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PREFACE

By the Grace and Blessings of Allah the Almighty, we would like to present, with great pleasure, the Volume 05 number 02 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, December 2023

Editorial Team

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Pigskin Treatment Using Different Food-Grade-Acids: Effects on The Physicochemical Characteristics of The By-Products

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ABSTRACT

Upcycling foods contributes to reducing food loss and waste and provides sustainable solutions to novel products. In the present work, it was studied the use of food-grade acids (Acetic(AH), Latic(AL), Citric(AC), and Ascorbic(AA) acid) to obtain pigskin by-products, acid-soluble collagen (ASC), and gelatin (G). The aim was to evaluate the effect of the use of different food-grade acids on pigskin by-product characteristics. The physicochemical and thermal features, including Hydroxyproline (Hyp), pH, Differential Scanning Calorimetry (DSC), and color, were evaluated on by-products. The ASC and G solutions pH´s showed relation with the acid solution pH used. The AH and AA ASC fractions, showed lower Hyp content than AC and AL-treatments. By contrast, G Hyp-content was higher for AH and AA than AC and AL-treatments. The dried ASC-AH and -AA thermal transition temperatures (Td) resulted lower than AL and AC. The four dried-G samples showed an endothermic signal around 120 °C but with differences on enthalpy values. Current results suggest that the acid used and the pH of the solution during the thermal process would affect the physical-chemical properties of the by-products. The possibility to obtain different pigskin by-products using food grade acid could be an option for obtaining novel ASC and G use. Independently of the treatment, the G by-product was the main yield. Likewise, further studies are required to understand the by-products chemical differences and their potential uses.

Keywords: Biopolymers, DSC, Upcycling food products, Loss and Waste Food

INTRODUCTION

The principal sources of collagen are skin, bones and tendons, which are the main slaughter waste, especially from pigs and bovines. These meat-processing industry losses and wastes are rich in collagen

proteins, a source of collagens and gelatins–their derivative by-products–, all of them used in numerous industries and processes.

Collagen Type-I is the type of collagen that animals mostly produce naturally (90%). Its most prominent functional roles in the skin and bone, and to a lesser of other tissues. This polymer is formed by two α-1 chains and one α-2 chain, which are joined into a triple helix, heterotrimeric α1(I)2–α2(I) (Amirrah, et al., 2022). The gelatin is a polypeptide product of the hydrolysis and thermal denaturation-disintegration of collagen fibres. The process to convert insoluble collagen in soluble gelatin requires a treatment to destroy the tertiary, secondary and partially primary structure by breaking non-covalent bonds (See et al., 2015). It is known that the non-covalent bonds disruption could cleave inter- and intra-molecular covalent crosslinks, without cleavage of any peptide bonds. This hydrolysis allows the conversion of collagen molecules (molecular weight ≈345,000-360,000) into small molecules (molecular weight ≈10,000-65,000).

The main methods used for collagen extraction are acid, enzymatic or alkali hydrolysis at low temperature (~4 °C) Matinong et al. (2022). The tissue treated in low concentration-acid allows the destabilization of the salt bonds between molecules and Schiff bases and causes collagen fibres to expand and dissolve Hua and Zibin (2014). Acetic, lactic, or citric acids (0.2-0.5M) have been used to study native collagen extractions of several tissues. For example, Liu et al. (2001) studied the effect of different organic acid solutions (acetic, citric and lactic) on the native collagen obtained from chicken feet. Kanwate &Kudre (2017) evaluated the influences of various acids (acetic, phosphoric, and propionic acids) used for gelatin extraction from the fish *Labeo rohita*, and observed that the acid affected the gelatin yields, the triple-helix loss and gelatin pH solubility. Moreover, Sompie et al. (2015) studied the influences of acetic acid concentration and extraction temperature on pigskin gelatin physical and chemical characteristics. The authors observed that the optimal gel strength, viscosity, protein concentration and pH of the gelatin solution were obtained using 4% acetic acid. More recently, Chakka et al. (2017) evaluated the gelatin extraction from chicken feet using different food-grade acids (acetic, citric and lactic acids) at different concentrations.

Nowadays, collagen and gelatin by-products present interest in food, pharmacology, cosmetic industries, and tissue bioengineering. Today, the by-products collagen and derived-peptide are considered as important components of innovative sustainable food systems. These animal by-products show novel impacts on the food system, processing, packaging, preservation, and functional foods (Changwei et al., 2021). Furthermore, several industries are interested in gelatin due to its property to form a three-dimensional network or gel at concentrations and temperatures conducive to chain entanglement. In addition, the new food implementations, like those used in bread, yogurts, drinks, etc., require new properties, such as a higher glass transition temperature, soft gels, etc. The yields and characteristics of the obtained by-products collagen and gelatin depend on the tissue source, extraction methods, and the extraction process conditions.

The steps to convert collagen to gelatin require hydrolysis as a necessary first step, following thermal denaturation. The denaturation temperature of pigskin collagen is ~65-67 °C (Li et al., 2020). The hydrolysis determines the gelatin types, the acid treatment allows to obtain Type-A gelatin, the alkali treatment, the Type-B gelatin. The acid treatment is widely used because the isoelectric point of range of gelatin type A is

pH 6-9, so presents a wide range of food, pharmaceutical and industrial uses Mariod, et al., 2013). The acid solutions used (concentration, time, temperature, etc.) have an effect on the non-covalent bond disruption and inter-and intra-molecular covalent crosslinks cleavage (Liu et al., 2001). The importance of this step is "opening up" the protein structure, breaking the intra- and intermolecular crosslinks (See et al., 2015), which is necessary for the following thermal collagen denaturation to obtain gelatin.

Thus, this research aimed to investigate the effect of the use of different food-grade acids to obtain pigskin by-products: i-acid soluble collagen and ii-gelatin. The by-products chemical and thermal characteristics were evaluated. Knowledge of the properties of by-products would allow assessment of potential uses in different and/or new applications.

MATERIALS AND METHODS

Material

The frozen skin from the pig carcass, was provided by slaughterhouse for pig habilitated. This was the object of this study. Before treatment, the pigskin –free from fat– and ears (approximately 5x5 mm) were washed with water (1:5 m:V; g:ml) during 2 hours at room temperature for globular protein extraction. Afterwards, the pigskin was filtered and, once clean, it was stored at 18 °C.

The pigskin tissue composition was analysed. Moisture content was determined using gravimetric method (drying in oven 80 °C during 48 h). Total Nitrogen by Kjeldahl method was performed according to method 992.15 of the AOAC International (2012), amount of total nitrogen in the raw materials were multiplied by a conversion factor of 6.25. Lipid content was evaluated using hexane:isopropanol extraction (3:2; v:v) (Saini et al., 2021) . Ash content was quantified as total dry matter residue obtained by burning in muffle at 550 ± 10 °C (20 h).

