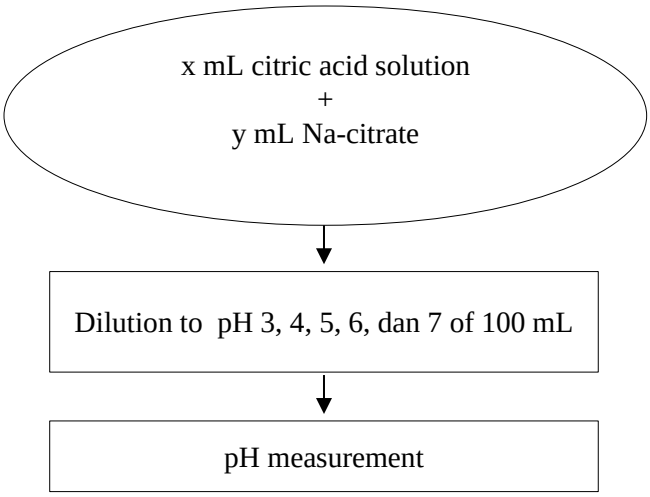

Figure 1. Preparation of citrate buffer solution

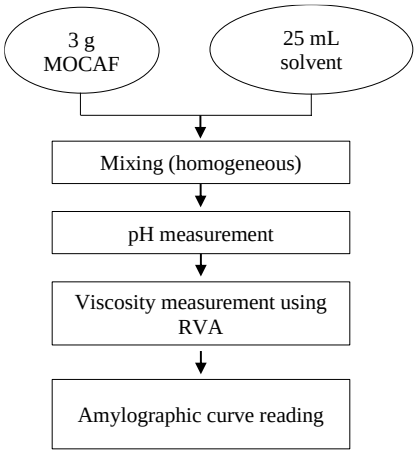

Figure 2. RVA running process stage

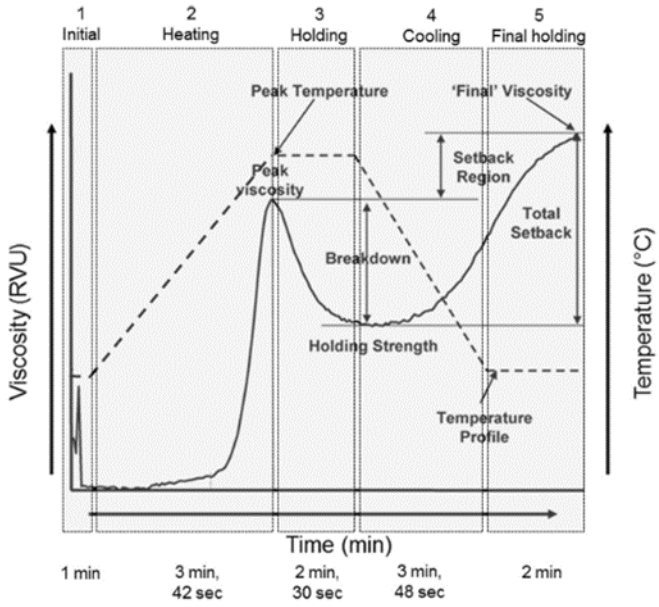

Figure 3. RVA temperature setting (Balet et al., 2019)

Time (hh:mm:ss)	Function Type	Value
00:00:00	Temp	50
00:00:00	Speed	960
00:00:10	Speed	160
00:01:00	Temp	50
00:04:42	Temp	95
00:07:12	Temp	95
00:11:00	Temp	50
00:13:00	End	

Figure 4. Flour methode on RVA setting

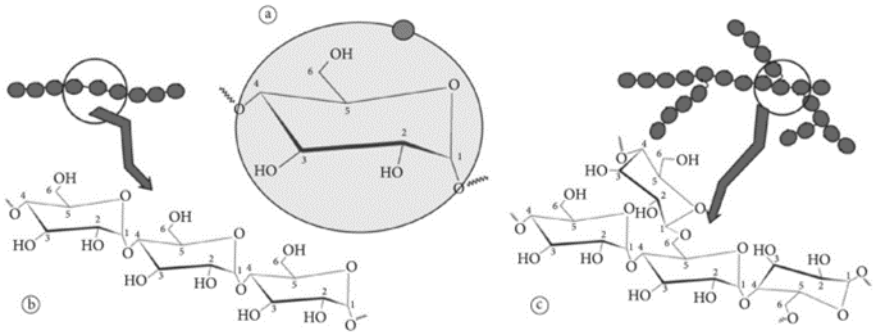

Figure 5. Starch chain structure (a), amylose (b), amylopectin (c) (Alcázar-Alay and Meireles, 2015)

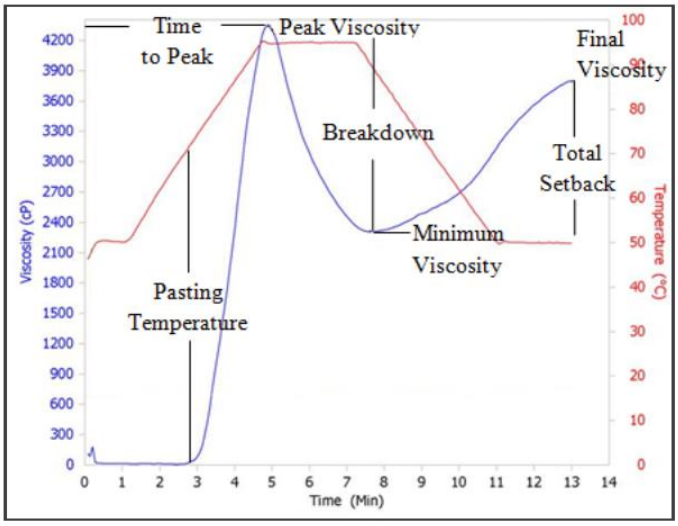

Figure 6. Amylographic curve of measurement results using RVA (Manaois, 2009)

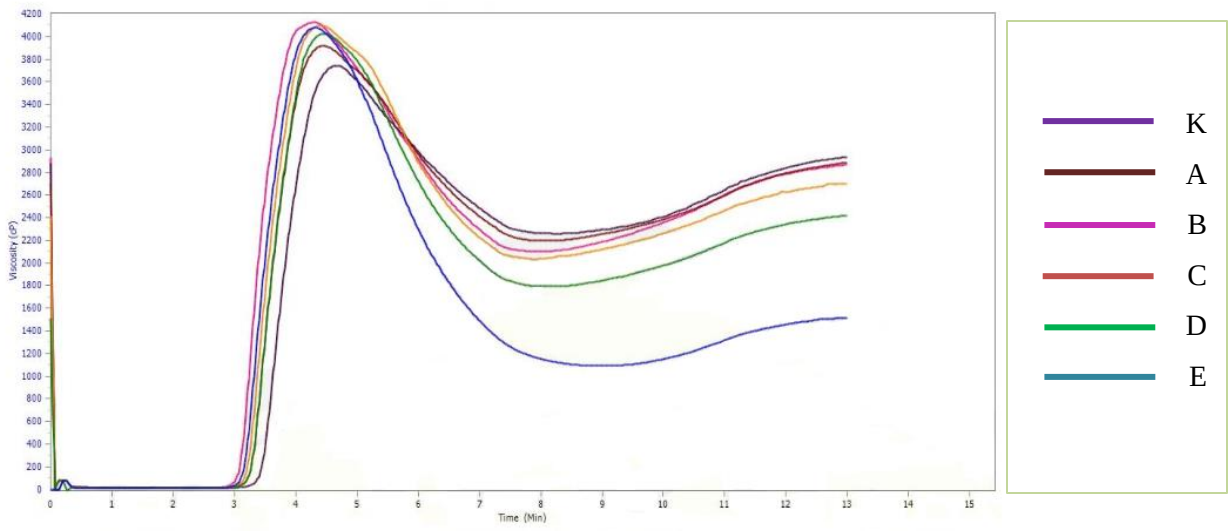

Figure 7. MOCAF amylograph curve with distilled water (K) and citrate buffer solution pH 7 (A), pH 6 (B), pH 5 (C), pH 4 (D), pH 3 (E)

Table 1. Chemical content of MOCAF

Components	Total (%)
Water	12,15 ± 0,07
Starch	83,63 ± 3,60
Amylose	16,22 ± 1,29
Amylopectin	83,78 ± 1,29

Table 2. MOCAF gelatinization profile with different types of solvents and acidity conditions

Sample	PV (cP)	MV (cP)	BD (cP)	FV (cP)	SB (cP)	Ptime (minute)	PT (°C)
K	3983.4 ± 129.16	1959.0 ± 84.90	2024.4 ± 76.75	2644.6 ± 125.62	685.6 ± 44.85	4.28 ± 0.03	73.65 ± 0.34
A	3741.0 ± 90.63	2248.6 ± 11.72	1492.4 ± 83.36	2924.0 ± 16.11	675.4 ± 12.66	4.60 ± 0.07	77.32 ± 0.38
B	3861,6 ± 33.95	2148.8 ± 9.65	1674.4 ± 27.84	2878.6 ± 7.83	674.6 ± 19.55	4.45 ± 0.04	75.65 ± 0.38
C	4106.8 ± 74.31	2038.4 ± 33.81	2068.4 ± 64.98	2706.4 ± 26.48	671.5 ± 9.14	4.40 ± 0.00	74.86 ± 0.34
D	4138.4 ± 95.66	1816.8 ± 22.47	2270.0 ± 59.46	2235.2 ± 13.41	631.8 ± 8.29	4.39 ± 0.11	74.74 ± 0.79
E	4206.4 ± 133.97	1158.6 ± 45.77	3047.6 ± 95.62	1604.0 ± 54.69	445.2 ± 11.17	4.31 ± 0.33	74.28 ± 0.06

Note: the value in the table is the average value of three measurements

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Waste Skin of Hawaiian Ladyfish (*Elops hawaiensis*) Utilization as Gelatin Raw Material with Immersion Solution Combination

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ABSTRACT

Bontot from Domas Village, Pontang District, Serang Regency is a fish jelly product. The manufacture of Bontot produces fish skin waste with a percentage of 10% of the whole weight of the fish. This waste can be reduced by applying the concept of zero waste in the processing of bontot which is reused as a raw material in the manufacture of gelatin. This research aims to determine the best NaOH concentration in the manufacture of gelatin from the skin of Hawaiian ladyfish (*Elops hawaiensis*) and to determine the effect of NaOH immersion on the organoleptic, chemical, and physical qualities of the gelatin. This study used an alkaline solution (NaOH) with a concentration of 0.1%; 0.3%; and 0.5%, which is then followed by a 6% acid solution immersion process. The production of gelatin from the skin of Hawaiian ladyfish was carried out using a one-factor completely randomized design (CRD) with 2 replications (duplo) accompanied by non-parametric analysis which was carried out for organoleptic testing with a hedonic scale using the Kruskal Wallis test. The results showed that the best combination was 0.1% NaOH and 6% HCl with a hedonic value of 3.7 with a whitish-yellow color; odor hedonic value 3.13; 11% yield; gel strength 280.43 g bloom; viscosity 36.95 cP; water content 8.75%; ash content 0.58%; and a pH value of 6.88.

Keywords: Hawaiian ladyfish, Fish skin, gelatin, HCl, NaOH

INTRODUCTION

Bontot is one of the mainstay home industry fish gel products from Domas Village, Pontang District, Serang Regency. The processing of bontot in the village of Domas can produce fish skin waste of 10% of the total weight of Hawaiian ladyfish (*Elops hawaiensis*) which is estimated to be 84.5 kg/week. The amount and intensity of waste that appears can be reduced by applying the concept of zero waste (Haryati and Munandar 2010). The best solution that can be given to support zero waste activities in utilizing Hawaiian ladyfish skin waste is to process into gelatin raw material.

Gelatin is a protein derived from collagen which has broad functions in the food and pharmaceutical industries,

including as a filler, emulsifier, binder, precipitant, and nutrient enrichment. Gelatin is flexible with a thin layer that is elastic, transparent, strong, and has high digestibility (Hastuti and Sumpe 2007). Most of the gelatin needs in Indonesia is supplied from foreign producers, including China, Thailand, Australia, Brazil, Bangladesh, and New Zealand (Nurilmala *et al.* 2017). Around 98.5% of gelatin traded globally comes from pork and beef (Karim *et al.* 2008). The gelatin raw material is known to contain non-halal ingredients, thus causing concern for Muslims to consume it. Raw materials from cattle will cause problems for people who follow the Hindu religion (Agustin 2013). In addition, the consumption of gelatin from cows in recent studies has resulted in reports of being able to transmit *Bovine Spongiform Encephalopathy* (BSE) or mad cow disease (Huang *et al.* 2019). Furthermore, Azziza *et al.* (2019) stated that the use of fish skin waste as a safe and halal raw material for gelatin is an alternative to increasing value-added in fishery industry waste while reducing pollution and reducing the dependence of industry in Indonesia on imported gelatin.

The manufacture of gelatin generally comes from the skin or bones that are rich in collagen soaked in acid or base. This research emphasizes the manufacture of gelatin using a combination solvent, starting with the immersion in alkaline solution (NaOH) and followed by immersion in acid solution (HCl). Wijaya *et al.* (2015) stated in their research that the immersion of NaOH solution before acid soaking was able to maximize the degreasing process, namely the process of eroding fat in the manufacture of tilapia gelatin. Puspawati *et al.* (2014) added that immersion with alkaline will trigger a deproteinization process that can dissolve non-collagenous proteins and remove color. For this reason, this research needs to be done by utilizing the waste skin of Hawaiian ladyfish as raw material for gelatin with a combination of soaking solutions.

The purpose of this study was to determine the best concentration of NaOH in the manufacture of gelatin from waste skin of Hawaiian ladyfish (and the effect of soaking NaOH on the organoleptic, chemical, and physical quality of gelatin from a waste of Hawaiian ladyfish skin.

MATERIALS AND METHODS

Methods

This study used the treatment of immersion in NaOH solution with a concentration of 0.1%; 0.3%; and 0.5% with a ratio of soaking the fish skin: solution = 1:3 for 18 hours, before being immersed again in an acid solution (6% HCl). Followed by the extraction process with a comparison (skin: water = 1:2) temperature 80˚C ± 3 for 2 hours, then filtered with calico cloth and dried using an oven ± 50˚C for 3 days. The best gelatin in this study was determined based on color, odor, yield, gel strength, viscosity, moisture content, ash content, and pH value.

Organoleptic test (Setyaningsih *et al.* 2010)

The hedonic scale is used for organoleptic tests with a scale of 1 (very dislike) to 5 (very much like). This test was conducted using a panel of 30 people. The parameters tested were odor and color. The test was carried out by giving a random code to the sample and assessed by the panelists using a score sheet.

Yield (Marzuki *et al.* 2011)

The yield is obtained from the dry weight of the gelatin flour produced by the weight of the fish skin used which is then entered into the formula

$$yield\ (\%) = \frac{dry\ weight\ of\ gelatin}{fish\ skin\ weight} \text{ x } 100\%$$

Gel strength (Tazwir *et al.* 2007)

The sample was weighed as much as 7.50 grams dissolved in 105 ml of water at a

temperature of 60°C and stirred using a magnetic stirrer for 15 minutes until homogeneous. Then the sample was allowed to stand at room temperature for 15 minutes and cooled at 10±0.1°C for 17 hours. The sample was then measured the strength of the gel using a texture analyzer and expressed in g bloom.

Viscosity (Tazwir *et al.* 2007)

The sample was weighed 6.67 g, then dissolved in distilled water to a volume of 100 ml. The sample was heated at a temperature of 60◦C and measured using a viscometer.

Water Content Analysis (AOAC 2005)

The water content is obtained through calculations using the following formula:

$$\text{Water Content (\%)} = \frac{B - C}{B - A} \times 100\%$$

Information:

A = weight of empty cups (g); B = weight of the cup filled with the sample (g); C = weight of the cup with dried sample (g).

Ash Content Analysis (AOAC 2005)

Determination of ash content was obtained by the dry ashing method. The principle of this analysis is to oxidize all organic substances at a high temperature (about 550°C), then proceed with the process of weighing the remaining substances (ash) after the combustion process.

$$\text{Ash Content (\%)} = \frac{B - A}{\textit{The initial weight of sample}} \times 100\%$$

Information:

A = weight of the cup (g); B = weight of cup with ash (g).

pH (Tazwir *et al*. 2007)

Determination of the pH value of the sample can be done using a pH meter. The sample was weighed 1 g then dissolved in 100 ml of distilled water at 80°C, homogenized, and analyzed using room temperature.

Data Analysis

The production of gelatin from the skin of Hawaiian ladyfish was carried out using a one-factor completely randomized design (CRD) with 2 replications (duplo). Data were analyzed by analysis of variance/univariate analysis (ANOVA). Non-parametric analysis was conducted for organoleptic testing with a hedonic scale using the Kruskal Wallis test. If the results of the analysis show a significant difference effect, the smallest significant difference test (LSD test) is carried out.

RESULTS AND DISCUSSION

Organoleptic Test

Color Value

The color hedonic value obtained in this study ranged from 2.77 to 3.7 with the appearance of the color obtained from cream to brown. These results indicate an increase in the concentration of NaOH used will significantly reduce the value of the hedonic color of gelatin. The color of the gelatin produced from the 0.1% NaOH concentration meets the BSN (1995) standard which states that the color of the gelatin is colored as expected. NaOH concentrations of 0.3% and 0.5% resulted in a brownish gelatinous cream color that did not meet these standards.

The brownish cream color is produced from the combination of yellow and black brown. Black brown color will appear with the higher concentration of NaOH. This is because the high concentration of NaOH solution will continue to hydrolyze until the time of extraction so that the skin is denatured and mixed in the gelatin solution. Ayudiarti *et al.* (2007) stated that the color of fish gelatin will be darker than commercial gelatin because it is influenced by pigments from fish skin that cannot be completely removed.

Based on the analysis of Kruskal Wallis, the immersion of different concentrations of NaOH solution given as

treatment was significantly different (P<0.05) (Figure 1). This shows that the concentration of NaOH treatment has a significant effect on the hedonic value of the gelatin color produced. The color of the gelatin is usually determined by the raw materials and the process used in the manufacture of gelatin. The whiter the gelatin color, the better and preferable it is because it will be easier to combine gelatin with other food ingredients without adding strong coloring agents to food products (Moranda *et al.* 2018; Shyni *et al*. 2014).

Odor Value

The results of organoleptic quality as indicated by the hedonic value, the smell of gelatin obtained in the study ranged from 3.03 to 3.13. Based on Kruskal Wallis analysis, each treatment was not significantly different (P>0.05). On average, the panelists assessed that the fish smell was still in the Hawaiian ladyfish gelatin in each treatment with the concentration of the given NaOH soaking solution. These results do not meet the standards of BSN (1995) which states that the smell of gelatin is normal. Choi and Regenstein (2000) stated that fish gelatin is rarely used and is not mass produced because of its dark size and fishy odor. Rahman and Jamalulail (2012) reported that fish gelatin does have a very strong fish odor compared to gelatin made from other raw materials such as gelatin made from chicken feet.

Yield

Yield is the percentage by weight of gelatin produced to the weight of the waste Hawaiian ladyfish skin used. The yield of gelatin from Hawaiian ladyfish skin waste is shown in Figure 3. Based on the analysis of variance, each treatment of immersion in NaOH solution with different concentrations was significantly different (P<0.05). The yield parameter value for each treatment is 11; 5.34; and 3.67%. The decrease in yield value occurred along with the increase in the concentration of NaOH given. Tazwir *et al* (2009) explained that the decrease in yield value was thought to be due to the gelatin washing process after immersion with NaOH was not optimal, so there was still NaOH solution left and the sample was hydrolyzed at the beginning. In addition, the reason that may occur again is the acid-base reaction between NaOH and HCl in the acid soaking process. This reaction can block the process of hydrolysis of collagen and the process of formation of ossein becomes less than perfect. Pangke *et al.* (2016) the extraction of tuna skin gelatin with a concentration of 0.6% NaOH solution got a yield of 4.14% while at a concentration of 0.3% the highest yield was 5.96%.

Gel Strength

The ability to form a gel (gel strength) is one of the physical properties that determine the quality of gelatin. Based on the analysis of variance, each treatment was significantly different (P<0.05). The gel strength obtained in this study ranged from 280.43 – 295.06 g bloom, with the highest gel strength at 0.5% NaOH concentration (Figure 4). Hawaiian ladyfish skin gelatin gel strength corresponds to type A gelatin and type B gelatin in the range of 50-300 bloom (GMIA 2012).

The gel strength in this study was included in the good category, where Rahmawati and Hasdar (2017) reported that a gel strength value below 50 bloom will make gelatin difficult to form a gel, while a gel strength value of more than 300 bloom will make gelatin difficult to form, so that it becomes stiffer and harder. Gelatinization (gelatinization) occurs due to immersion with alkaline solutions in the gelatin manufacturing process which can combine amino acid monomer chains and form a three-dimensional structure that will bind air to form a compact gel structure (Said *et al*.

2011). Gudmundsson and Hafsteinsson (1997) stated that gel strength can depend on the isoelectric point and can be controlled to some extent by adjusting the pH. Wijaya *et al.* (2015) reported in their research, that the highest average gel strength was obtained in the 0.6% NaOH immersion treatment, which was 86.47 blooms.

Viscosity

The viscosity obtained in this study ranged from 27.15 to 37.85 cP. Based on the analysis of variance, each treatment was significantly different ($P<0.05$). This shows that the concentration of the NaOH soaking solution affects the viscosity of the gelatin produced. The concentration of 0.5% NaOH solution resulted in a significantly different viscosity from the concentrations of 0.1% and 0.3%. However, this Hawaiian ladyfish skin gelatin viscosity value is not in accordance with the value of type A gelatin with a value of 1.50-7.50 cP and type B gelatin with a viscosity value of 2.00-7.50 cP in GMIA (2012).

Viscosity values that fall into the high category can be caused by the large molecular weight of the Hawaiian ladyfish skin gelatin. Tazwir *et al. (*2009) explained that the immersion treatment with NaOH solution was able to close the space around the polymer protein left by fats and other minerals, so as not to break the existing amino acid chain, this caused the gelatin obtained to have a larger molecular weight, this is shown by high viscosity value. Siburian *et al.* (2020) explained that the viscosity of gelatin is related to the average molecular weight and molecular distribution. The molecular weight of gelatin is directly related to the length of the amino acid chain. That is, the longer the amino acid chain, the higher the viscosity. Astawan and Avian (2003) reported that the alkaline immersion treatment resulted in greater quality than the acid treatment. This is because the soaking process with alkaline produces gelatin with long peptide chains so that the molecular weight becomes larger and the excessive viscosity becomes larger.

Water Content

Parameters of water content in gelatin will relate to shelf life, rancidity, and color of the gelatin produced. The water content obtained in this study ranged from 8.75 to 9.23%. The water content is related to the process of soaking with an acid solution after the alkaline solution is carried out. Ulfah (2011) reported that the acid solution given during immersion will diffuse into the raw material network so that the collagen structure contained will be more open and weaker, as well as produce a gelatin structure with weak bonds and make the water-binding capacity of gelatin less strong. The weak water holding capacity of gelatin will make water evaporate easily during the drying process.

Based on the analysis of variance, each treatment was not significantly different ($P>0.05$). The water content of gelatin will affect shelf life because it is closely related to metabolic activities that occur during the storage of gelatin such as enzyme, microbial, and chemical activity, namely the occurrence of rancidity and non-enzymatic reactions, causing changes in organoleptic properties and quality values (*Rachmania et al.* 2013).

Ash Content

The ash content obtained in this study ranged from 0.53 to 0.75% with the highest value at a concentration of 0.5% NaOH immersion solution. Based on the analysis of variance, each treatment was not significantly different ($P>0.05$). However, the content of ash content in the gelatin of Hawaiian ladyfish skin is still in accordance with the criteria of the GMIA (2012), type A gelatin ranging from 0.3-2.0% and type B gelatin ranging from 0.5-2.0%. The range of

ash content of the Hawaiian ladyfish skin gelatin also still meets the quality standard of BSN (1995) with a maximum value of 3.25%.

The content of ash content in the gelatin of Hawaiian ladyfish skin is due to the treatment of immersion in NaOH solution will release Na^+ ions and soaking with HCl acid solution releases Cl^- ions. Na^+ and Cl^- ions trapped in the skin will bind to form NaCl salts which will affect the ash content of gelatin. Oktaviani *et al.* (2012) stated that the increase in ash content was caused by the presence of salt formed from Na^+ and Cl^- ions in a food product. The size of the ash content value is influenced by the washing and filtering process. Mineral components that have not been released during the washing and filtering process will be carried over to the ashing process (Febryana *et al.* 2018)

pH Values

The pH values obtained in this study ranged from 6.88 to 7.10. Based on the analysis of variance, each treatment was significantly different ($P<0.05$). Treatment of 0.1% NaOH solution concentration resulted in a significantly different pH value with 0.3% and 0.5% NaOH concentration solutions. The pH value of the Hawaiian ladyfish skin gelatin proves that there is a combined effect of NaOH and HCl when soaking which causes the pH of the Hawaiian ladyfish skin gelatin to be neutral. The pH range of fish skin gelatin is still in accordance with the GMIA (2012) standard for type B gelatin with a pH range of 5.00-7.50. Sompi *et al*. (2015) reported that the pH value of gelatin is caused by the small amount of acid that binds to skin proteins. The range of pH values indicates the process of penetration and washing of the skin before the extraction process runs perfectly so that contamination can be minimized. Gunawan *et al.* (2017) explained that a good washing process will cause less acid to be trapped in the skin so that the pH value will approach neutral.

CONCLUSION

The treatment of soaking fish skin with NaOH base soaking solution before soaking it with HCl acid soaking solution resulted in values that had a significant effect on color, yield, gel strength, viscosity, and pH. The best treatment combination produced was 0.1% NaOH and 6% HCl with a hedonic value of 3.7 with a whitish-yellow color; 11% yield; gel strength 280.43 g bloom; viscosity 36.95 cP; and a pH value of 6.88.

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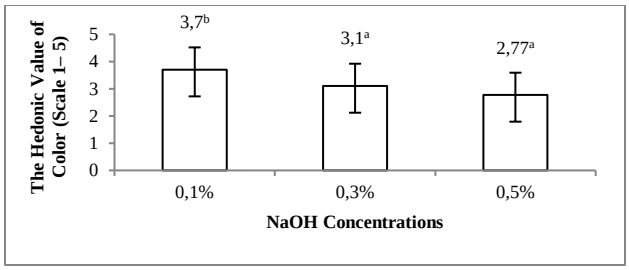

Figure 1. Histogram of the hedonic value of gelatin color with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

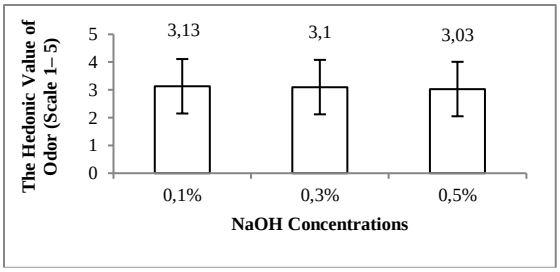

Figure 2. Histogram of hedonic odor of gelatin with different concentrations of NaOH solutions

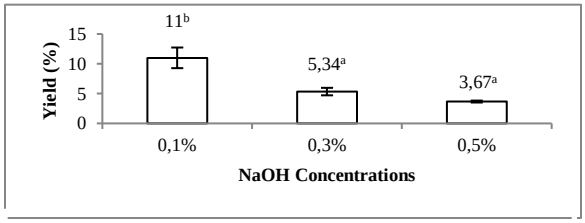

Figure 3. Histogram of gelatin yield with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

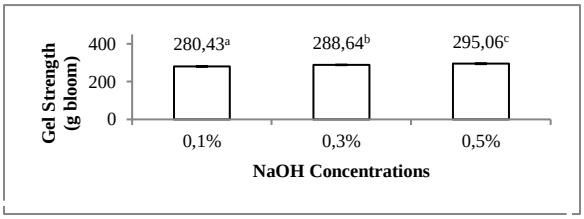

Figure 4. Histogram of gelatin gel strength with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

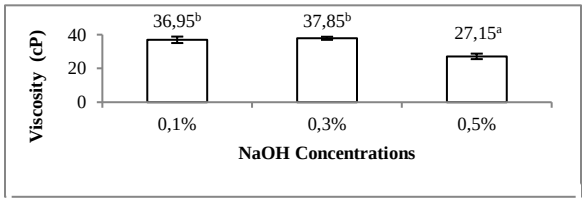

Figure 5. Histogram of gelatin viscosity with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$)

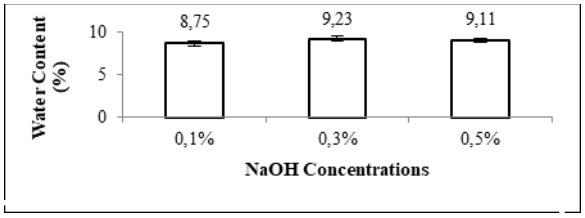

Figure 6. Histogram of water content of gelatin with different concentrations of NaOH solutions

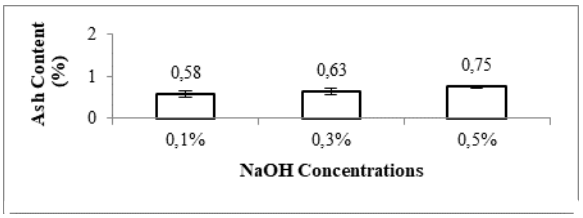

Figure 7. Histogram of gelatin ash content with different concentrations of NaOH solutions

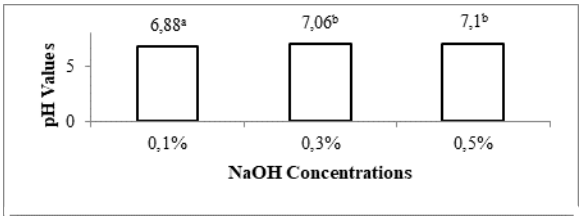

Figure 7. Histogram of gelatin pH values with different concentrations of NaOH solutions. Different superscript letters showed significantly different results ($P < 0.05$).