Acid and Thermal treatments

The pigskin frozen samples were treated with different food-grade acid solutions (0.5M) –Acetic acid (AH), Lactic Acid (AL), Citric Acid (AC) and Ascorbic Acid (AA)–. Figure 1 outlines the overall process employed for obtaining the by-products from the pigskin. The frozen tissues (wet base [w.b.]) were soaked in each acid solution at a ratio 1:5 (m:v; g:ml) during 24 h at 4-8 °C, with stirring. Acid soluble collagen (ASC) obtained after the acid treatment was filtered and separated. The liquid ASC was precipitated with NaCl at 4 °C during 48 h. ASC was centrifuged at 5,000 rpm during 10 min (Jouan®, BR 4i Centrifuge, Saint Herblain, France) and the pellet was separated and dried at 37°C 24 h. The swelling pigskin acid insoluble fraction (solid residue) was re-suspended in water (1:5 m:v) and heated at 85-90 °C during 90min (collagen thermal denaturation process) with the aim of obtaining gelatin (G) solutions. Each G solution was filtered at 45 °C and separated from the thermal insoluble fraction. Glycerol was added in each G solution (0.8% Glycerol w/v), previously to the drying process. The solution G-glycerol was heated at 60 °C with stirring (125 rpm) during 30 min. Finally, each solution was fractioned (15 ml) on silicone plates and dried during 48 h at 37 °C. The dried samples (dried G in Fig.1) were stored in desiccators 7 days at 25 °C with blue silica gel (cobalt chloride, indicator). The insoluble residue, resistant collagen (RC), was dried at 37 °C during 24h (dried RC in **Fig.1**). All treatments were performed three times. Figure 1 outlines the overall process employed to obtain the pigskin by-products, ASC and G, by physical-chemical treatment.

Previously to the drying process, the pH was measured in each solution (ASC and

G). Total Hydroxyproline (Hyp) was quantified in ASC and G solutions, dried RC and raw tissue (pigskin wet base (w.b.)).
Dried ASC and dried G samples were used for DSC analysis. The color was evaluated on dried G samples.

Analysis of pH

The pH of the acid solution and ASC and G solutions was measured using a pHmeter (HANNA Edge®; HI5222, made in Romania, Woonsocket, RI 02895 USA).

Chemical analysis. Quantification of Hydroxyproline.

The samples were hydrolyzed in HCl (6N) at 110 °C for 16 h (m:V; 1:10; g:ml). After hydrolysis, samples were neutralized and the Hydroxyproline (Hyp) concentration was determined according to Velazquez & Latorre (2019). The total collagen content was calculated by using a correction factor of 7.55. The values were expressed as mg Hyp and mg Collagen per gram of pigskin (w.b.). The yield of each fraction (ASC and G) was calculated by the following equation:

$$\text{Yield \%} = \frac{\text{Hyp content of fraction*}}{\text{Total Hyp content**}} \times 100$$

*Hyp content in each collagen fractions, ASC, G and RC. **Sum of ASC, G and RC Hyp content of each treatment.

Differential Scanning Calorimetry (DSC)

Dried ASC and dried G samples were analysed using a DSC Setaram Evo 131. Samples of ~10 mg mass and encapsulated in small aluminium sample pans were evaluated. Non-isothermal DSC curves were obtained using heating rates of 10 °C min-1, from 25 °C to 300 °C using Ar as sweeping gas, and an empty pan as reference. After proper baseline correction using a polynomial function, enthalpies (ΔH) and mean transition temperatures (Td) were determined from the curves.

Color

The dried G samples were placed on a white tile and CIE color space coordinates L*, a* and b* values were acquired three times, using a Minolta Chroma meter CR-400 (Minolta Co. Ltd., Osaka, Japan) with illuminate D65 and α: 2° observer angle.

Statistical analysis

All experiments were performed at least three times. The results are reported as mean, standard deviation (±sd) and standard error (SE). Comparisons among the results of each treatment were performed by one-way ANOVA with Post-hoc Tuckey´s post-test (α 0.05). The statistical analysis was carried out using Graph-Pad Prism version 5.00 for Windows, Graph-Pad Software, San Diego, California USA http://www.graphpad.com

RESULTS AND DISCUSSION

Pigskin tissue characteristics

The chemical composition of the pigskin raw tissue is shown in **Table 1.** The results showed high protein content (Total Nitrogen with a Factor 6.25). The total collagen content confirms that this protein is the major protein in pigskin tissue. The composition characteristics (55% water, 35% connective tissue (collagen), 5–10% fat) (Feiner, 2006) have allow to use as effective alternative ingredients and components the lower protein in non-protein ingredients (Alves et al., 2016; Olivera et al., 2017). The knowledge of the total lipid content shows the importance of a previous lipid extraction step for future studies. The lipid extraction allows obtaining purer collagen by-products.

Physicochemical characteristics of the by-products, collagen and gelatin.

The pH of the food-grade acid solution, ASC and G solutions, soluble fractions obtained from the acid and thermal

treatments, are shown in **Table 2.** The pH in the ASC solutions (soluble fraction) as expected, showed relation with the values of the acid solution used. The hydrolysis step (acid treatment or enzymatic) is necessary in the collagen extraction protocols (Xu, et al., 2021).

During this process the tissue is swollen and the electrostatic intra- and intermolecular collagen interactions are weakened and some collagen molecules (ASC) may be solubilized. The laboratory experiment showed that the four insoluble fractions after acid treatments AH, AL, AC and AA (24 hours at 4 °C) exhibited good and equal swellings. Choe & Kim (2018) indicated that the optimum swelling times were observed when the soaking solution had a constant pH (1.68-1.88) during 24 h at 4 °C. During this first step (hydrolysis) the polypeptide chains and the cross-linkages are broken (hydrogen bonds are destroyed) allowing to the denaturation of the collagen protein (triple helix) during heat treatment (Gorgieva and Kokol 2011). According to Choe and Kin (2018), for pig and chicken skin, acid processing is the most suitable treatment. Acid process is applied in the industry to obtain Type A-gelatin.

In this work, the neutralization step previous to the thermal process was not done because the interest was to evaluate the acid effect during the denaturation of proteins. The pH values of solution G ranged from 2.4 to 3.6 (**Table 2**). The pH values of G-solution would indicate the effects of the swelling processes (collagen structure and interactions of acid molecules) and the possible presence of acids molecules in the thermo-soluble fractions. Knowing the gelatin pH is important since it might affect other properties, such as gel strength, viscosity, etc., and its application.

The neutralization step is relevant and necessary to obtain normal gelatin according to the standards of Gelatin Manufacture Institute of American (GMIA) (2019) (gelatin powder reconstituted in water, pH values 4.5 to 6.6). According to Yudhistira et al. (2019) the neutral gelatin pH is commonly used in meat products, pharmaceuticals, photography, painting, etc., whereas low gelatine pH is used in products like juices, mayonnaise, soups.

According to Donald (2001), 1% w/v is the minimal concentration at which the nucleation of the triplex helix occurs. Thus, the helixes overlap resulting in gel formation. For all the thermo-soluble fractions, the G-solutions obtained (**Table 2,** not significant differences p=0.1934), resulted in higher concentration than the minimum required (1% w/v) for nucleation of the triplex helix. Before drying the G-solutions, the samples were kept 24h at 4 °C to observe the gelling properties. It was observed that AH and AA turned into a firm gel (jellified), AL turned into "weak" gel (poor gel) and AC did not jellify (cloudy solution). This difference could be due to the effect of the pH values on the hydrolysis of the collagen-chains s and/or on the proteins charges. Kaewruang et al. (2013) worked on gelatin extracted from unicorn leatherjacket skin and indicated that the gelatin with the lowest hydrolysis was more likely to present the longest chains and that the maintenance of chain length was a prerequisite for a better gelation. Moreover, Koli et al. (2013) indicated that the differences in the pH treatment could modify the amphoteric nature and the hydrophobic zones on the peptide chain of gelatin, limiting functional protein properties.