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Physical-Mechanical Properties of Edible Film Based on Beneng Taro (*Xanthosoma Undipes* K.Koch) Starch with Plasticizer

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ABSTRACT

Edible film is a thin layer as a coating for foodstuffs made from edible materials such as taro. One of the taro tubers that can be used as edible film is Beneng from Banten. Edible films made from starch have a weakness that is easy to tear and have low elasticity, to change the physical properties of the film from starch it is necessary to add a plasticizer. Plasticizers that are often added to the production of edible films are xylitol, sorbitol, polyethylene glycol, and glycerol. The purpose of this study was to determine the effect of the type of treatment and concentration of plasticizer on the physical-mechanical properties of edible film made from taro beneng starch. The treatment in this study was divided into 2 factors, the first factor was the type of plasticizer (sorbitol, glycerol and PEG), while the second factor was the concentration of the plasticizer (1%, 3%, and 5%). The tests carried out in this study were the tensile strength, elongation, thickness, water vapor transmission, and water solubility tests. The results showed that the type and concentration of plasticizer have a significant effect on the characteristics of the edible film. The interaction between the type and concentration of plasticizer has no significant effect on the water vapor transmission test. The best edible film in this study was the edible film with the addition of the plasticizer sorbitol with a concentration of 3%.

Keywords: Edible film; plasticizer; Beneng; starch

INTRODUCTION

Packaging on food products is an activity aimed at protecting and maintaining the quality and safety of food or beverages to the hands of consumers. The types of packaging circulating in Indonesia are very diverse, ranging from canned packaging, paper, plastic, to leaves. Plastic is one of the packaging materials that are often used, the use of plastic can pollute the soil and contribute to waste that is difficult to decompose also contains chemicals that are quite dangerous (Salamah, 2018). Currently, there have been many alternative packaging materials that have been developed such as edible film.

Edible film is a thin layer as a coating for food ingredients made from materials

suitable for consumption. Edible film, in addition to being used as a packaging material, can also be used as a food coating (coating) or as a film placed between foods. Edible film can function as a barrier to inhibit moisture, oxygen, lipids, light and solutes (Ariska and Suyatno, 2015).

Edible film is a packaging made with starch-based ingredients from the type of tubers so that it is environmentally friendly (Saleh et al., 2017). One of the tubers that can be used as edible film is taro beneng which comes from Banten. According to Budiarto and Rahayuningsih (2017), taro beneng has a high carbohydrate content as a source to produce starch. According to the research of Rostianti et al. (2018), the starch content contained in taro beneng Kampung Pagerbatu is 84.96% while in the study of Kusumasari et al. (2019), taro beneng from Gapoktan Juhut has a starch content of 56.29%.

Edible film made of starch has the disadvantage of being easily torn and has low elasticity, to change the physical properties of the film from starch, it is necessary to add a plasticizer. Plasticizer is a non-volatile material that has a high boiling point so that if mixed with other materials, it can change the physical properties of the material (Marpongahtun, 2013). If a plasticizer is added to a material, it will make the polymer chain edible film produced have elasticity and flexibility properties so that it is not easily broken (Wattimena et al., 2016).

Plasticizers that are often added to the manufacture of edible film are xylitol, sorbitol, polyethyleneglycol (Marpongahtun, 2013) and glycerol (Afifah et al., 2018). The purpose of this study is to determine the effect of the type treatment and concentration of plasticizers on the physical-mechanical properties of edible film based on taro beneng starch.

MATERIALS AND METHODS

Tools and Materials

The materials used for made edible film were beneng taro from Juhut village, carrageenan (Moli), salt (Dolpin), water, sorbitol (Teknis), glycerol (Teknis), polyethylene glycol (PEG) 400 (Teknis), aquadest, silica gel, and vaseline. The tools used in this research include tools for made edible film were analytical balance sheet, thermometer (Goto), hot plate stirrer (Thermo Scientific), beaker glass (Pyrex), measuring cup (Pyrex), refrigerator (freezer), film mold, moisture content oven (Memmert), cabinet dryer, knife, filter paper, blender (Phillips), stirring rod, scissors (Joyko), desiccator, centrifugation, chopper (Mitochiba), blacu cloth, 100 mesh sieve, clamp rods, universal testing machine (MCT 2150), and micrometers (Mitutoyo). Procedures and formulations for made edible film refer to Sitompul and Zubaidah's research (2017). The treatment formulation in this research consisted of four formulations. Formulations are presented in Table 1.

Methods

The research had been carried out in three stages, namely beneng taro starch production, edible film production, and physico-mechanical properties testing. The physical tests carried out were the solubility in water and the rate of transmission of water vapor, while the mechanical tests carried out are tensile strength, elongation, and film thickness.

Beneng Taro Starch Production

The production of beneng taro starch refers to Jacoeb et al. (2014) which have been modified in raw materials. This process began with stripping, cutting, washing and soaking with saline solutions. Beneng taro cleaned again and continued with reducing the size. The beneng taro filtered using

clothes and the water produced from the process precipitated for 6 hours at a temperature of 4°C. The water resulting from the settling process discharged. Washing of the starch produced by re-settling by adding 1: 1 water by volume at a temperature of 4°C for 12 hours. The starch dried at 50°C for 8-12 hours. The dried starch flakes mashed and sifted with a 100 mesh sieve to produce fine grains of beneng taro starch.

Edible Film Production

Edible film production refers to Sitompul and Zubaidah (2017) with modified raw materials. The process began with weighing a 5.25 grams (75% b/v) beneng taor starch and 1.75 grams (2.5% b/v) carragenan then dissolved with 150 ml aquadest and then stirred until a solution suspension was formed. The solution transferred into a 250 ml Beaker glass then heated over the *hotplate* at a temperature of ±70°C while stirring at a speed of 60 rpm for 15 minutes until it forms a gel. After the solution forms a gel, a plasticizer (sorbitol, glycerol and polyethylene glycol) with a concentration of 1, 3 and 5% (v/v) were added. The solution reheated at a temperature of ±70°C on the magnetic stirrer while stirring at a speed of 60 rpm for 15 minutes. Next the solution was allowed to stand to remove the air contained in the solution for 10 minutes. The solution then poured into edible film container with a size of 30 x 30 cm around 120 ml, then put in cabinet dryer for 15 hours with a temperature of 50°C. After drying, edible film allowed to stand for 1 hour to make it easier when removed from the container.

Water Vapor Transmission

Water vapor transmission test using samples in saucer cover containing saline solution. Then the saucer was put into a desiccator with a temperature of 25°C and RH 50%. The sample was weighed periodically for 7 hours with a weighing time interval of 1 hour (Rhim and Wang, 2013). The value of the rate of transmission of water vapor can be calculated using the formula:

$$WVTR = \frac{\Delta W}{t \times A}$$

Description:
WVTR = Water Vapor Transmission Rate ($g/m^2\,h$)
ΔW = Change in film weight (g)
t = Time (hours)
A = Film area (m^2)

Solubility in Water

The solubility of the film in water was obtained by drying film sample and filter paper in 105°C oven for 24 hours. Furthermore, the film sample and filter paper were weighed separately (W1) then soaked in aquadest 50 mL for 24 hours and stirred. After that, the film sample was dried again using a 105°C oven for 24 hours followed by weighing (W2) (Lismawati, 2017). The data obtained is then calculated using the formula:

$$\%\, Solubility = \frac{W1 - W2}{W1}\, X\ 100\%$$

Description:
Solubility = Solubility edible film in water (%)
W1 = Initial weight (g)
W2 = Final weight (g)

Tensile Strength

The sample was cut according to the specification of length x width listed on the universal testing machine MCT 2150 with a size of 10 x 4 cm. Then the sample was observed at length initially by being placed on an analyzer (Sitompul and Zubaidah 2017). Based on Rhim and Wang (2013), the engine setting is carried out with a pull speed of 50 mm / min with an initial distance between clamps of 100 mm. The data obtained is then calculated using the formula:

$$Tensile\ Strength = \frac{F}{A}$$

Description:
Tensile Strength = Tensile strength edible

film (N/mm2)
F = Tensile strength force (N)
A = Cross-sectional area (mm^2)

The obtained data then converted to MPa unit.

Elongation

Elongation testing was obtained by the same procedure as tensile strength (Sitompul and Zubaidah, 2017). Elongation is expressed in percentages and is calculated using the formula:

$$Elongation = \frac{P2 - P1}{P1} X\ 100\%$$

Description:
Elongation = Extension of material (%)
P1 = Initial length (mm)
P2 = Final length (mm)

Thickness

The thickness of edible film was measured using a micrometer with an accuracy of 0.01 mm at five different points on the surface of the film. The data obtained were then averaged and expressed as film thicknesses with mm units (Zuwanna et al., 2017).

Data Analysis

The results of testing the physical-mechanical properties of edible films were statistically analyzed using the SPSS application to look for Analysis of Variance (ANOVA), to determine the effect of the treatment. The results of the analysis that show a significant effect will be analyzed by further testing using the Duncan's Multiple Range Test (DMRT) test at a 95% confidence level.

RESULTS AND DISCUSSION

From its physical appearance, edible film with the addition of PEG plasticizer was stiffer, harder and not transparent compared to edible film with sorbitol and glycerol plasticizer added. Edible film with the addition of sorbitol plasticizer had a smoother surface appearance compared to edible film with the addition of glycerol and PEG plasticizers. When compared with control edible films, edible films with the addition of the plasticizer sorbitol had a transparent film surface like the film from the control. The surface of the film with the addition of glycerol plasticizer tended to be transparent with a slightly thin yellow color, while the film with the addition of PEG plasticizer is white like matte (Figure 1). Physically, edible film with the addition of sorbitol and PEG plasticizer was not stiff and dry, so it did not stick to paper or other objects when in contact. While the edible film with the addition of glycerol plasticizer had elastic properties, soft, and moist, so it was easy to stick and difficult to remove.

The response of edible film analysis to the type and concentration of plasticizers can be seen in Table 2.

The water vapor transmission test results from Table 2 showed that the increased concentration of the plasticizer could increase the transmission value of *edible film* water vapor. Based on the results of the research conducted, the range of water vapor transmission values of sorbitol plasticizers, glycerol and PEG was successively 0.1210 - 0.1317 g / m^2h, 0.1750 - 0.2046 g / m^2h, and 0.1242 - 0.1732 g / m^2h. The largest water vapor transmission value in the study was produced by a 5% glycerol sample and the smallest water vapor transmission value was produced by a 1% sorbitol sample. Analysis of variance showed that the interaction between the type and concentration of plasticizer did not have a significant effect on the response of water vapor transmission. The best water vapor transmission value was obtained in sample 1% sorbitol, but this value was not significantly different from the other samples.

The transmission of moisture depends on the properties of the constituent materials used in the production of edible film. *Edible film* that has a high vapor transmission value is generally made of polysaccharide and protein materials. Proteins can absorb high water moisture because they are included in polar polyners (Herliany *et al.,* 2013). Water vapor transmission that has the ability to absorb the smallest moisture is the best packaging. This is due to the smaller the product that is packaged to be exposed to water and experience damage caused by air. The main role *of edible film* as a packer is to inhibit moisture, light, oxygen, lipids and solutes (Dwimayasanti and Kumayanjati, 2019).

The value of water vapor transmission of edible film according to the Japanese Industrial Standard (JIS) is a maximum of 7 g/m^2h. In this study, the highest water vapor transmission of edible film was 0.2046 g/m^2h, which means it has met the JIS standard. In the research of Pangesti et al. (2014), the water vapor transmission of the taro starch edible film was 5.75 g/m^2h, which means that the water vapor transmission value of the taro starch edible film was lower than previous studies.

The results of the solubility test in water from Table 2 showed that an increase in the concentration of plasticizers in the type of sorbitol plasticizer could increase the solubility value in edible film water, while in the PEG type of plasticizer, the addition of a plasticizer decreased the solubility value of edible film the resulting. Based on the results of the research conducted, the range of solubility values in sorbitol, glycerol and PEG plasticizer water was 62.8 –74.8 %, 77.53 – 91.57%, and 41.94 – 49%, respectively. The largest solubility value in water in the study was produced by a 5% glycerol sample and the smallest solubility value was produced by a 5% PEG sample. Analysis of variance showed that the interaction between the type and concentration of plasticizer had a significant effect on the solubility in water response. The best value from the analysis of solubility in water was obtained in sample 5% glycerol with analysis letters that were not the same as other samples

The high solubility of the film indicates the ease with which the film is dissolved in water and the poor resistance to water. The solubility of the film is influenced by the nature of the film-forming compounds. Plasticizers interact with matrix film by facilitating the migration of water into the film and increasing the space between the film chains, so that the solubility of the film can be improved (Lagos et al., 2015). Edible film with high solubility is good to use for ready-to-eat products due to its easily soluble properties (Dwimayasanti and Kumayanjati, 2019).

The tensile strength test results from Table 2 showed that an increase in the concentration of the plasticizer cannot always raise the tensile strength value of edible film. Based on the results of the research conducted, the range of tensile strength values of sorbitol, glycerol and PEG plasticizers was 10.8660 - 17.7567 MPa, 8.7533 - 11.9967 MPa, and 8.3100 - 9.2100 MPa. The largest tensile strength value in this study was generated by a 3% sorbitol sample and the smallest tensile strength value was generated by a 5% PEG sample. Analysis of variance showed that the interaction between the type and concentration of plasticizer had a significant effect on the tensile strength response. The best value of the tensile strength analysis is obtained in the 1% sorbitol sample with the analysis letter that is not the same as the other samples

In general, the higher the concentration of plasticizers added will make the tensile strength value of edible film decrease. However, in this study there were several treatments that showed the opposite trend

where the tensile strength increased higher (Dwimayasanti and Kumayanjati, 2019). The higher the concentration of the added plasticizer will reduce the tensile strength value. The cause of this is the nature of the plasticizer which will reduce the internal hydrogen bond in the intermolecular bond, as a result of which the resulting edible film will have weak physical properties and can reduce the tensile strength value of a film (Sitompul and Zubaidah, 2017).