Hydroxyproline content and extraction yield

The Hyp content (mg Hyp/100 g pigskin [w.b.]) of ASC, G and RC fractions is shown in **Table 3.** The ASC Hyp content was significantly different between treatments ($p \leq 0.05$). The AH and AA ASC fractions,

showed lower Hyp content than AC and AL treatments.

The G Hyp content resulted higher in AH and AA than in AC and AL treatments. Several studies analysed the use of different acids to obtain native collagen (acid soluble collagen) and gelatin from different matrixes (waste tissue), but separately. The ASC collagen content results, calculated by using a correction factor of 7.55, were 3.9; 6.8, 6.2 and 3,6 mg Collagen/g pigskin (w.b.) to AH; AL, AC and AA treatment, respectively. acid soluble collagens (ASC) Oslan et al., (2022) studied the acid soluble collagens (ASC) from skin of the purple-spotted bigeye snapper (*Priacanthus tayenus*) using acetic, lactic and citric acid. In this study the highest ASC content were obtained by acetic (5.79%), then in citric acid (4.15%), the lowest by lactic (3.19%).

On the other hand, Kanwate& Kudre (2017) studied the gelatin characteristics from fish (*Labeo rohita*). The authors showed that the gelatin extracted with propionic acid showed higher Hyp content when compared with acetic and phosphoric acid.

It is known, that the Hyp contents of "pure" gelatins is variable according to the specie, race, age, et. and it suggest used as a valid criterion of purity for mammalian collagens and gelatins. According to Sompie et al. (2015), hydroxyproline in gelatin stabilizes the hydrogen bonds between free hydroxyl groups and water molecules. Moreover, Kaewruang et al. (2013) proposed that the iminoacid (Hyp) determine the gel strength by introducing pyrrolidine rings for bridging between chains, apart from H-bonding. This could explain the differences observed in the G-Hyp content and the jellified characteristic from AH, AA vs. AL and AC. However, other studies, including FTIR, amino acid profile, isoelectric point, etc., are required for further understanding.

RC fractions exhibited a number of losses in the drying process. In particular, the AL and AC samples resulted highly sticky, which affected the full recover of the dried CR. According to this and avoiding to infer into an error, the RC Hyp content has not been analyzed by ANOVA-analysis. This results are novel, because at the moment nor study reported the Hyp (collagen equivalent) content in the solid remained after gelatin obtaining process.

Nevertheless, the sum of ASC, G and RC Hyp content (Total Hyp content mg/g) allowed confirming these losses. The Total Hyp content resulted lower in AL and AC than in AH and AA treatments. This observation is supported by the Hyp content in the raw pigskin (43.6 mg/g pigskin w.b.) *vs* the total Hyp (sum of fractions) in AH and AA (43.4 and 41.4 mg/g pigskin w.b., respectively) and AL and AC (35.7 and 36.2 mg/g pigskin w.b., respectively). Likewise, estimated yields are presented in **Figure 2**. For all treatments, G is the main percentage of the by-product.

Differential Scanning Calorimetry (DSC)

DSC is widely used to study the thermal transitions of proteins. In this study, the thermal characteristics of dried ASC and G samples were studied by DSC. Thermal transition temperatures and enthalpy changes were determined in order to evaluate the effect of using different food-grade acids on the ASC and G protein by-products. The samples were measured after conditioning at 25 °C, as described in section 2.2.

The collagen thermal transition temperature (T_d) is the temperature at which collagen triple-helix is converted to randomized coil structures (corresponding to the irreversible unfolded-denatured step) (Latorre et al., 2018). The ASC-DSC results showed differences between treatment. The AH and AA presented similar T_d (59 and 48 °C, respectively) between them, whereas AL and AC showed similar T_d, 124 and 139 °C, respectively, both higher than AH and AA.

These differences could be due to an increase of inter and intra fibrillar interactions with higher Hyp content (**Table 3**).

Moreover, it is known that multi-processes are involved in collagen crosslinking. For example, the reaction between collagen and hydrolysed vegetable tannins, which confers moderate hydrothermal stability. According to Cass &Burg (2012), tannic acid functions as a collagen cross-linking agent through hydrogen-bonding mechanisms and hydrophobic effects. The authors observed that thermal denaturation temperatures of the cross-linked scaffolds (68 °C) resulted significantly higher than those of uncross-linked scaffolds (55 °C). Furthermore, collagen stabilization involves water molecules, hence the water activity resulting an important factor on the thermal characteristics.

The enthalpy results showed discrepancy between samples (data not shown). The drying procedure could be responsible for differences in the samples. This may be due to non-uniform distribution of the water in the samples (Latorre & Velazquez, 2020). Unfortunately, in the present work water activity was not measured.

Thus, based on these observations, more studies such as, water activity, scanning electron microscopy images, FTIR, etc., are necessary to explain collagen (ASC) structural characteristics obtained by the different acid treatments.

Table 4 shows the results of the glass thermal transition temperature (T_g) and total enthalpies corresponding to dried G samples determined from DSC curves. The endothermic signals resulted around 120 °C for all treatments. However, differences on ΔH results (**Table 4**) suggest that the process used affected the protein hydrophobic and/or hydrogen binding. The enthalpy values resulted higher for AA and AC than for AL and AH in dried G.

As mentioned before, DSC curves show a single endothermic signal around 120 ºC for all treatments. This could correspond to the superposition of more than one thermal process, such as evaporation, structural reorganization, non-equilibrium Tg of the rigid blocks, etc. In addition, polymers take time to crystallize because crystallization requires movement of the chains to order them in the crystalline phase (Vlasova 2019). Gelatin films can be considered as a semicrystalline polymer, with crystalline domains, which during drying could form triple helix structures that act as physical cross-links (Mosleh et al., 2020).

These structural changes could affect the thermal behaviour of the gelatins studied in this work. According to Mosleh et al. (2020), the glass transition is a time and temperature dependent transition (amorphous regions of the random coil string). For that reason, parameters such as thermal history (eg, drying time and temperature) and moisture content are relevant in investigations of gelatin glass transition.

For other side, Tsereteli & Smirnova (1992) observed that, depending on the type of gelatin (extraction process and/or nature of gelatin), the “melting heat” presents a stronger relation with the number of cross-links present in the starting gelatin. The authors indicated that gelatin (gel or crystalline state) forms metastable collagen-like structures and that the resulting thermodynamic parameters depend on the production conditions.

Additionally, in the present results the acid-protein interaction might be affecting the thermal properties. For example, Xu et al. (2013) studied the citric acid use to cross-link wheat-derived gliadin at low temperatures. The study reported that when more than one carboxyl group is involved in the reaction, further inter and/or intra-molecular crosslinking can be possible.

The current ΔH results may also suggest that the process used to obtain the gelatin may affect protein interactions. In addition, despite all G samples were dried under the same conditions, water activities were not quantified in the present work. Therefore, different amounts of protein-protein and water-protein interactions could be present in each sample studied.

Unfortunately, this study did not assess water activities neither the FTIR study of the dried-G samples was performed to explain the differences observed. These studies will be included in detail in future research.