The tensile strength of edible film according to the Japanese Industrial Standard (JIS) is a minimum of 0.39 MPa. In this study, the value of the lowest edible film tensile strength was 7.4867 MPa, which means that all edible film treatments had met the JIS standard. In the research of Putri et al. (2021), the tensile strength of the kimpul taro starch edible film was 0.361 MPa, while in Handayani and Nurzanah (2018), the tensile strength of the taro starch edible film was 1.198 MPa, which means that the tensile strength value of the taro starch edible film is higher than previous studies.

The elongation test results from Table 2 show that an increase in the concentration of plasticizers can increase the elongation value of edible film. Based on the results of the research conducted, the range of elongation values of sorbitol, glycerol and PEG plasticizers was 5.18 - 11.02 %, 8.33 - 13.86 %, and 2.78 - 5.01 %. The largest elongation value in this study was generated by a 5% glycerol sample and the smallest elongation value was generated by a 1% PEG sample. Analysis of variance showed that the interaction between the type and concentration of plasticizer had a significant effect on the elongation response. The best value from the results of the elongation analysis was obtained in the 5% glycerol sample with analysis letters that were not the same as the other samples.

The increase in the concentration of plasticizers will affect the elongation value obtained, the higher the plasticizer added, the higher the percent value of the resulting lengthening. This can be attributed to the treatment of increasing the concentration of plasticizers. According to Nandika et al. (2021) with the increasing concentration of plasticizers added, it will cause a reduction in the intermolecular hydrogen bonds and the intramolecular polymer chains adjacent to it will be weak, so that the resulting film will be more flexible and the percentage of elongation will increase.

The elongation of the edible film according to the Japanese Industrial Standard (JIS) is minimal 70%. In this study, the highest edible film elongation is 13.8600%, which means that it does not meet the JIS standard. In the research of Putri et al. (2021), the elongation of the taro kimpul starch edible film was 76.70%, while in Handayani and Nurzanah (2018), the elongation of the taro starch edible film was 55.13%, which means that the elongation value of the taro starch edible film was lower than previous studies.

The thickness test results from Table 2 show that the increase in the concentration of the plasticizer increases the thickness of the edible film produced. Based on the results of the research conducted, the range of thickness values of sorbitol, glycerol and PEG *plasticizers* was 0.0403 – 0.1027 mm, 0.0590 – 0.0843 mm, and 0.0673 – 0.0904 mm respectively. The thickest edible film value in this study was produced by a 5% sorbitol sample and the smallest thickness value was produced by a 1% sorbitol sample. Analysis of variance showed that the interaction between the type and concentration of plasticizer had a significant effect on the thickness response. The best value from the thickness analysis was obtained in the 5% sorbitol sample with the analysis letters that were not the same as the other samples

The thickness of edible film increased along with the increase in the concentration

of plasticizers. This could be happened because the plasticizer has properties that can increase the viscosity of edible film solutions. The thickness of edible film is influenced by several factors such as the length of time the film is dried, the size of the film container, and the properties of the constituent materials used. If there are many materials used in making edible film, the resulting film will be thicker because the constituent components are diverse (Maharani et al., 2017).

The thickness of the edible film according to the Japanese Industrial Standard (JIS) is a maximum of 0,25 mm. In this study, the highest thickness of edible film was 0,1027 mm so that it met the JIS standard. In the research of Putri et al. (2021), the thickness of the taro kimpul starch edible film is 0,22-0,26 mm, while in Handayani and Nurzanah (2018), the thickness of the taro starch edible film is 0,3 mm, which means the thickness of the taro starch edible film is thinner than in previous studies.

The effectiveness of beneng taro as food packaging is not yet known, because until now there has been no research that discusses the use of taro beneng starch as a basic material for making edible films, edible coatings and microencapsulations. Further research is needed regarding the use and application of taro beng starch edible film as food packaging.

CONCLUSION

Based on the research that has been done, it can be concluded that the treatment of type and concentration of plasticizer had a significant effect on the characteristics of tensile strength, elongation, thickness, water vapor transmission, and water solubility of edible film. The interaction had a significant effect on the characteristics of tensile strength, elongation, thickness, and water solubility of the edible film, but had no significant effect on the value of the water vapor transmission rate. The best edible film in this study was the edible film with the addition of the plasticizer sorbitol with a concentration of 3%. The test value of the water vapor transmission rate was 0.1214 g/m2-hour, 67.7205% water solubility, 17.7567 MPa tensile strength, 9.0367% elongation, and 0.0683 mm thick. The edible film produced in this study met the JIS standard on the variable rate of water vapor transmission, tensile strength, and thickness, but did not meet the JIS standard on the elongation variable.

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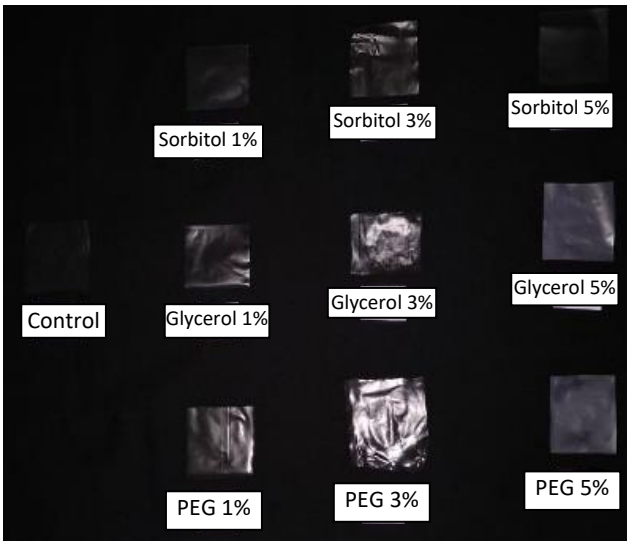

Figure 1. Edible Film appearence

Table 1. Edible Film Formulation

Formulation per 150 ml			
Plasticizer type	Beneng starch (grams)	Carrag-eenan (grams)	Plasticizer concentra-tion (v/v)
Sorbitol	5,25	1,75	1%
Glycerol	5,25	1,75	3%
PEG	5,25	1,75	5%

Table 2. Results of analysis

Types of Plasticizers	Plasticizer Concentration	Analysis of physical and mechanical properties				
		Water Vapor Transmission	Solubility in Water	Tensile Stength	Elongation	Thickness
	%	g/m^{2}h	%	MPa	%	Mm
Control		0,1248	37.8794	19,9200	1,4400	0,0900
Sorbitol	1	0,1210^{a}	62.8047^{f}	10.8660^{b}	5.1867^{d}	0.0403^{e}
	3	0,1214^{a}	67.7205^{e}	17.7567^{a}	9.0367^{c}	0.0683^{cde}
	5	0,1317^{a}	74.8029^{d}	11,1700^{b}	11.0233^{b}	0.1027^{a}
Glycerol	1	0,1750^{a}	77.5366^{c}	8.7533^{c}	8.3300^{c}	0.0590^{de}
	3	0,2022^{a}	85.0058^{b}	11.9967^{b}	10.4867^{b}	0.0700^{cde}
	5	0,2046^{a}	91.5766^{a}	8.9467^{c}	13.8600^{a}	0.0843^{bc}
PEG	1	0,1242^{a}	49.7507^{g}	8.3100^{cd}	2.7867^{e}	0.0673^{cde}
	3	0,1550^{a}	48.2629^{g}	9.2100^{c}	3.7167^{e}	0.0743^{cd}
	5	0,1732^{a}	41.9469^{h}	7.4867^{d}	5.0100d	0.0904^{b}

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Sago Starch Bagea Cookies with Moringa Leaf Powder Composite as a Fiber and Antioxidant Enhancer

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ABSTRACT

Bagea is a traditional food typical of Eastern Indonesia such as the Ternate and Maluku regions. The addition of moringa leaf powder to bagea cookies can increase the nutritional content, so that it can meet the needs of dietary fiber and the antioxidant properties of bagea cookies. This study aims to determine the effect of adding moringa leaf powder to the physical, chemical and sensory characteristics of bagea cookies and to determine the best formulation of bagea cookies with sago starch with the addition of moringa leaf powder. This study used an experimental method with a completely randomized design (CRD) with a single factor, namely the addition of moringa leaf powder 3%, 6%, 9%, 12%, 15%. The tests included physical characteristics (color lightness, texture), chemical characteristics (moisture content, crude fiber content and antioxidant activity), sensory tests (color, aroma, texture, taste and overall) and effectiveness tests. The results showed that the addition of moringa leaf powder to bagea sago starch cookies had a significant effect on physical, chemical, sensory characteristics and gave no significant effect on the moisture content of bagea cookies. The best formulation of bagea cookies was in the L3 treatment which resulted in a color lightness characteristic of 76.42; texture; 262.92 g/2mm; moisture content 4.82%; crude fiber content 1.71%; antioxidant activity 8.52%; color preference 5.97 (slightly like-like); aroma preference 4.90 (neutral-slightly like); texture preference 5.33 (slightly like-like); taste preference 4.83 (neutral-slightly like); and overall preference of 5.57 (slightly like-like). The nutritional content of bagea cookies per 100 g is protein, fat, ash, and carbohydrates of 9.32%, 9.40%, 0,13%, and 76.30% respectively.

Keywords: Bagea cookies; moringa leaf powder; fiber; antioxidant activity

INTRODUCTION

Bagea is a traditional food typical of Eastern Indonesia such as the Ternate and Maluku regions. In addition, bagea are also found in Papua and Sulawesi. These cookies have a dry, crispy texture and are pale brown in color. The sweet taste causes bagea to be liked by children to adults (Hasriani et al., 2018). The main ingredients for making bagea are sago starch and other ingredients in the form of sugar, vegetable oil and eggs. Bagea are generally topped with peanuts or

walnuts (Metaragakusuma et al., 2015). Bagea made from sago starch produces a fairly high carbohydrate content, but the content of other nutrients is still quite low such as protein and fiber so that those who consume it can cause obesity.

Modern society today expects bagea not only to have a delicious and filling taste but also to have functional properties for health (Anggraeni, 2019). Cookies can be functional if in their manufacture are added ingredients that have a positive effect on the body such as fiber, calcium and provitamin A (Purba et al., 2017). Bagea cookies can be developed into food products with high nutritional value by adding potential food ingredients such as Moringa leaves.

Moringa is a tropical plant that has many benefits as food, has high nutritional value and also has medicinal properties. Various literatures state that Moringa leaves are rich in essential amino acids, fats, vitamins and minerals (Liu et al., 2018). Moringa leaves are known as plants that are high in protein content of 29.4 g/100g dry leaf weight and fiber around 18.1-21.1 g/100g dry leaf weight (Milla et al., 2021). Moringa leaves contain antioxidants in the form of flavonoids, polyphenols and ascorbic acid. The total phenolic content in Moringa leaves ranges from 20-122 mg GAE/g (Yazeed, 2019).

The use of Moringa leaves in food fortification programs has begun to be developed, because it has potential as a functional food product. Based on research by Azizah (2015), the substitution of 5%, 10% and 15% Moringa leaf powder in biscuit products with wheat flour substitution will affect the color, aroma and taste of the biscuits. The Moringa leaf biscuits produce a green color that is getting darker with greater substitution. The unpleasant smell of biscuits is typical of dry leaves and the more concentrated the aroma is at the higher the percentage addition. The taste of the biscuits is getting bitter when the substitution of Moringa leaf powder is getting bigger. Based on the research of Dachana et al., (2010), the manufacture of flour-based cookies with the addition of 5%, 10% and 15% Moringa leaf powder affects the physical, sensory and chemical characteristics of cookies, The increase in the addition of Moringa leaf powder from 5% to 15% increases the hardness value of the cookie texture because moringa leaf powder has high protein and fiber content, resulting in a harder dough. Sensory evaluation showed that cookies with the addition of 10% Moringa leaf powder were acceptable, while above 10% would result in unacceptable cookies. The addition of 10% Moringa leaf powder significantly increased the protein content of 11.6%, dietary fiber 4.3%, iron 5.09 mg%, calcium 272.3 mg% and b-carotene 1,600 mg% cookies. In the study of Sartina et al., (2018), sago chips products with the addition of 15% Moringa leaf powder resulted in antioxidant activity of 84.27 µg/mL.

Making bagea cookies with the addition of moringa leaf powder has never been done, but the potential for adding moringa leaf powder can increase its nutritional content, so that it can meet the needs of food fiber and antioxidant properties of bagea cookies. In addition, the addition of moringa leaf powder can also improve physical characteristics such as the color and texture of bagea cookies. Therefore, it is necessary to do research on the addition of moringa leaf powder composite in bagea cookies which can improve the quality of bagea cookies and be accepted by the community.

MATERIALS AND METHODS

Tools and materials

The tools used in this research are digital balance, mixer, oven, baking sheet, wooden rolling pin and cookie mold. Other equipment used in this test are Minolta

Colorimeter (CR-400, Japan), Rheotex type SDA-700, spectrophotometer (Thermo Sientific Genesys 10S UV-VIS, China), vortex, volumetric flask, beaker glass, volume pipette, desiccator, porcelain exchange rate, weighing bottle, erlenmeyer and filter paper.

The ingredients used in making bagea cookies are sago starch from Tani's sago brand, Moringa leaf powder from Yusron Herbamart Jember store (moisture content = 6,30%), Rose Brand refined sugar, Dorang Mas fish vegetable oil, Cap Kapal salt and domestic chicken eggs. The materials used for the analysis were distilled water, NaOH, H_2SO_4, K_2SO_4, ethanol 96%, methanol and DPPH (1,1 diphenyl-1-2-Picrylhydrazil).

Bagea Cookies Production

The process of producing bagea cookies refers to Hasriani et al (2018) and (Milla et al., 2021) with modifications. The first stage is mixing 50 grams of eggs and 60 grams of powdered sugar using a mixer at high speed for 5 minutes until it forms a cream so that the sugar dissolves in the eggs and the eggs bind the air. Then add 30 ml of vegetable oil and 1 gram of salt and mix again using a mixer 3 minutes at high speed until the resulting dough is soft and well mixed. Furthermore, the addition of dry ingredients in the form of sago starch and moringa powder, control (200 grams of sago starch), L1 (194 grams of sago starch: 6 grams of moringa leaf powder), L2 (188 grams of sago starch:12 grams of moringa leaf powder), L3 (182 grams of sago starch) grams of sago starch: 18 grams of moringa leaf powder), L4 (176 grams of sago starch: 24 grams of moringa leaf powder), L1 (170 grams of sago starch: 30 grams of Moringa leaf powder), mixed manually for 3 minutes until the dough is soft, smooth and easy to shape.

The second mixing is done manually so that the dough is mixed evenly and does not experience premature maturity which makes the dough harden. The next step, the dough is flattened using a rolling pin with the aim of flattening the surface of the dough and making it easier for printing. Furthermore, printing using a cookie cutter is round, 3 cm in diameter and 0.5 cm thick and then placed on a baking sheet that has been lined with baking paper. The last stage is roasting in the oven at 150°C for 60 minutes.

Research design

This study used an experimental method with a completely randomized design (CRD) with a single factor, namely the addition of moringa leaf powder 3%, 6%, 9%, 12%, 15%. The experiment was carried out with 3 repetitions.

Analysis Method

Parameters of analysis include physical, chemical and sensory characteristics. Physical characteristics include color lightness and texture, chemical characteristics include moisture content (BSN, 2011), crude fiber content (Fajri et al., 2018) and antioxidant activity (DPPH method) (Visita and Putri, 2014), sensory characteristics is hedonic test include color, aroma, texture, taste and overall (Setyaningsih et al., 2010) and effectiveness (De Garmo, 1984).

Sensory test data were analyzed using the Chi square method with a 95% confidence level and analyzed descriptively. Meanwhile, the data from the physical and chemical tests were analyzed using analysis of variance (ANOVA) at the test level ($\alpha \leq 0.05$) to determine the effect of treatment on the measured parameters. If the difference is significant, it is continued with Duncan's New Multiple Range Test (DNMRT).

RESULTS AND DISCUSSION

Physical Characteristics

Color Lightness (L)

Color testing is carried out to determine the color of bagea cookies products objectively. The parameter measured in the color test is L for the lightness value. The lighter the sample being measured, the L value approaches 100. On the other hand, the darker the sample, the L value approaches 0 (Saputri, 2014). The average value of the color lightness test for bagea starch sago cookies at various percentages of addition of moringa leaf powder ranged from 70.45±0.35 to 82.00±0.34, presented in Table 1.

The results of variance showed that the percentage of addition of moringa leaf powder had a significant effect on the 95% confidence level. ($\alpha \leq 0.05$)on the color lightness of sago starch bagea cookies. Duncan further test results showed that bagea cookies treatment L1 (was significantly different from treatment L2, significantly different from L3, significantly different from L4 and significantly different from the L5 treatment.

Table 1. shows that the highest lightness value of bagea cookies is found in the L1 treatment which is 82.00±0.34 while the lowest value is found in the percentage of 15% moringa leaf powder is 70.45±0.35. *Cookies* the treatment of L1, L2, L3, L4 and L5 resulted in the lightness value is lower than the control bagea cookies, namely 92.11±0.34. The lightness level of bagea cookies samples is influenced by the percentage of raw materials used. Enhancement the percentage of sago starch resulted in the lightness value of bagea cookies which tended to increase (light). The color of bagea cookies produced is greenish white to dark green (dark). This is influenced by the white color obtained from sago starch, where the lightness value of sago starch is 96.00 and the green color is obtained from the addition of moringa leaf powder with a brightness value of 63.32. Moringa leaf powder contains chlorophyll or a green pigment that causes a green color. The chlorophyll content in moringa leaves reaches 6.89 mg/kg dry matter, while 10 grams of moringa leaf powder contains 202.5 mg of chlorophyll (Krisnadi, 2012).The higher the percentage level of moringa leaf powder used will affect the color of bagea cookies to be greener (dark). This is in accordance with research (Ardianti et al., 2019), that the more use of moringa leaf powder in taro cookies, the greener (darker) cookies are produced.

Texture

The texture is one of the parameters that determine the quality and consumer acceptance of food products. Testing the texture of bagea cookies was carried out using a rheotex tool. Rheotex has the principle of product hardness level which is expressed in g/mm units, namely the amount of compressive force required to deform the product to a certain depth according to user settings. The higher the texture test value, the harder the cookie texture, on the other hand, the lower the texture value, the less hard the cookie texture. The results of the average value of texture testing of sago starch bagea cookies at various percentages of addition of moringa leaf powder ranged from 233.43±0.3g/2mm up to 340.87±0.51 g/2mm, can be seen in Table 2.

The results of variance showed that the percentage of addition of Moringa leaf powder had a significant effect on the 95% confidence level. ($\alpha \leq 0.05$)on the texture of bagea sago starch cookies. Duncan's further test results showed that bagea cookies in L1 treatment were significantly different from L2 treatment, significantly different from L3, significantly different from L4 treatment. and significantly different from L5.

Table 2 shows that the texture value of bagea cookies in treatment L5 has the

highest value of 340.87±0.51 g/2mm while the lowest texture value is found in treatment L1 which is 233.43±0.59 g/2mm, it shows that bagea starch sago cookies with the addition of 3% Moringa leaf powder are crunchier than other treatments. Bagea *cookies* the treatment of L1, L2, L3, L4 and L5 resulted in The hardness value was higher than the control bagea cookies, which was 180.63±0.31 g/2mm. The higher the percentage of sago starch, the lower the texture value of bagea cookies produced, where the product with a low texture value results in a less hard (crispy) product.This is influenced by the amount of starch content in bagea cookies. Sago starch has a starch content of about 97.68% with an amylopectin content of 75.01% and an amylose content of 24.99% (Larasati et al., 2017). Starch with a high amylopectin content will stimulate the puffing process, so that the food product will give it a light, porous, crunchy and crunchy nature (Kusnandar, 2010). The high starch content in the ingredients can increase the crispness of the resulting cookies because the amylose in the ingredients forms hydrogen bonds with more water, so that when roasting the water will evaporate and leave an empty space in the ingredients and make cookies crispier (Rosida et al., 2020).

The texture of bagea cookies is also influenced by the high protein and fiber content in moringa leaf powder. Moringa leaves contain about 29.4 g/100 g protein and 18.1-21.1 g/100 g dry leaf weight (Milla et al., 2021). High levels of protein can form a harder structure as a result of the strong bond between protein and starch (Giuberti et al., 2021). Proteins that bind to starch cause cookies to become hard because of the interaction between protein and starch through hydrogen bonds. According to Kusnandar (2010), proteins bind to water in the presence of hydrophilic hydrogen groups. The hardness of bagea cookies is caused by hydrogen bonds between the amino groups of Moringa leaf powder and the hydroxyl groups of sago starch to form a complex.

The hardness of the bagea cookie products produced is also caused by the fiber content contained in the raw material for making bagea cookies. According to Astuti et al. (2019), crude fiber content causes a decrease in water absorption in starch granules. Decreased water absorption resulted in the starch gelatinization process being imperfect and causing the texture to become hard. Fiber as a compound that is not soluble in water and streng thens the material network, in food serves as a texture reinforcement. The higher the fiber content in the raw material, the resulting product with a sturdier and stronger texture will result in a harder product. During the texture formation process, starch, fiber and protein components compete with each other to bind water (Astuti et al.,2019).

Chemical Characteristics

Moisture content

Moisture content is a chemical characteristic that is very influential on food ingredients because it can affect texture, taste and shelf life food stuffs (Kusnandar, 2010). Moisture content can affect physical properties such as hardness. The results of the average value of testing the moisture content of sago starch bagea cookies at various percentages of addition of moringa leaf powder ranged from 4.58±0.28% up to 4.97±1.66% (Table 3).

The results of variance showed that the percentage of addition of moringa leaf powder had no significant effect at the 95% confidence level. ($\alpha \leq 0.05$)on water content of sago starch bagea cookies. This is due to the amount of moisture content contained in the dough does not differ much in all treatments, so that the moisture content of bagea cookies products is not significantly different. The difference in moisture content is influenced by the composition of the raw

material. Heryani and Silitonga (2017) stated moisture content of sago starch is 14% and the moisture content of moringa leaf powder is 7.5% (Rani et al., 2019).

Table 3. showed the lowest moisture content of bagea cookies was found in the L1 treatment (the addition of 3% Moringa leaf powder) which was 4.73±1.58%, while the highest water content was found in treatment L5 (addition of 15% Moringa leaf powder) which was 4.97±1.66%. *Cookies*Bagea treatment L1, L2, L3, L4 and L5 produced a higher moisture content than the control bagea cookies, which was equal to4.58±0.28%. The decrease in the moisture content of bagea cookies is caused by the composition of the raw materials, where the higher the percentage of sago starch, the lower the moisture content produced. This is because the moisture content is closely related to the starch content. According to Winarno (2004), carbohydrates (starch) is one of the important components in determining the value of water absorption. Starch is a hydrophilic compound. Starch granules have the ability to absorb very large water because of the very large hydroxyl group of starch, therefore the higher the amount of starch, the lower the water content (Rosida et al., 2020).

The increase in moisture content was caused by the higher percentage of moringa leaf powder resulting in an increasing moisture content. This is in accordance with the research of Ardianti et al., (2019) The higher concentration of Moringa leaf powder added to taro cookies, the higher the moisture content value. The increase in moisture content can be influenced by the fiber content in the material. Moringa leaf powder contains fiber around 19.2 g/100 grams (Rani et al., 2019).Fiber has water binding properties with a strong enough bond so that the more percentage of moringa leaf powder added, the higher the moisture content of the cookies produced. This is supported by the statement of Mozin et al., (2019)that fiber has the ability to bind water, water that is tightly bound in dietary fiber is difficult to be re-evaporated even with the heating process.

According to the quality standard of SNI 01-2973-2011, the maximum moisture content of cookies is 5%. Bagea cookies products with the addition of moringa leaf powder have SNI quality standards. Cookies produced must meet the specified quality requirements to be safe for consumption and have a long shelf life. The low water content in food stuffs will make the product less hard (crispy) (Nuraini, 2013).

Crude Fiber Content

Crude fiber is a part of food that cannot be hydrolyzed by strong acids or bases (Yulianti, 2016). The results of the average value of testing the crude fiber content of sago starch bagea cookies at various percentages of addition of moringa leaf powder ranged from 0.50±0.0% up to 2.80±0.03%, can be seen in Table 4.

The results of variance showed that the percentage of addition of moringa leaf powder had a significant effect on the 95% confidence level. ($\alpha \leq 0.05$) on crude fiber content of sago starch bagea cookies. Duncan's further test results showed that bagea cookies in L1 treatment were significantly different from L2 treatment, significantly different from L3 (9% addition of moringa leaf powder), significantly different from L4 treatment. (12% addition of Moringa leaf powder) and significantly different from L5 (15% addition of Moringa leaf powder).

Table 4 shows that the highest crude fiber content in the L5 treatment was 2.80±0.03% and the lowest in the L1 treatment was 0.50±0.05%. *Cookies* Bagea treatment L1, L2, L3, L4 and L5 produced higher crude fiber content than control bagea cookies, namely as big as 0.39±0.28%. The results obtained indicate that the higher the

addition of Moringa leaf powder, the crude fiber content of bagea cookies tends to increase. The increase in crude fiber content was accompanied by an increase in the percentage of Moringa leaf powder and a decrease in the percentage of sago starch. This is because moringa leaf powder has a higher fiber content about 18.1-21.1 g/100 g dry leaf weight (Milla et al., 2021) than sago starch 0.08-0.50% (Rinto et al., 2017).

According to SNI 01-2973-2011, the content of fiber in cookies maximum is 0.5%. The results showed that the crude fiber content of the L1 treatment met the SNI, but the L2, L3, L4 and L5 treatments did not meet the SNI requirements because it was more than 0.5%. Suryani et al., (2018), according the higher the fiber content, the better for digestion, so cookies with a high fiber content can be used as a snack for the diet.

Antioxidant Activity

Antioxidants are compounds that work by inhibiting the rate of oxidation of other molecules or neutralizing free radicals. The results of the average antioxidant activity of bagea starch sago cookies at various percentages of addition of Moringa leaf powder ranged from 2.67±0.21% up to 15.25±0.32%, can be seen in Table 5.

The results of variance showed that the percentage of addition of Moringa leaf powder had a significant effect on the 95% confidence level. ($\alpha \leq 0.05$)on antioxidant activity of bagea starch sago cookies. Duncan's further test results showed that bagea cookies in L1 treatment were significantly different from L2 treatment (66% addition of moringa leaf powder), significantly different from L3, significantly different from L4 and significantly different from L5.

Table 5 shows that the highest antioxidant activity was found in the L5 treatment reaching 15.25±0.32% and the lowest was in the L1 treatment bagea cookies which was 2.67±0.21%. Bagea *cookies* treatment L1, L2, L3, L4 and L5 resulted in higher antioxidant activity than the control bagea cookies, which was equal to 1.56±0.24%. The more addition of moringa leaf powder, the antioxidant activity of bagea cookies tends to increase.This is in accordance with what was reported by Sartina et al. (2018), the antioxidant activity of chips increased along with the addition of moringa leaf powder.This is because moringa leaf powder contains antioxidants such as flavonoid compounds,polyphenols and ascorbic acid.The antioxidant activity of dried moringa leaves in 100 grams is about 78.98% (Yazeed, 2019). Bioactive compounds that provide activity as antioxidants are feluric acid, gallic acid and ellagic acid, sitosterol, myricetin, niazimycin, vanillin, kaempferol, quercetin, carotene, catechin, astragalin and isoquercetin (Milla et al., 2021). Antioxidants in Moringa leaves have free radical neutralizing activity thereby preventing oxidative damage to most biomolecules and providing significant protection against oxidative damage.(Ardianti et al., 2019).

Sensory Characteristics

Sensory testing is a way of evaluating a product using the senses with sensory abilities. Rating score with 7 test scales (1 = very dislike, 2 = dislike, 3 = slightly dislike, 4 = neutral, 5 = slightly like, 6 = like, 7 = very like).

Color

Color is one of the first visual analyzes of a food product so that it greatly determines consumer preferences for the product (Winarno, 2004). The results of the analysis show that the average value of the level of preference panelists on the color of sago starch bagea cookies with various percentages of moringa leaf powder were on

a score of 4.47 - 5.97, meaning that acceptance was on the criteria of neutral-like (Table 6).

Chi-square calculation results test level (α≤0.05) arithmetic value (50.872)>table value (43.773) showed that the percentage of addition of moringa leaf powder had a significant effect on the panelists' preference for the color of bagea cookies. Panelists' assessment of the color of cookies with the lowest score was in treatment L5 with a value of 4.47 and the highest score was obtained in treatment L3 with a value of 5.97 which means the panelist acceptance rate is in the neutral - like criteria. Cookies with L3 treatment had a higher rating score than control cookies of 4.73 (neutral-slightly like). This is because the control bagea cookies produce a bright white color that is not like the color of cookies in general, the panelists' preference level decreases. Cookie products from L1 and L2 treatments produced slightly paler colors, L3 treatment resulted in light green so preferably,the L4 and L5 treatments had a dark green color which was less favored by the panelists. This is in accordance with the research of Sartina et al (2018), which states that the more number of Moringa leaves added to the manufacture of chips, the lower the panelists' preference level because the color of the chips produced is getting darker. The green color of bagea cookies is obtained from Moringa leaf powder which contains chlorophyll. Chlorophyll is a natural green leaf pigment that is generally found in leaves, so it is often called leaf green matter. Moringa leaves contain chlorophyll 6.89 mg/kg chlorophyll material (Krisnadi, 2012).