Gelatin color

Gelatin color has proven to influence acceptability and food application. The color of dried G obtained from pigskin by different acid pre-treatments is shown in **Table 5**. The results demonstrated that the different pre-treatments used to obtain gelatin influenced ($p < 0.05$) the values of the films: lightness (L*), redness (a*) and yellowness (b*). The AH-dried G presented the highest L* value, while the AA-dried G resulted in the lowest L*value and the highest browning (<b*). The L* values of AL and AC-dried G were lower as compared with AH treatment and higher than AA treatment. In the present work, the acids used at the same concentration during the pigskin hydrolysis don't present the same pH (data not sowed). Musso et al., (2016) work with commercial gelatin, adjusted different pH and the color of gelatin films (G) obtained resulted clear and colorless for all pHs tested. Significant differences between treatments were found in red (a*) and (b*) yellow hues (both $p < 0.0001$). The AC-dried G a* value obtained by AL<AA< AH acids-treatment was higher as compared with dried G. However, the highest values of yellow hue, analyzed by b* parameter, were observed on AC- and AA-dried G. This and lightness differences could be at different Mailliard and or (AA and AC) oxidation reaction during the thermal process.

More studies, like low acid concentration or acid mix, are necessary to improve the gelatin color.

CONCLUSION

Results indicated that ASC and G by-products of pigskin could be obtained using different food grade acids. The acid and thermal processes showed differences in Hyp content of the ASC and G by-products. The AC and AL treatments had a higher Hyp content than the AH and AA ASC fractions G Hyp content resulted higher in AH and AA than in AC and AL treatments. The DSC results showed that the acid treatment affected the thermal properties of the by-products. The current results suggest that the acid used and the pH of the solution during the thermal process affects the ASC and G protein interactions and/or their structures (cross-linking). Dried-G color was affected by the different acid treatments. Results suggest that obtaining different pigskin by-products using acid-food grade could be an option for the rendering industry. The results presented invite an opportunity to deepen future research.

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Table 1- The chemical composition of Pigskin, raw tissue (g /100 g pigskin wet base (w.b.)).

Raw tissue (pigskin)	g/100 g (w.b.)*
Moisture	37.5 ± 0.68
Total Protein	45.4 ± 1.45
Total Lipid	12.0 ± 1.91
Total Ash	0.26 ± 0.10
Total Collagen	32.9 ± 5.41

*Value are given as mean ± standard deviation (n=3).

Table 2- pH solutions values; food grade acid solutions (0.5M), ASC and G solutions (soluble fractions obtained after the acid and thermal treatments, respectively). Total collagen protein content (Hyp quantification) in G soluble fraction after thermal treatment (90 °C-90 min).

	AH	**AL**	**AC**	**AA**
pH Value*				
Food-grade acid Solution	2.55 ± 0.07[a]	1.98 ± 0.04[b]	1.70 ± 0.14[c]	2.39 ± 0.13[a]
ASC solution	2.60 ± 0.10[a]	2.00 ± 0.30[b]	1.85 ± 0.13[b]	2.40 ± 0.22[a]
G solution	3.55 ± 0.15[a]	2.80 ± 0.2[bc]	2.35 ± 0.10[c]	3.20 ± 0.25[a]
G-solution**				
g Gelatin/100 ml	2.59 ± 0.21 (0.10)	2.27 ± 0.12 (0.06)	2.66 ± 0.06 (0.03)	2.26 ± 0.57 (0.28)

*Value are given as mean ± standard deviation (n=3). The different letters in the same column indicate significant differences (P<0.05). One-way ANOVA (Tukey's Multiple Comparison Test).
** Gelatin corresponds to Hyp content (g/100 ml) x Conversion Factor (7.55). Value are given as mean ± standard deviation and (SE) (n=3).

Table 3- Hydroxyproline content (mg Hyp/ g pigskin w.b.) in ASC, G and RC fractions by treatment.

	mg Hyp /g pigskin (w.b)*		
Treatment	ASC	G	RC
AH	0.48 ± 0.07(0.04)[a]	41.40 ± 5.19 (2.32)[a]	1.47 ± 0.02 (0.01)
AL	0.91 ± 0.02(0.14)[b]	34.44 ± 2.73 (1.22)[b]	0.31 ± 0.02 (0.01)
AC	0.82 ± 0.01(0.10)[b]	33.75 ± 2.62 (1.17)[b]	1.66 ± 0.15 (0.11)
AA	0.48 ± 0.15(0.09)[a]	40.37 ± 2.57 (1.49)[a]	0.52 ± 0.04 (0.03)
p-Value	0.005	0.0094	

*Value are given as mean ± standard deviation and (SE) (n=3). The different letters in the same column indicate significant differences (P<0.05). One-way ANOVA (Tuckey's Multiple Comparison Test).

Table 4- Dried G thermal transition temperatures (T; °C) and changes in enthalpy (ΔH; J/g)

	T_g (°C)*	ΔH (J/g)*
AH	122.4 ± 6.4 (3.7)	121.2 ± 26.8 (16)[a]
AL	120.1 ± 1.8 (1.3)	169.2 ± 9.1 (6.4)[b]
AC	119.9 ± 3.5 (2.5)	244.2 ± 12.0 (8.5)[c]
AA	120.3 ± 0.5 (0.4)	298.7 ± 13.4 (9.5)[c]

*Value are given as mean ± standard deviation and (SE) (n=3). The different letters in the same column indicate significant differences (P<0.05) One-way ANOVA (Tukey's Multiple Comparison Test).

Table 5- Dried G CIELab parameters.

Acid Treatment*	L*	a*	b*
AH	83.68 ± 1.09 (0.03)[a]	-0.83±0.05(0.03)[a]	7.40 ± 0.41 (0.24)[a]
AL	69.52 ± 2.92 (1.69)[b]	4.18 ± 1.54 (0.89)[b]	25.01±3.07 (1.77)[b]
AC	64.93 ± 0.80 (0.46)[c]	9.86 ± 1.36 (0.79)[c]	39.01 ± 2.52 (1.46)[c]
AA	35.86 ± 1.16 (0.82)[d]	0.90 ± 0.21 (0.15)[d]	-0.30 ± 0.04 (0.03)[e]

*Value are given as mean ± standard deviation and (SE) (n=3). The different letters in the same column indicate significant differences (P<0.05) One-way ANOVA (Tukey's Multiple Comparison Test).

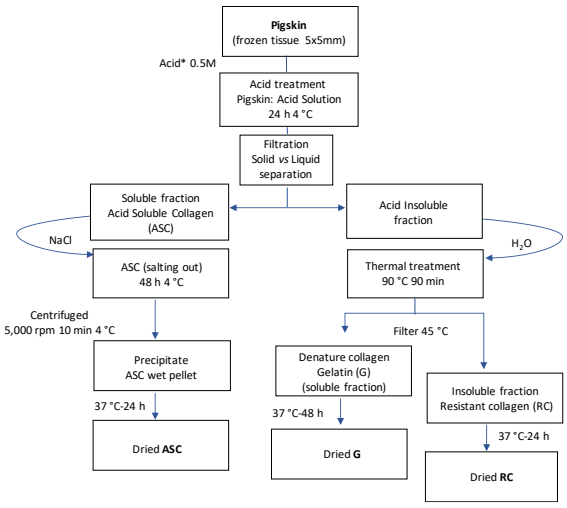

Fig 1- Schematic diagram of the process used to obtain the pigskin by-products. *Acid solutions Acetic acid (AH); Lactic acid (AL); Citric acid (AC) and Ascorbic acid (AA) 0.5M

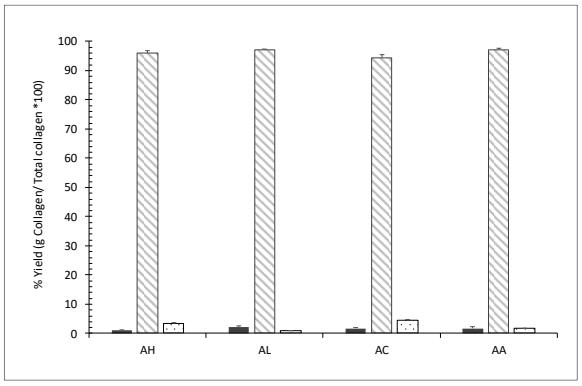

Fig 2- Yield of collagen extracted from pigskin after different acid-thermal treatments. Black: ASC-yield, striped: G-yield and spotted: RC-yield; respectively.