Aroma

In the food industry, odor testing is considered important because it can provide an assessment of the product regarding whether the product is acceptable or not. The average value of the panelists' preference for the aroma of cookies bagea starch sago at various percentages of addition of Moringa leaf powder is at a score of 3.87-4.93, which means that product acceptance is on the criteria of slightly dislike to like, can be seen in Table 7.

Chi-square calculation results test level (α≤0.05) arithmetic value (66.956)>table value (43.773) showed that the percentage of addition of Moringa leaf powder had a significant effect on the panelists' preference for the aroma of bagea cookies. Panelists' assessment of the aroma of cookies with the lowest value is in product L5 with a value of 3.87 and the highest value is obtained on product L1 with a value of 4.93 which means the panelist acceptance rate is in the criteria of somewhat dislike to like. Cookies bagea treatment L1, L2, L3, L4 and L5 had a lower score than the control bagea cookies of 5.47 (Slightly like-like). This is because the aroma of the control bagea cookies produces a distinctive aroma of cookies so that it is preferred by the panelists

The highest panelist acceptance rate was found in the L1 treatment because the highest percentage of sago starch, which was 97%, still produced a distinctive aroma of cookies. The addition of moringa leaf powder in the L5. The decrease in panelists' preference was due to the large percentage of addition of Moringa leaf powder resulting in a very sharp distinctive aroma of Moringa leaves so that the level of preference for aroma parameters decreased.

This is in accordance with what was reported by Sartina et al (2018), that the higher the concentration of Moringa leaves used in making chips, the more it affects the panelists' preference for the aroma of chips. Moringa leaves have a distinctive unpleasant aroma. The unpleasant smell of Moringa leaf powder is caused by moringa leaves containing lipoxidase enzymes, which are enzymes found in green vegetables, lipoxidase enzymes hydrolyze or decompose

fats into compounds that cause unpleasant odors belonging to the hexanal 7 and hexanol groups (Astutik, 2020).

Texture

Food texture is something related to the structure of the food that can be detected by tasting the food in the mouth. According to Rosida et al (2010), crispness is a the driving factor for consumers to prefer the resulting product because the crispness of dry food products shows the quality and quality of the product, so that it will attract consumers to like it more.The average value of the panelists' preference for the texture of bagea starch sago cookies with various percentages of addition of Moringa leaf powder is on a score of 4.60 to 5.40, which means that the panelists' acceptance is on the criteria of neutral to like, can be seen in Table 8.

Chi-square calculation results test level ($\alpha \leq 0.05$) arithmetic value (38.979) <table value (43.773) whichshowed that the percentage of addition of moringa leaf powder had no significant effect on the panelists' preference for bagea cookies texture. This is because the resulting bagea cookies have a crunchy texture that can still be accepted by the panelists.This is in accordance with the research of Sartina et al (2018), which states that chips with the addition of moringa leaf powder produce a crunchy texture so that it does not affect the panelists' preference for the texture of chips.

Panelists' assessment of the texture of cookies with the lowest score is on product L5 with a value of 4.60 and the highest score is obtained on product L1 with a value of 5.40 which means the panelist acceptance rate is in the neutral to like criteria. The L1 treatment bagea cookies had the same score as the control bagea cookies. This is because the percentage of sago starch in the L1 and control treatments did not differ much, resulting in a crunchy cookie texture.The crispness of a food product can be related to the water content. This is because the more water that is evaporated during roasting will form air cavities so that the resulting product is more crispy (Rosida et al., 2020). The decrease in panelists' acceptance of the L5 treatment was due to the high fiber content of moringa leaf powder which made the cookies texture harder.

Taste

Taste is one of the important factors to determine whether or not a food or food is accepted. The results of the analysis show that the average value of the panelists' preference for the taste of bagea starch sago cookies with various percentages of addition of moringa leaf powder is at a score of 3.43 to 5.43, which means that the panelists' acceptance is in the criteria of slightly dislike to like (Table 9).

Chi-square calculation results test level ($\alpha \leq 0.05$) arithmetic value (56.814)>table value (43.773) which showed that the percentage of addition of Moringa leaf powder had a significant effect on the panelists' preference for the taste of bagea cookies. Panelists' assessment of the taste of cookies, the lowest score was in treatment L5 with a value of 3.43 and the highest score on product L1 with a value of 5.43, which means that the panelists' acceptance was at the criteria somewhat dislike to like. Cookies bagea treatment L1, L2, L3, L4 and L5 had a lower score than the control bagea cookies of 5.83 (somewhat like-like). This is because the control bagea cookies produce a sweet and savory taste like cookies in general so that the panelists prefer it.

The results obtained indicate that the higher the addition of Moringa leaf powder, the panelist acceptance rate tends to decrease. This is in line with research Sartina et al (2018), that the more addition of moringa leaves will affect the taste of the chips because the nature of Moringa leaves gives

an after taste so that the taste quality of the chips decreases. This happens because of the distinctive taste caused by moringa leaves. This taste arises because in Moringa leaves there are tannin compounds which give the effect of a bitter taste. Tannins can cause a suffocating taste because when consumed, cross-links are formed between tannins and proteins or glycoproteins in the oral cavity, causing a dry and fibrous feeling or a suffocating taste.(Ardianti et al., 2019).

Overall

Rating of overall preference is accumulated from all parameters, namely color, aroma, texture and taste. The results of the analysis showed that the average value of the panelists' overall preference for bagea starch sago cookies with various percentages of addition of moringa leaf powder was on a score of 4.00 to 5.57, which means that the panelists' acceptance was in the neutral to like criteria (Table 10).

Chi-square calculation results test level ($\alpha \leq 0.05$) arithmetic value (69.568)> table value (43.773) whichshowed that the percentage of addition of Moringa leaf powder had a significant effect on the overall preference of the panelists for bagea cookies. Panelists' assessment of the taste of cookies with the lowest score is on product L5 with a value of 4.00 and the highest score is obtained on product L3 with a value of 5.57 which means the panelist acceptance rate is in the neutral to like criteria. This is because the appearance of the L3 treatment product a light green bagea cookie color, the aroma produced is still typical of cookies and a bit unpleasant but still acceptable to the panelists, the texture is crunchy and the resulting taste is sweet, savory and delicious. a little distinctive taste of moringa so that it gives the impression of liking by the panelists.

Cookies The L3 treatment bagea still had a lower score than the control bagea cookies of 5.70 (somewhat like it). This is because the control bagea cookies are still better and preferred by the panelists on the parameters of texture, aroma and taste.

Effectiveness Test

The effectiveness test is used in this study to determine the best treatment. Parameters of effectiveness testing include water content, crude fiber content, antioxidant activity and sensory test. The results of the analysis of the effectiveness of the best treatment of bagea cookies can be seen in Figure 1.

The results of the effectiveness test showed that the best formulation of bagea cookies was in the L3 treatment with an effectiveness value of 0.76. The addition of 9% Moringa leaf powder has an average value ofwater content of 4.82%, crude fiber content of 1.71%, antioxidant activity of 8.52%, preference color 5.97 (slightly like-like), aroma preference 4.90 (neutral-slightly like), texture preference of 5.33 (slightly like-like), taste preference of 4.83 (neutral-slightly like) and overall liking of 5.57 (slightly like - like).

CONCLUSION

The addition of moringa leaf powder to bagea sago starch cookies had a significant effect on physical, chemical, sensory characteristics and gave an insignificant effect on the moisture content of bagea cookies. The best treatment chosen was the L3 treatment with a color lightness of 76.42; texture; 262.92 g/2mm; water content 4.82%; crude fiber content 1.71%; antioxidant activity 8.52%. The nutrional content of bagea cookies per 100 g is protein, fat, ash, and carbohydrates of 9.32%, 9.40%, 0,13%, and 76.30% respectively.

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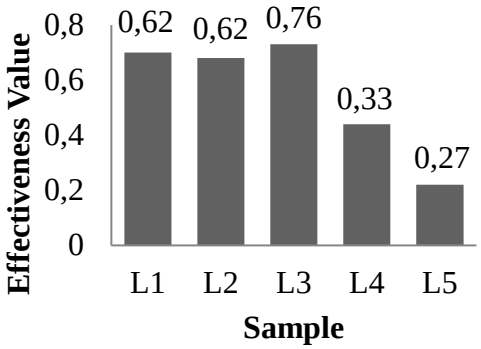

Figure 1. Effectiveness of sago starch bagea cookies with the addition of Moringa leaf powder 3% (L1), 6% (L2), 9% (L3), 12% (L4), 15% (L5)

Table 1. Color lightness of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: Moringa leaf powder)	Color Lightness	Notation
Control (100:0)	92.11 ± 0.34	
L1 (97 : 3)	82.00 ± 0.32	a
L2 (94 : 6)	81.06 ± 0.33	b
L3 (91 : 9)	76.42 ± 0.37	c
L4 (88:12)	73.92 ± 0.39	d
L5 (85:15)	70.45 ± 0.35	e

Note: Different letters show a significant effect on the 95% confidence level (α≤0.05)

Table 2. Texture of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: Moringa leaf powder)	Texture (g/2mm)	Notation
Control (100:0)	180.63± 0.31	
L1 (97 : 3)	233.43± 0.59	a
L2 (94 : 6)	242.11± 0.47	b
L3 (91 : 9)	262.92± 0.45	c
L4 (88:12)	270.58± 0.42	d
L5 (85:15)	340.87± 0.51	e

Note: Different letters show a significant effect on the 95% confidence level (α≤0.05)

Table 3. Moisture content of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: Moringa leaf powder)	Moisture content (%)	Notation
Control (100:0)	4.58 ± 0.28	
L1 (97 : 3)	4.73 ± 1.58	a
L2 (94 : 6)	4.77 ± 1.59	a
L3 (91 : 9)	4.82 ± 1.61	a
L4 (88:12)	4.92 ± 1.64	a
L5 (85:15)	4.97 ± 1.66	

Note: Different letters show a significant effect on the 95% confidence level (α≤0,05)

Table 4. Crude fiber content of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: Moringa leaf powder)	Crude fiber content (%)	Notation
Control (100:0)	0.39±0.28	
L1 (97 : 3)	0.50±0.05	a
L2 (94 : 6)	1.13±0.02	b
L3 (91 : 9)	1.71±0.09	c
L4 (88:12)	2.28 ± 0.02	d
L5 (85:15)	2.80±0.03	e

Note: Different letters show a significant effect on the 95% confidence level (α≤0.05)

Table 5. Antioxidant activity of sago starch bagea cookies at various percentages of Moringa leaf powder

Treatment (%) (Sago starch: Moringa leaf powder)	Antioxidant activity (%)	Notation
Control (100:0)	1.56±0.24	
L1 (97 : 3)	2.67±0.21	a
L2 (94 : 6)	5.34±0.28	b
L3 (91 : 9)	8.52±0.29	c
L4 (88:12)	10.18±0.21	d
L5 (85:15)	15.25±0.32	e

Note: Different letters show a significant effect on the 95% confidence level (α≤0,05)

Table 6. Panelists' level of preference for the color of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: moringa leaf powder)	Color Value	Criteria
Control (100:0)	4.73	Neutral-Slightly like
L1 (97 : 3)	4.70	Neutral-Slightly like
L2 (94 : 6)	5.50	Slightly like-like
L3 (91 : 9)	5.97	Slightly like-like
L4 (88:12)	4.87	Neutral-Slightly like
L5 (85:15)	4.47	Neutral-Slightly like

Table 7. Panelists' level of preference for the aroma of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: moringa leaf powder)	Aroma Value	Criteria
Control (100:0)	5.47	Slightly like-like
L1 (97 : 3)	4.93	Neutral-Slightly like
L2 (94 : 6)	4.77	Neutral-Slightly like
L3 (91 : 9)	4.90	Neutral-Slightly like
L4 (88:12)	4.00	Neutral
L5 (85:15)	3.87	Slightly like-dislike

Table 8. Panelists' level of preference for the texture of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: moringa leaf powder)	Texture Value	Criteria
Control (100:0)	5.40	Slightly like-like
L1 (97 : 3)	5.40	Slightly like-like
L2 (94 : 6)	5.17	Slightly like-like
L3 (91 : 9)	5.33	Slightly like-like
L4 (88:12)	4.70	Neutral-Slightly like
L5 (85:15)	4.60	Neutral-Slightly like

Table 9. Panelists' level of preference for the taste of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: moringa leaf powder)	Taste Value	Criteria
Control (100:0)	5.83	Slightly like-like
L1 (97 : 3)	5.43	Slightly like-like
L2 (94 : 6)	4.67	Neutral-Slightly like
L3 (91 : 9)	4.83	Neutral-Slightly like
L4 (88:12)	3.77	Slightly dislike- neutral
L5 (85:15)	3.43	Slightly dislike-dislike

Table 10. Panelists' level of preference for the overall of sago starch bagea cookies at various percentages of moringa leaf powder

Treatment (%) (Sago starch: moringa leaf powder)	Overall Value	Criteria
Control (100:0)	5.70	Slightly like-like
L1 (97 : 3)	5.43	Slightly like-like
L2 (94 : 6)	5.40	Slightly like-like
L3 (91 : 9)	5.57	Slightly like-like
L4 (88:12)	4.50	Neutral-Slightly like
L5 (85:15)	4.00	Neutral

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Purple Corn (*Zea mays indurata*) Ice Cream as an Immune Booster in The Pandemic Era

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ABSTRACT

The COVID-19 pandemic has had an impact on public health. Immune resistance was a crucial factor in the pandemic era considering all diseases that arise as self-limiting diseases. Food as a source of nutrients had a vital role as an immunomodulator. As an agricultural country with food production commodities that were still massive, agricultural products are needed to develop food innovations, such as purple corn. One of the nutritional contents of purple corn was the flavonoid group in the form of anthocyanin compounds as a source of antioxidants to increase the body's immunity and prevent diseases caused by viruses, fungi, and bacteria, as well as prevent atherosclerosis, gastric damage, cholesterol, obesity, and others. Anthocyanin compounds in purple corn 70mg/100 grams. Anthocyanins in purple corn are processed into healthy food in ice cream with the product label "Purple Corn Ice Cream". This research aims to help people choose food innovations that can help them increase their immunity in the Covid-19 pandemic era. This ice cream product was formulated and has gone through several tests. The anthocyanin test results showed that the sample contained anthocyanins. The sensory test results stated that this product had a soft texture, milky smell, light purple colour, and sweet taste, so it was accepted and received a positive response from the community. Based on the total questionnaire assessment results, 88% of respondents accepted, it was expected to be a nutritious food innovation, popular with people regardless of age, practical, and durable with the content of flavonoid anthocyanin compounds in purple corn, which can improve the body's immune system during a pandemic.

Keywords: Pandemic, Immune, Anthocyanin, Purple Corn, *Zea mays indurata*, Ice Cream

INTRODUCTION

Nowadays, The Corona Virus Disease nineteen (COVID-19) pandemic has overwhelmed healthcare systems around the world, especially in Indonesia. This situation appears to be a crisis for the country and

requires the government to manage the pandemic as soon as possible before it worsens. Instead, the government of Indonesia and many countries worldwide have taken steps to solve the impact of COVID-19 by creating sequence policies such as local lockdown and imploring people to do healthy lifestyle, physical distancing, using a mask, take a vaccine, and so forth. Nevertheless, COVID-19 is an infectious disease caused by a virus called SARS-CoV-2, and just like other infectious diseases, COVID-19 is classified into a self- limiting disease that is typically not affected by any medication and tends to persist. How long our body would take to recover from the COVID-19 is depending on how strong our immunity is and whether we have any serious health condition or not. That is why we need an immunomodulator intake as an immune booster to fight against the virus (Hidayah et al., 2014). Immunomodulator are substance that can modulate (alter or affect) the body's immune system in an average direction (Praworo, 2011).

One of the best ways to increase immunity and help to make it stronger is with some particular food. Certain nutrients in foods are vital in maintaining our health and boosting the immune system, such as flavonoids (Devagaran and Diantini, 2012). Purple corn contains high amounts of anthocyanin flavonoids, which is 70mg/100gram (PT. Advanta Seeds Indonesia, 2019). As an agrarian country, purple corn is one of the commodities cultivated quite massively in Indonesia, especially in the last decade. However, the utilization of purple corn into innovative food products, efficacious, and high selling value's products is still relatively rare. The population of purple corn in Indonesia is not as much as yellow and white corn, but the nutritional content is much higher than other genotypes (Nursa'adah et al., 2017). Purple corn contains anthocyanin, an antioxidant to boost the immune system, prevent atherosclerosis, disease blockage of blood vessels, lose the stomach to damage, inhibit tumour cells, improve eye vision ability, and serve as anti-inflammatory compounds that protect the brain from violence (Nursa'adah et al., 2017). The research results from Pamandungan and Ogie (2017) also reinforce that purple corn with a high anthocyanin content acts as an antioxidant compound in improving immunity and prevents several diseases such as cancer, cholesterol, and coronary heart.

The nutritional content of purple corn needs to be innovated through food to introduce purple corn as an alternative intake rich in efficacy, especially as an immunomodulator. This innovative food can be applied in products favoured by most society: children, adolescents, and adults, one of them is ice cream. Referring to the current research results, the consumption of ice cream continues to increase every year and make ice cream the most favourite food in all circles (Sianipar et al., 2016). Departing from above, we are interested in creating a food innovation in purple corn-based ice cream as a processed food that can be consumed to increase immunity during the COVID-19 pandemic.

MATERIALS AND METHODS

Research Design

This study used a descriptive research design with a qualitative approach. The data were collected using previous research related to the topic, laboratory tests, questionnaires, and several literature sources.

Tools and Materials

The ice cream ingredients were purple corn, fresh milk, water, sweetened milk, sugar, whip cream powder, vanilla extract, and SP.

All ingredients except purple corn were bought from mini market. Purple corn is

obtained from agricultural land owned by PT. Advanta Seeds Indonesia in Papar Village, Kediri City, East Java Province, Indonesia.

The tools used in making ice cream include mixer, blender, knife, freezer, pan, basin, strainer, stoves, and ice cream package. While the tools for the anthocyanin test were test tubes, bunsen, pipette, measuring glass, and beaker glass.

Ice Cream Formulation

The first step in making ice cream was to peel and wash purple corn with clean water. Then the purple corn was blended with fresh milk and sweetened condensed milk until smooth. Next, the mixture was filtered and boiled until thicked. Next, the mixture cooled at room temperature. After the mixture cooled, it was mixed by adding whip cream and SP using a mixer for 10 minutes. Put the mixture in the freezer at a temperature of 1°C for 24 hours. Then remove the ice cream mixture from the freezer and mix again for 5 minutes until the mixture becomes smooth. Put the ice cream mixture into the package. Finally, put the ice cream into the freezer until frozen. The formulations of the ingredients for ice cream making ca n be seen in Table 1.

Research Variables

The independent variable used in this research was purple corn, while the dependent variables were anthocyanin, HCl reagent, and NaOH 2M.

Research Sample

The total panellist needed to try "Purple Corn Ice Cream" are 50 males and females of various ages, based in Malang, East Java, Indo. Panellist are randomly selected.

Organoleptic Test

The organoleptic test is a how-to test using the senses of humans as the primary tool for measurement receptivity to the product (Ningrum et al., 2017). An organoleptic test was carried out by preparing a test sample of purple corn and ice cream products. The senses used in assessing the nature of senses are the senses sight, touch, smell, and taste. Panelists were asked to explain purple corn and ice cream products' organoleptic (texture, color, smell, and flavor).

Hedonic Test

The hedonic test is used to measure the level of preference for a product. This preference is called the hedonic scale. Panelist shows their level of preference for each sample by choosing the category in accordance (Ningrum et al., 2017). Panellists used in the hedonic test was an untrained panellist of 50 people in the Malang area. Panellists were chosen randomly and came from various age groups. A sensory test was carried out using the questionnaire Google Form. Parameter tested in the form of texture, colour, aroma, taste, and total acceptance. The panellist's hedonic test scores started from (immensely dislike) to 5 (very much like).

Anthocyanin Test

Anthocyanin test used to determine the anthocyanin content in purple corn and product "Purple Corn Ice cream." Firstly, the sample was heated with 2M HCl for 2 minutes using a temperature of 100 °C, the colour of the sample was observed. If the red colour in the sample does not change (constant), it indicates a positive of anthocyanins. In the second step, the sample was mix by adding 2M NaOH dropwise. When the red colour changes to green blue and fades slowly, it indicates a positive of anthocyanins (Lestario et al., 2011).

RESULTS AND DISCUSSION

The organoleptic test is used to examine the physical appearance of the raw

material for purple corn and ice cream products, including texture, smell, colour, and taste (Table 2, 3).

The hedonic test was used to check the panelists' preferences regarding ice cream products. Thepopulation in this hedonic test is men and women who live in the Malang area with an age range from toddlers (3 years) to old adults (44 years), total of 50 panelists. The percentage of male panelists is 62%, and the percentage of female panelists is 38%.

Parameters tested in the hedonic test include texture, colour, smell, taste, and overallassessment of ice cream. Parameters were assessed using a score of 1-5 (very dislike-very much like) with a total of 50 panellists from various age groupsthrough the google form media (Table 4).

Based on the test results for filling out the question number 1 questionnaire, one panellist did not like it, five panellists were neutral, 21 panellists liked it, and 23 panellists liked the texture of the product "Purple Corn Ice Cream." The results of filling out the number 2 questionnaire, two panellists do not like it, eight panellists are neutral, 17panellists like it, and 23 panellists like the colour of the product "Purple Corn Ice Cream." The results of filling out the number 3 questionnaire show that one panellist does not like, nine panellists are neutral, 18panellists like, and 22 panellists like the smell of the product"Purple Corn Ice Cream." The results offilling out the number 4 questionnaire show that one panellist strongly dislikes it, twopanellists do not like it, 17 are neutral, 15 panellists like it, and 15 panellists like the taste of the product "Purple Corn Ice Cream." The processed product frompurple corn "Purple Corn Ice Cream" has good organoleptic (texture, colour, smell, and taste) to enjoy itby various ages, from children to old adults. Based on the results of filling out questionnaire number 5,there are six neutral panellists, 23panellists like, and 21 panellists who like the product "Purple Corn Ice Cream" as a whole. It proves that the processed purple corn product "Purple Corn Ice Cream" has received a positive response from the widercommunity.

Anthocyanin Test

Anthocyanin test was conducted to determine the secondary metabolite compoundsof anthocyanins in purple corn. The anthocyanin test was carried out using a phytochemical screening method. The samples tested were purple corn powder and ice cream products. The anthocyanin test results can be seen in the Figure 2.

Based on the anthocyanin test results using thephytochemical screening method, purple corn taken from thecultivation of the Malang area waspositive for anthocyanin. This positive result was indicated by the samples' colour changing (a and b) from purple to red on the heating reaction and the 2M HCl reagent. Meanwhile, the addition of 2M NaOH (c and d) has a positive result indicated by a change in the colour of the samplefrom purple to green. However,there is a difference in the positiveresults in the purple corn sample and the ice cream product.

The ice cream product had a lighter cheerful colour than the purple corn sample caused by reduced levels of anthocyanins while making ice cream products. Although the content of anthocyanin compounds in purple corn is quite large, processing treatments such as heating can reduce anthocyanin content in processed products. The heating result is the loss of some nutrients, especially labile ones such as ascorbic acid, anthocyanins, and beta-carotene (Budiarto, 1991).

Anthocyanins are natural dyes belonging to the flavonoid group with three carbon atoms bonded by an oxygen atom to connect two benzene aromatic rings (C6H6) in the main structure. As a bioactive compound, the arrangement of conjugated double bonds in the anthocyanin structure

makes anthocyanins function as natural antioxidant compounds in humans (Barrowclough, 2015). Anthocyanins can scavenge various types of reactive oxygen-derived free radicals, such as hydroxyl (OH*), peroxyl (ROO*), and single oxygen (O2*) (Azima et al., 2014). These free radicals in the body are formed by pro-oxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, nicotine, other chemicals, and air pollution.

Antioxidants from anthocyanins have benefits in preventing various degenerative diseases, such as cardiovascular diseases, such as atherosclerosis, by inhibiting and reducing cholesterol levels in the blood caused by LDL oxidation (Wallace, 2011). The anthocyanin acylation process can increase antioxidant activity (Sari et al., 2015). Research says that anthocyanin is a valuable antioxidant to protect the body from free radical attacks and boost the immune system, prevent atherosclerosis, disease blockage of blood vessels, lose the stomach from damage, inhibit tumour cells, improve eye vision ability, and serve as anti-inflammatory compounds that protect the brain from violence (Nursa'adah et al., 2017).

According to laboratory results conducted by PT Advanta Seeds, the anthocyanin content in purple corn is 70 mg/100 gram, which means that in the total ice cream formulation, which is 500 grams of purple corn, there are 350 mg anthocyanins. This amount is quite large, so it has the potential to be processed into food products amid the urgency of the COVID-19 pandemic.

In addition, anthocyanins have high anti-viral, anti-fungal, and anti-bacterial activities (Hidayah et al., 2014; Saira and Kamran, 2017). Several types of flavonoids, such as anthocyanins, are thought to have biological activity in inhibiting several coronavirus proteins or preventing lung inflammation and cytokine storms which are severe consequences of SARS-CoV-2 infection (Tutunchi et al., 2020). Because it has many benefits, theflavonoid anthocyanin compoundscontained in purple corn have the potential to be used to improve the body's immune system during the COVID-19 pandemic.

CONCLUSION

Purple corn and "Purple Corn Ice Cream" products were positive for anthocyanins in Acid and Base test. In addition, "Purple Corn Ice Cream" products received a positive response from the public that can be seen from the total acceptance of ice cream by 88%.

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Figure 1. "*Purple Corn Ice Cream*" and purple corn

Table 1. The ice cream ingredients

Ingredients	Unit
Fresh milk	1000 ml
Purple corn	500 g
Water	300 ml
Sweetened condensed milk	200 g
Sugar	200 g
Whipe cream powder	150 g
Vanilli extract	1 tsp
SP	1 tsp

Table 2. Purple Corn Organoleptic Test Results

Parameter	Description
Texture	Hard seeds
Smells	No smell
Color	Blackish purple
Flavor	Tasteless

Table 3. "*Purple Corn Ice Cream"* Organoleptic Test Results

Parameter	Description
Texture	Soft
Smells	Milky
Color	Violet
Flavor	Sweet

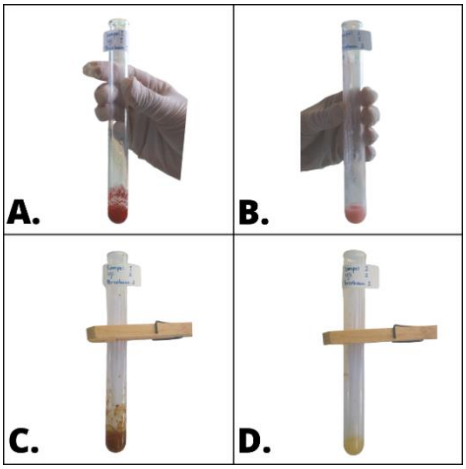

Figure 2. Anthocyanin test 1 of purple corn sample (a), Anthocyanin test 1 of Purple Corn ice cream sample (b), Anthocyanin test 2 of purple corn sample (c), Anthocyanin test 2 of Purple Corn ice cream sample (d)

Table 4. Hedonic Test Results

Questions	1	2	3	4	5
Texture of the product	0 (0%)	1 (2,2%)	5 (10%)	21 (42%)	23 (46%)
Color of the product	0 (0%)	2 (4%)	8 (16%)	17 (34%)	23 (46%)
Smell of the product	0 (0%)	1 (2,2%)	9 (18%)	18 (36%)	22 (44%)
Taste of the product	1 (2%)	2 (4%)	17 (34%)	15 (30%)	15 (30%)
Rate of the product	0 (0%)	0 (0%)	6 (12%)	23 (46%)	21 (42%)

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Sodium Cyclamate Identification and Determination of Dawet Ice Sold in Wedi District Indonesia

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ABSTRACT

Sweeteners are food additives that are added to food or beverages to create a sweet taste, improve taste, aroma as well as a source of calories for the body. Sodium cyclamate is a sweetener food additive that is permitted to be used in food products but is often misused by food manufacturers. Excessive consumption of sodium cyclamate can cause bladder cancer, stomach pain, headache, and fever. This study aims to determine the content of sodium cyclamate in dawet ice sold in Wedi District. This study uses descriptive methods for both qualitative and quantitative. Identification of sodium cyclamate content in dawet ice sold in Wedi District using the precipitation test method, determination of sodium cyclamate content was determined quantitatively using UV-visible spectrophotometry. The results of the precipitation test showed that 5 positive samples contained artificial sodium cyclamate, with sodium cyclamate levels of 58.683 mg/L, 79.466 mg/L, 95.066 mg/L, 94.116 mg/L, and 79.5 mg/L, respectively. The conclusion is that the sodium cyclamate content in dawet ice sold in Wedi District from all samples analyzed does not exceed the maximum usage limit set by BPOM Regulation of the Republic of Indonesia No. 4 of 2014, concerning the Maximum Limit for the Use of Sweetener Food Additives, amounted to 250 mg/kg.
Keywords: sodium cyclamate, dawet ice, wedi district

INTRODUCTION

Food safety is an important requirement that must be attached to food that will be consumed by all Indonesian people. Safe and quality food can be produced from household kitchens and the food industry (Siregar et al., 2013). Food is one of the basic human needs. Therefore, the problem of food procurement from the production stage to the consumption stage must be handled from post-harvest until consumption is greatly assisted by food technology which is closely related to the properties of the food itself (Simarmata, 2018).