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Reliability of Time-Temperature Indicator From Corn and Red Palm Oil Blending For Monitoring Microbial Growth of Pasteurized Milk

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ABSTRACT

Time-Temperature Indicator made from corn oil and red palm oil had potential to be used in food cold chain. However, the evaluation of its reliability was required. The aim of this study was to evaluate the reliability of the indicator to monitor the changes of pasteurized milk quality based on microbial growth at several storage temperatures. This study was conducted in three stages. Stage 1 was making corn oil and red palm oil blending with 70:30 (%v/v) ratio. Stage 2 was measurement of diffusion length, coefficient, kinetics and activation energy at five storage temperatures (4, 29, 37, 44, and 51 ºC). Stage 3 was counting the total microbes at three different storage temperatures (8, 29, and 40 ºC). The result showed that the activation energy of corn oil and red palm oil Time-Temperature indicator was 36.796 kJ/mol. Meanwhile, the activation energy of pasteurized milk microbial growth kinetics was 44.021 kJ/mol. The reliability of the indicator was good, because the activation energy difference value between microbial growth and the indicator was lower than 25 kJ/mol.

Keywords: Corn oil, Pasteurization milk, Red palm oil, Time-temperature indicator

INTRODUCTION

Pasteurized milk is one of the products that requires cold chain distribution system. The product safety and quality might change as temperature changes during distribution. To monitor changes in temperature during distribution and storage, irreversible indicators such as TTI (Time-Temperature Indicator) are needed.

The TTI could be produced by using the principle of fluid diffusion. The fluid should have low melting point and stable viscosity (Khairunnisa, 2018). Also, good commercial TTI should have the activation energy between 34-50 kJ/mol (Pocas et al., 2008).

Corn oil and red palm oil blending could be the best candidate to produce the fluid diffusion based TTI. Corn oil had -11 ºC of melting point (Strayer, 2016). On other hand, red palm oil had 20.7 ºC of melting point (Ulfah et al., 2016). Based on the previous research, corn oil and red palm oil blending resulted the best energy activation in 70:30 (%v/v) ratio (Widyasaputra et al.,

2022). Also, this blending had 2.47 ºC of melting point (Widyasaputra et al., 2022).

The objective of this research was to evaluate the reliability of indicator to monitor the changes of pasteurized milk quality based on microbial growth at several storage temperatures. This evaluation was obtained based on checking the activation energy microbial growth kinetics of pasteurization milk against the activation energy of the TTI indicator label.

MATERIALS AND METHODS

Tools and Materials

The materials of this research were waterproof glossy photo paper 15 cm x 1.0 cm x 0.01 cm as the medium of diffusion (Printech), corn oil (Mazola, Moi Foods, Selangor) and red palm oil (Salmira, PT. Nutripalma Abadi, Bogor), pasteurization milk (Greenfields, PT. Greenfields Indonesia, Malang), PCA (*Plate Count Agar*).

The tools were refrigerator Samsung RT20 (Samsung Electronics Co., Ltd), incubator oven (Memmert, GmbH, Schwabach, DE), beaker glass 250 mL (Iwaki pyrex), beaker glass 500 mL (Iwaki pyrex), petri dish (Iwaki pyrex), infrared thermometer GM320 (Shenzen Capital Electronics Co., Ltd.), hotplate.

Methods

This study was performed in three stages. First, blending the corn oil and red palm oil with 70:30 (%v/v) ratio. The process was conducted with 40 ºC of heating and stirring for 10 minutes (Widyasaputra et al., 2022). Second stage, measurement of diffusion length, coefficient, kinetics and activation energy at five storage temperature (4, 29, 37, 44, and 51 ºC). The diffusion length measurement (cm) was conducted with soaking the medium (photo paper) in 2 mL of oil blend for 30 hours. The diffusion length measurement was performed after 30 hours. Diffusion coefficient (D) was calculated by equation:

$D = \frac{x^2}{2t}$ .....(1) (Khairunnisa et al., 2018)

D was diffusion coefficient (cm^2/ hour), x was diffusion length (cm), and t was time (hour).

Diffusion kinetics was calculated by using Arrhenius equation with modification:

$\text{Ln } D = -\left(\frac{Ea}{R}\right)\frac{1}{T} + \ln \text{ Do}$... (2) (Li et al., 2008)

D was diffusion coefficient (m^2/s), Ea was activation energy (KJ/mol), R was gas constant (8.314 J mol^{-1} K^{-1}) and T was temperature (K). The diffusion kinetics measurement was conducted with Arrhenius equation.

Third stage was measurement of total viable (microbial) count at three different storage temperatures (8, 29, and 40 ºC). Total viable count was performed with plate count analysis at 0, 2, 4, 6, 24, 26, and 28 hours (modification from Khairunnisa et al., 2018). 10 mL pasteurization milk sample was diluted in 90 mL physiological solution (10^{-1}). The dilution was continued until 10^{-4} (for 0, 2, 4, 6 hours) and 10^{-7} (for 24, 26, 28 hours). 1 mL sample of each of the last four dilutions was poured into petri dish (duplicate). Then, plate count agar media was poured into the petri dish and slowly shaken to form a figure eight. The incubation was conducted at 30 ºC for 48 hours. The analysis was repeated two times. Total viable count was calculated by equation:

$N = \frac{\Sigma C}{(1\ x\ n1)+(0.1\ x\ n2)+d}$ .....(3)

The kinetics of microbial growth was calculated by equation:

$Ln\frac{No}{N} = k.t$ .....(4)

$\text{Ln } k = -\left(\frac{Ea}{R}\right)\frac{1}{T} + \ln \text{ ko}$ .....(5)

N was total viable count (CFU/mL); ΣC was countable colony; n1 was number of petri dish in dilution 1; n2 was number of petri dish

in dilution 2; d was the lowest dilution, k was coefficient; R was gas constant (8.314 J mol^{-1} K^{-1}).

The statistical analysis was performed by Analysis of Variance with SPSS v.22. The kinetics calculation was performed by Microsoft Excel 2019.

RESULTS AND DISCUSSION

The good TTI should be sensitive to changes in temperature. The diffusion coefficient of corn oil and red palm oil based TTI was studied under five different temperatures. Figure 1 showed that increasing temperature could increase the diffusion coefficient. Diffusion coefficient was affected by time and temperature (Setiawan, 2012). The movement of molecules in oil was triggered by changing potential energy to kinetic energy during temperature increase (Khairunnisa et al., 2018).

The activation energy could be calculated by connecting 1/T (K^{-1}) and ln k (Figure 2). The regression equation used was $y = -4425.8x - 3.1187$ with R^2 value 0.9718. From regression equation and Arrhenius equation (2), the activation energy of 36.696 kJ/mol was obtained.

Total microbial growth of pasteurized milk (Figure 3.) followed first-order Arrhenius equation, so that the activation energy value was determined by connecting time (t, hours) and ln (N/No, CFU/mL). The growth in 8 ºC storage temperature was the lowest than other. Pasteurization process could only kill 95% of microbes, therefore pasteurized milk need to be stored in low temperature (Fromm & Boor, 2004). In low temperatures, pasteurized milk had longer shelf life (Ziyaina et al., 2018).

Kinetics of total microbial growth in pasteurized milk showed in Figure 4. The Ea value could be determined by connecting 1/T (K^{-1}) and ln k. An equation of straight line could be rendered into a kinetics equation (5). The regression equation used was $y = -5294.9x + 16.176$ with $R^2 = 0.9195$. From the calculation, the Ea value of pasteurized milk (stored in 8, 29, and 40 ºC) was 44.021 kJ/mol.

The reliability of corn oil and red palm oil based TTI could be obtained by calculating the difference between Pasteurized milk Ea value and TTI Ea value. From table 1, the Ea difference was 7.225 kJ/mol. The reliability of indicator was good because the difference was lower than commercial TTI (25 kJ/mol) (Ellouze & Augustin, 2010; Khairunnisa et al., 2018; Park et al., 2013).

The Ea difference of the blending of corn oil and red palm oil based TTI was better than the blending of palm oil, canola oil and soybean oil. The Blending with 70:30 ratio of corn oil and red palm oil had 7.225 kJ/mol activation energy difference. The blending of palm oil, canola oil and soybean oil with 50:40:10, 50:25:25, and 50:10:40 had 23.816, 22.811, and 17.459 kJ/mol activation energy, respectively (Khairunnisa, 2018). Corn oil had -11 ºC of melting point, the melting point was lower than canola oil (-10 ºC), olive oil (-6 ºC), but higher than soybean oil (-16 ºC)(Strayer, 2016). The melting point of oil had close relation with diffusion rate which also affected the activation energy. The lower melting point could increase the diffusion rate (Widyasaputra, et al., 2022).

The blending of corn oil and red palm oil was easy to produce. But the blending ratio might be needing modification for cold chain food system with below 0 ºC storage temperature.

CONCLUSION

The TTI had 36.796 kJ/mol of activation energy. On other hand, pasteurized milk microbial growth kinetics had 44.021 kJ/mol of activation energy. The difference between the two was 7.225 kJ/mol (<25

kJ/mol), shown that indicator had good reliability.

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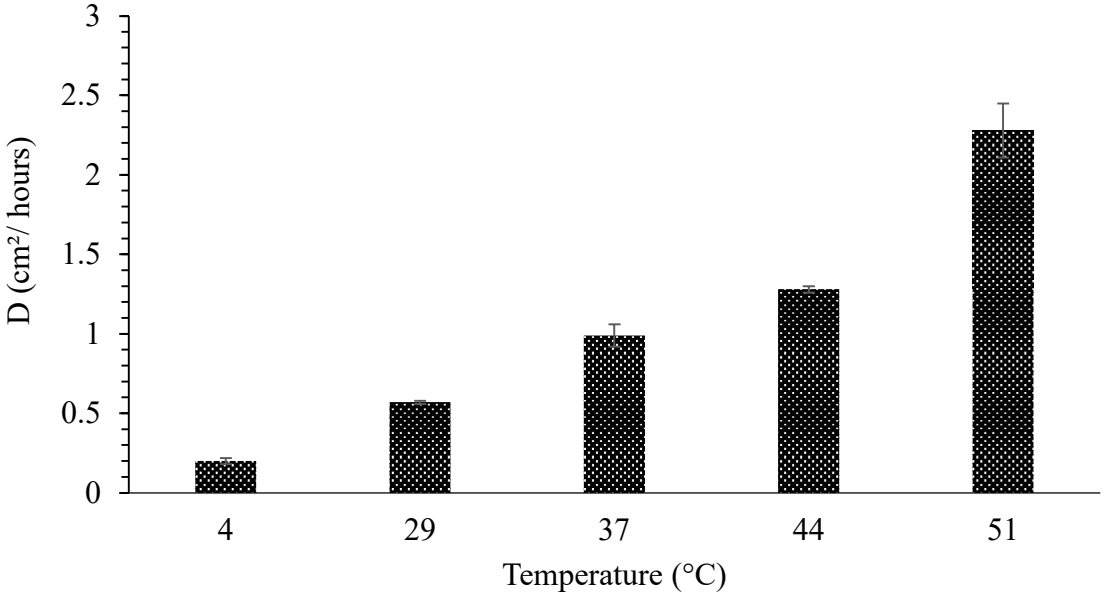