Today's, food products have various forms, both in terms of type and taste and processing methods. The rapid development on food processing techniques, the addition of addictive ingredients on food products is difficult to avoid. Fresh drinks which are sold on the roadside is an alternative for small and medium-sized traders in Indonesia. The drinks sold on the roadside are suspected of using synthetic sweeteners (cyclamate) to make the drinks taste. Based on instructions from the Ministry of Health of the Republic of Indonesia, the use of cyclamate is only intended for patients or for people who need

low-calorie food, but in fact, the use of cyclamate is increasingly widespread from the small and medium business sector with a variety of snacks. This is because the price is cheaper, and has a sweetness level of 30 times of sugar (Sarumaha, 2019).

Food additives in purer forms and commercially available at relatively low prices will encourage increased use of food additives, which means increasing consumption of these materials for each individual. The development of technology in the production of artificial sweeteners in processed food, both food, and beverages, is very popular with large industries and home industries because the price is cheaper, and the intensity of sweetness is higher than natural sugar(Nurjannah, 2012). The role of food additives is very important in line with advances in technology for the production of synthetic food additives. Many food additives are available in pure form which is commercially available at low prices so that they can encourage increased consumption of food additives, this has started to happen since the middle of the 20th century (Utomo et al., 2012).

The use of chemicals as one of the additives in food and beverages is currently common. Additional ingredients are ingredients that are intentionally added to food and beverages to get better quality. Additional ingredients known as addictive substances in food or beverages can be in the form of dyes, flavorings, aromas, stabilizers, antioxidants, preservatives, emulsifiers, bleaches, thickeners, and sweeteners (Handayani & Agustina, 2015). Sweeteners are food additives that are added to food or beverages to create a sweet taste, improve taste, aroma, improve physical and chemical properties as well a source of calories for the body. Sweeteners based on the source are divided into natural sweeteners and artificial (synthetic) sweeteners (Handayani & Agustina, 2015).

Cyclamate (Cyclamate) is a Food Additive (BTP) sweetener that is permitted to be used in food products with strict regulations but is often misused by food manufacturers and other communities. Cyclamate is heat resistant, so it is often used in foods that are processed at high temperatures, for example in food and beverages (Marlina, 2016). The maximum limit for the use of cyclamate in food is determined based on the food category. Dawet ice drink is included in the dessert food category with a maximum limit of 250 mg/kg (BPOM, 2014). The use of cyclamate in Indonesia is still permitted, but, the product of cyclamate metabolism, namely cyclohexamin, is a carcinogenic compound. Excretion of cyclohexamin through urine can stimulate the growth of bladder tumors in rats. Excessive consumption of cyclamate can also cause cyclamate metabolism in the stomach to produce cyclohexamin compounds which are carcinogens. This compound is capable of causing bladder cancer and is capable of causing atrophy, namely testicular shrinkage and chromosomal damage. The carcinogenic potential of cyclamate occurs when it is converted to cyclohexylamine in the digestive tract. Cyclohexylamine is toxic and is a tumor stimulant (promoter), therefore the Acceptable Daily Intake (ADI) of cyclamate must be determined (Sarumaha, 2019).

Research on the use of cyclamate in food and beverages has been widely carried out. Research conducted by (Simarmata, 2018), in the analysis of cyclamate levels in dawet ice was found to contain cyclamate sweeteners. Meanwhile, the results of research by (Misrawati et al., 2020), in the analysis of artificial sweeteners in snacks sold in traditional markets in Manado City, found cyclamate sweetener in 2 samples with the lowest level of 848.65 mg/kg and the highest level of 931.98 mg/kg. The sample exceeds the set limit.

The addition of cyclamate is usually used by beverage traders, such as dawet ice. Snack drinks such as dawet ice are small-scale industries that generally pay less attention to sanitation and food safety. This drink has an attractive appearance, delicious taste, and fresh making this product much liked by the public. In the manufacturing process, manufacturers often use artificial sweeteners that aim to replace natural sweeteners to reduce production costs (Marliza et al., 2020). Based on this description, research was conducted on the Identification and Determination of Sodium Cyclamate Levels in Dawet Ice Sold in Wedi District. This study aims to determine the content of sodium cyclamate in dawet ice sold in Wedi District.

MATERIALS AND METHODS

Tools and Materials

The materials used in this study were dawet ice samples, 10% hydrochloric acid solution, 10% barium chloride solution, 10% nitrite solution, acetic acid, ethyl acetate, 10N sodium hydroxide, cyclohexane, 30% sulfuric acid, 1% free chlorine, standard solution of sodium cyclamate, concentrated sulfuric acid, 0.5 N NaOH, aquades. The tools used in this research are analytical balance, blender, volume pipette, test tube, measuring cup, filter paper, Erlenmeyer flask, separating funnel, hotplate, water bath, and UV-Vis spectrophotometer.

Method

This research is descriptive research with a total sampling technique. There are 5 samples of dawet ice from 5 trader sold in Wedi District, Klaten, Central Java, Indonesia. Sample preparation was carried out by homogenizing the dawet ice sample, then filtering it with filter paper. The filtrate solution was then used for qualitative and quantitative analysis. Qualitative analysis of cyclamate using precipitation method ([BSN] Badan Standardisasi Nasional, 1996). Quantitative analysis of cyclamate levels using the UV-Vis spectrophotometric method (Kurnia, 2017). The data obtained from the results of research in the laboratory were analyzed descriptively in the form of tables and graphs.

RESULTS AND DISCUSSION

This study aims to determine the content of sodium cyclamate in dawet ice sold in Wedi District. Sampling was carried out based on total sampling. In this study, the precipitation test method was used for qualitative analysis and the UV-Visible spectrophotometric method for quantitative analysis.

The dawet ice sample obtained from Wedi District contained 5 samples, namely sample A, sample B, sample C, sample D, and sample E. The samples obtained were then identified qualitatively with sodium cyclamate by precipitation test. The results of qualitative analysis of sodium cyclamate content can be seen in the Table 1. Based on Table 1, it can be seen that of the 5 samples tested, all positive samples contained sodium cyclamate which was characterized by the formation of a white precipitate.

The principle of the precipitation method is that the sample which is proven to contain artificial sweetener sodium cyclamate is seen by the formation of a white crystalline precipitate from the reaction between 10% BaCl2 and 10% NaNO2 which produces a BaSO4 precipitate (Sudjadi, 2012). Qualitative testing of sodium cyclamate started with homogenized the sample between solids and liquids then filtered to get the filtrate. Sodium cyclamate reacts with hydrochloric acid to produce primary amines (cyclohexamine), sulfuric acid, and sodium chloride. The sulfuric acid formed reacts with barium chloride to form a precipitate of barium sulfate which is suspended in the mixture. The addition of

10% HCl serves to acidify the solution. The solution is made in an acidic state so that the reaction that will occur can be more easily reacted. The addition of 10% BaCl2 aims to precipitate impurities in the solution, such as the presence of carbonate ions. The addition of 10% NaNO2 serves to break the sulfate bond in the cyclamate. The heating process serves to form nitrogen gas. When the sulfate bond has been broken, the ions will react with sulfate ions and produce a precipitate of barium sulfate (BaSO4) (Rosdayani, 2018). The nitrogen gas produced from this reaction can be identified by the presence of a pungent odor during the heating process. The white precipitate cyclamate in the sample (Suliati, 2020). The 5 samples on this study indicated the positive contain of artificial sweetener sodium cyclamate.

These positive samples, then analyzed quantitatively using UV-Visible spectrophotometry. Quantitative analysis was carried out on positive samples to determine the amount of cyclamate sweetener content in the sample. Quantitative analysis was performed using the UV-Visible spectrophotometric method. This method is used because of the presence of a chromophore group in the chemical structure so that it can be detected by a UV-Visible detector. Solvents and chemical molecular structures containing chromophore groups are the ones that affect the maximum wavelength (Susanti, 2013).

The preparation of standard solutions is the first step in determining the levels of cyclamate sweeteners. A standard solution is a solution that contains a precisely known concentration. Determination of the maximum wavelength using a standard solution with a concentration of 1800 ppm and measured at a wavelength of 200-400 nm. The determination of the maximum wavelength aims to determine the maximum absorption area that can be produced in the form of the absorbance value of a test solution. The maximum wavelength of sodium cyclamate solution is obtained at a wavelength of 252 nm by giving an absorption or absorbance of 0.672 and is still in the range of the optimum absorption area of sodium cyclamate, which is 200-400 nm so that it can be said that the measurement results meet the requirements for use for analysis. The absorbance value of the cyclamate standard solution quantitatively can be seen in the Table 2.

The calibration curve was obtained by measuring the absorbance of standard solutions with concentrations of 1000, 1200, 1400, 1600, 1800 ppm at a wavelength of 252 nm. From this measurement, the regression equation is obtained, namely $y = 0.0006x – 0.3602$ with an R^2 value of 0.9966. The R^2 value indicates that there is a very strong correlation between concentration and absorbance (Hidayat, 2019).

The absorbance value of the sodium cyclamate standard solution above can be used to determine the linearity test with the correlation (r) in the linear regression equation $y = ax + b$. The graph of the calibration curve and the linear regression equation can be seen in Figure 1.

Determination of sodium cyclamate levels is done by first homogenizing the sample between the solid and the liquid. Sodium cyclamate is reacted with H_2SO_4 to convert cyclamate into cyclamic acid, then the cyclamic acid solution is reacted with ethyl acetate to form cyclamic acid in the organic phase and there are two colorless layers. Extraction of cyclamic acid with distilled water aims to bind the cyclamate compound in the sample thoroughly so that it is separated from other sample components. The addition of NaOH solution serves to provide an alkaline atmosphere while the addition of cyclohexane functions as a cyclamate extractor. The extract from the cyclamate was reacted again with H_2SO_4, cyclohexane, and Na-hypochlorite to form

two layers. The cyclohexane layer was taken and washed with NaOH to form a colorless solution. In this cyclohexane layer, the cyclamate has been extracted in it, then diluted with distilled water, and then the absorbance was measured using a UV-Visible spectrophotometer (Padmaningrum & Marwati, 2015).

The absorbance obtained from each sample is then entered into the linear regression equation (y = 0.0006x – 0.3602) to obtain the sample concentration (x). The average levels of sodium cyclamate in the samples were 58.683 mg/L, 79.466 mg/L, 95.066 mg/L, 94.116 mg/mL and 79.5 mg/L.

Based on the quantitative tests carried out and shown in Tabel 3, the sodium cyclamate levels in sample A are 58.683 mg/L, sample B is 79.466 mg/L, sample C is 95.066 mg/L, sample D is 94.116 mg/L and sample E is 79,5 mg/L. These data indicate that the levels of the dawet ice samples tested have not exceeded the maximum usage limit set by the BPOM Regulation of the Republic of Indonesia Number 4 of 2014 concerning the maximum limit for the use of sweetener food additives, which is 250 mg/kg. The low levels obtained are still safe for consumption but the use of artificial sweeteners is more specifically for people such as diabetics whose aim is to control excess sugar levels or obese people, but also must be within certain limits and must be supervised by a doctor or health expert (Musiam, 2016).

The results of this study showed lower levels than the results of previous studies. Research by Misrawati et al. (2019), it is known that the results of the cyclamate test on mixed ice snacks circulating in elementary schools in Kendari City, 4 positive samples contained cyclamate artificial sweeteners with the highest cyclamate content of 300 mg/kg. According to (Hadju et al., 2013), showed that the results of testing cyclamate on snack drinks in the traditional market of Manado city that two positive samples contained cyclamate sweeteners with cyclamate levels of 931.98 mg/kg and 848.65 mg/kg. These levels indicate that the sample has exceeded the maximum limit for the use of sodium cyclamate that has been set, which is 250 mg/kg.

Based on the results of the study, consuming sodium cyclamate above the specified limits can trigger health problems, including tremors (neural diseases), migraines, headaches, memory loss, confusion, insomnia, irritation, asthma, hypertension, diarrhea, stomach pain, allergies. , impotence, baldness, and brain cancer (Hadju et al., 2013). Other problems related to sodium cyclamate consumption are cardiovascular and nervous system problems, decreased growth rate, bladder cancer, thyroid adenoma, abnormalities in red blood cells, leukocytes, monolayers, spinal cord, and bacterial infections (Hidayat, 2019). Therefore, it is recommended for the community to consume beverages, especially dawet ice, in moderation.

CONCLUSION

This study concluded that the 5 samples of dawet ice drinks sold in Wedi District are contain the artificial sweetener sodium cyclamate. The content of sodium cyclamate in the sample is under the maximum limit of the regulation which is set by BPOM of the Republic of Indonesia.

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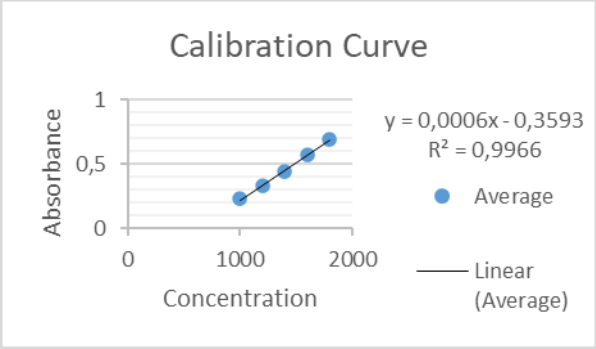

Figure 1. Sodium Cyclamate Standard Solution Calibration Curve

Table 1. Results of Qualitative Analysis of Sodium Cyclamate Precipitation Method

No	Sample	Observation Result	Interpretation
1.	A	Presence of white precipitate	Positive (+)
2.	B	Presence of white precipitate	Positive (+)
3.	C	Presence of white precipitate	Positive (+)
4.	D	Presence of white precipitate	Positive (+)
5.	E	Presence of white precipitate	Positive (+)

Table 2. Absorbance Value of Standard Solution of Sodium Cyclamate at Maximum Wavelength of 252 nm

Concentration (ppm)	Absorbance
1000	0,233
1200	0,331
1400	0,439
1600	0,570
1800	0,694

Table 3. Sodium Cyclamate Levels in Dawet Ice based on UV-Visible Spectrophotometer

No	Sample	Average Absorbance	Average rate ± SD (mg/L)
1.	A	0,344	58,683 ± 0,0577
2.	B	0,593	79,466 ± 0,2753
3.	C	0,780	95,066 ± 0,0577
4.	D	0,769	94,116 ± 0,0288
5.	E	0,594	79,5 ± 0,4582

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