Figure 1. The diffusion coefficient of indicator (D) at some storage temperature

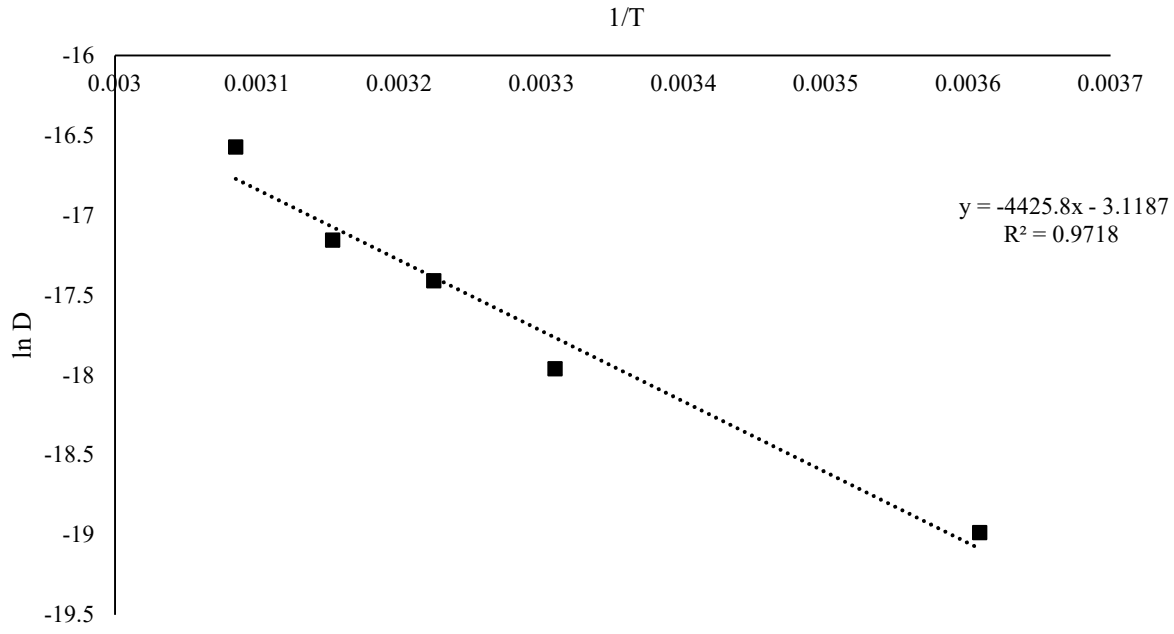

Figure 2. ln D and 1/T plot for corn oil and red palm oil TTI

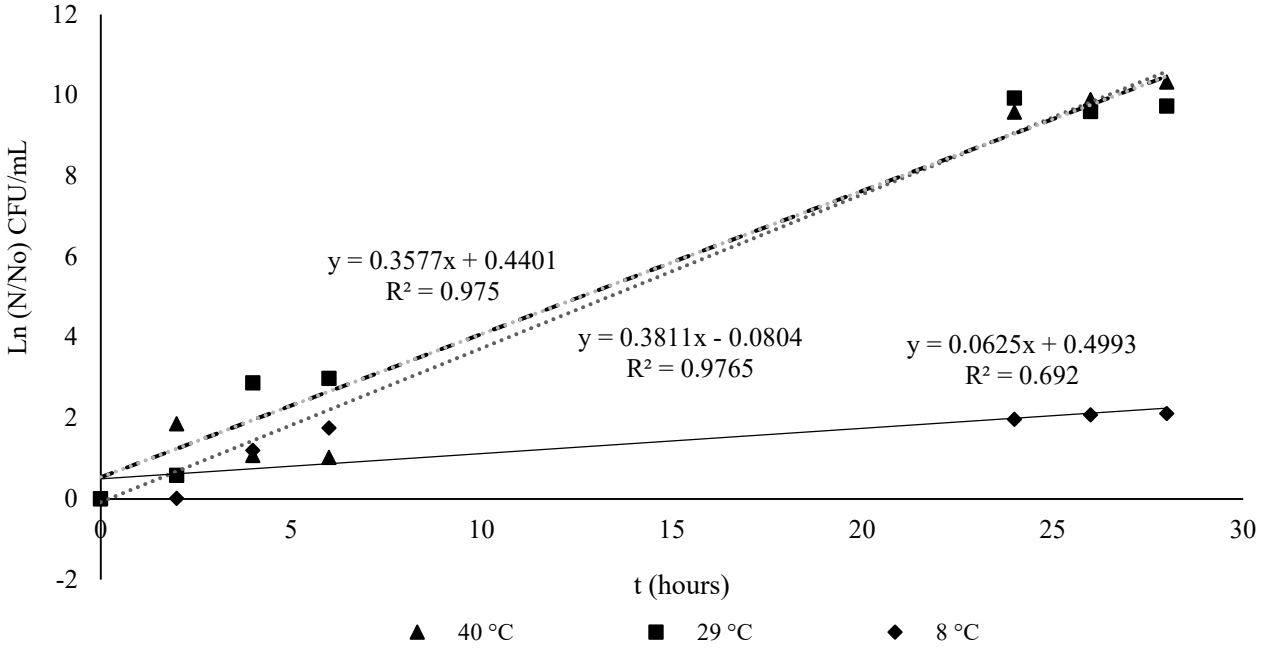

Figure 1. Ln (N/No) and t plot for total microbial growth in pasteurized milk

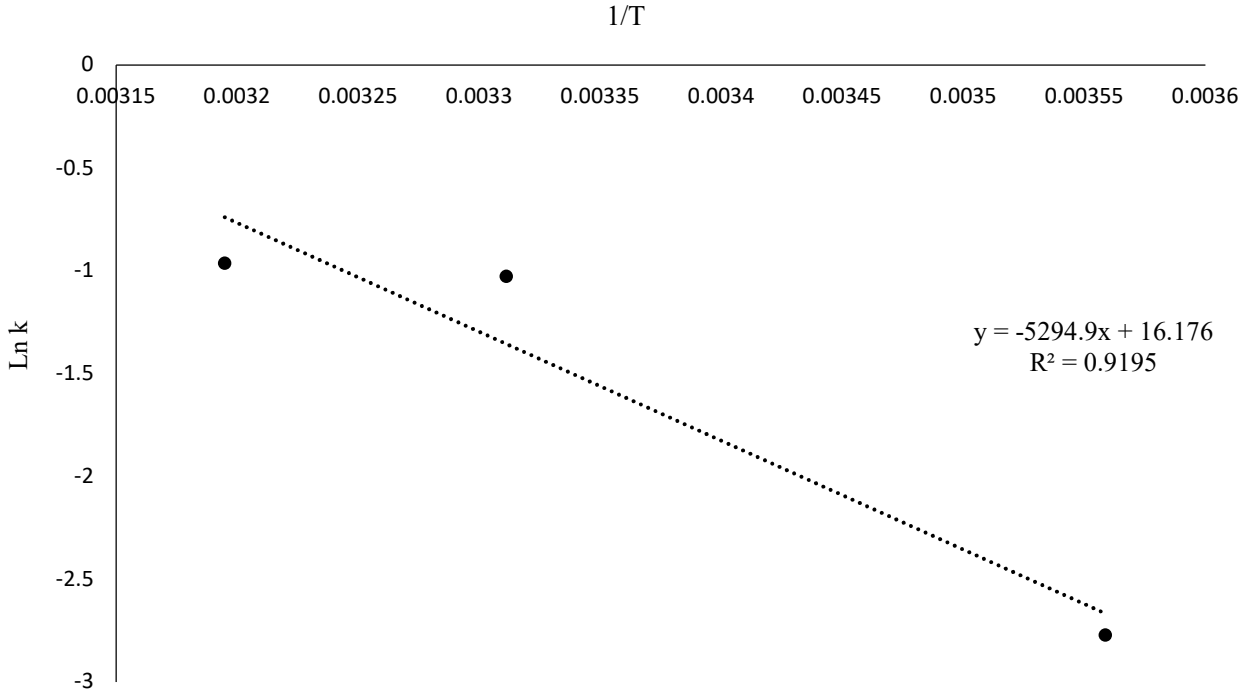

Figure 2. 1/T and ln K plot of total microbial growth in pasteurization milk

Table 1. Activation energy (Ea) of corn oil and red palm oil blend TTI and pasteurization milk

TTI Ea (KJ/mol)	Pasteurized milk Ea (KJ/mol)	Pasteurization milk Ea – TTI Ea (KJ/mol)
36.796	44.021	**7.225**

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Effect of Coriander (*Coriandrum sativum*) Smoke on Physicochemical and Sensory Characteristics of Mudaffara Cheese

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ABSTRACT

This study was done to compare physiochemical and sensory attributes of smoked and non smoked Mudaffara cheese using *Coriandrum sativum* seed. Mudaffara cheese was prepared from fresh cow's milk and the resulted cheese was divided into two portions; the treated cheese, which was smoked by *Coriandrum sativum* in a close smoker for about 2 hours. The second portion was left as a control. Both cheeses were packed into labeled plastic containers and stored for five weeks in the refrigerator. The analysis was carried out in the first day, then every week. The result indicated that the smoked Mudaffara cheese was significantly ($P<0.05$) higher in total solids, protein and fat contents compared to the control cheese, which revealed significantly ($P<0.05$) higher acidity compared to the smoked Mudaffara cheese. The study also found significant ($P<0.05$) variations in protein and total solids content and the acidity of Mudaffara cheese during the storage period. The smoked Mudaffara cheese showed higher scores for flavor, salt and overall acceptability as was stated by the panelists, however the better color was recorded for unsmoked Mudaffara cheese. Hence the study concluded that smoking using *Coriandrum sativum* seed improve the keeping quality and acceptability of Mudaffara cheese.

Keywords: Coriandrum sativum; Mudaffara cheese; Smoking; Storage

INTRODUCTION

Most of the cheese varieties are manufactured by common steps that include formation followed by the whey expulsion, acid production salting and ripening (Beresford et al., 2001).

In Sudan, *Gibna bayda*; the white cheese; is the major type that is produced traditionally especially during rainy seasons in rural areas (El Owni & Osman, 2009; Elkhider et al., 2012). The second major type of the local cheese produced is *Gibna mudaffara* (Hamid & El Owni, 2007; Nour El Diam & El Zubeir, 2007; Farah & El Zubeir, 2020). *Gibna mudaffara* is characterized by its ability to withstand for a long period when immersed in the whey (Mohammed Salih et al., 2011). Moreover, the storage of the braided (Mudaffara) cheese in good suitable conditions could be one of the practical methods for preserving the milk into a product with high nutritional value and long shelf life (Farah & El Zubeir, 2020).

Among the recommendations for manufacture of good quality cheese, high quality milk produced by healthy animals, good manufacturing procedures and maintenance of the product quality during both storage and marketing are necessary (Warsma et al., 2006). Also for the improvement of the Sudanese white cheese, it was suggested that the cheese be further processed in order to increase its hygienic quality and the shelf life (Nour El Diam & El Zubeir, 2006).

Ahmed (1995) reported a titratable titriatable acidity of 0.29–0.69%, pH of 3.29–4.30, fat of 6.02–20%, protein of 28.29–31.92%, total solids of 53.63–63.83% and ash of 5.40–7.29% for Mudaffara cheese. The fat, protein, total solids, ash and acidity content of of the same cheese were reported as 17.2±2.13, 21.49±2.53%, 51.89±12.25%, 10.96±9.32 and 0.48±0.06,' respectively (Harun et al., 2015). The mean values were 4.17±0.06%, 22.00±0.28%, 61.69±0.12%, 4.17±0.06% and 0.46±0.29%, respectively (Farah & El Zubeir, 2020).

Currently the focus of food industry is the development of novel functional foods that contain natural ingredients with health promoting properties in order to replace food additives and to avoid their potential hazards (Caleja et al., 2015). The antimicrobial properties of wood smoke were found effective in many foods, moreover. when the cheese was smoked the chance of spoiling by molds growth is less compared to that of unsmoked natural cheeses (Wendorff et al., 1993). The cheese producers' are able to process natural smoked cheeses without any detectable benzo(a)pyrene using the electrostatic precipitation of tars from wood smoke or cold smoke treatment (Riha et al., 1992). Three types of local wood material most commonly used in cheese smoking in Yaman include *Zizyphus spina christi, Acacia asak,* and *Dodonia viscose* (Shaiban et al., 2006). Coriander (*Coriandrum sativum* L.) is one of valuable medicinal plants that is belong to the *Apiaceae* family, which is grown annually worldwide (Duarte et al., 2012; Hassanen et al., 2015).

Coriander (*Coriandrum sativum L.*) uses include flavoring of food products, medicine and cosmetics Darughe et al., 2012; Nadeem et al., 2013). Also, coriander prevents food degradation because of its antibacterial, antifungal properties and anti–oxidative activities (Sriti et al., 2011). It is added to the mixtures of other aromatic herbs for the production of herbed cheese and herbed cottage (Kaptan & Sivri, 2018). It is also added to yoghurt, fresh and cream cheese to give aroma and taste (Kaptan & Sivri, 2018). Also the addition of coriander may increase the consumption of cheese and improve consumer health (Turgut & Diler, 2020). Because of its seed popularity and extensive uses in Sudan in many foods, it was selected in this study to produce smoked Mudaffara cheese.

MATERIALS AND METHODS

Sources of materials

About 16 liters of fresh cow's milk were brought from University of Khartoum farm. Rennet tablets (Chr– Hansen's Laboratory, Denmark) were obtained from a private veterinary centre, while the table salt (commercial grade), *Coriandrum sativum*, black cumin (*Nagella sativa*) seed and charcoal were purchased from a local market. The starter culture was a product of CHR–HANSEN, YoFlex® Express 1.0).

Preparation and storage of cheese

The cheese was manufactured at Dairy Production Laboratory in the Faculty of Animal Production, University of Khartoum.

Analysis of milk

The milk which was used for preparation of the cheese, was analyzed using the Lactoscan (Milkotronic LTD, Bulgaria).

Processing of Mudaffara cheese

The cheese was made using the available cheese making equipment at the laboratory as described by Farah & El Zubeir (2020). Briefly, the milk was filtered through a muslin cloth, heated (62^{o} C for 15 minutes) and then cooled to 38.5^{o} C using ice water. The starter culture was added to the milk at a rate of 2%. After mixing gently, the mixture was left to stand for 5 minutes before the addition of rennet. The rennet tablets (2/ 100 kg milk) were dissolved and added to the milk with gentle mixing. It was thoroughly stirred for five minutes and left to stand for 45–60 minutes to allow the coagulation of the milk. A sterile knife was used for cutting the curd to allow whey drainage. Then the cut curd was placed in an incubator at 47 ºC for 2–3 hours to reach 0.67% acidity and the required elasticity. The elasticity test was done by putting small piece of the cheese into warm water at 85 °C for 5 minutes. If the curd stretched, then the rest of the curd was cooking in the warm water (85 ºC) for 5 minutes. The elastic curd was formed into balls and transferred to clean table for stretching into a 4 meters long rope. Black cumin was added at a rate of 0.5% w⁄w to the hot curd before braiding. The braiding of the curd was done using hands by pulling it into long ropes, which were then braided. The braided curd was washed using cold water and then preserved in 10% w⁄w salted whey for 24 hours. The cheese was divided into two parts: one was subjected to smoking using *Coriandrum sativum*, while the second was the control.

The smoking process

Commercial dried sample of *Coriandrum sativum* (coriander) was obtained from a local market, washed and roasted before it was used in smoking of Mudaffara cheese.
Mudaffara cheese was brought to room temperature for 2–3 hours for the formation of a layer outside the cheese to facilitate penetration of the smoke inside the cheese and prevent its melting. Pieces of charcoal were put in the periphery of smoking chamber and *Coriandrum sativum* seeds were put on it. Then Mudaffara cheese was hang on sticks inside the smoking chamber (Figure 1).

The smoking chamber was covered by muslin cloth. The smoking was done for 2-3 hours until the surface of the cheese sample had a nice brown colour all over and imparted a characteristic aroma and flavor (Michalski & Germuska 2003; Shaiban et al. 2006). After that the smoked cheese (Figure 2) was left at room temperature for 12 hours and packed in a plastic package. The smoked Mudaffara cheese was stored in the refrigerator at 5 °C together with the control Mudaffara cheese before analysis.

Examination of Mudaffara cheese

Coagulation time, yield, chemical composition and sensory characteristics of Mudaffara cheese were estimated at the laboratory at day 1, 8, 15, 22, 29, and 35.

Mudaffara cheese yield

Cheese yield was calculated by dividing the weight of cheese over the weight of milk and expressed on a percentage basis.

Chemical analysis of the cheese

The Gerber method was used for the determination of the crude fat, while Kjeldahl method was used for the estimation of crude protein of Mudaffara cheese (AOAC, 2003). The modified method of AOAC was used for the determination of the total solids. The ash content was determined by gravimetric method and the titratable acidity was determined by the titration determined described in AOAC (2003).

Sensory evaluation

A panel of 10 semi–trained staff and students from the Dairy Department that were familiar with cheese evaluated the sensory attributes of Mudaffara cheese using

5 point hedonic scale as described by Lim (2011). Where 5 was for *excellent*, 4 was for *very good*, 3 was for *good*, 2 was for *acceptable* and 1 was for *poor*. The sensory results were reported as the sum of scores of individual evaluators**.**

Statistical analysis

Statistical Package for Social Sciences (SPSS, version 16) was used. Analysis of variance (ANOVA). The means were separated by Duncan´s Multiple Range Test.

RESULTS AND DISCUSSION

Effect of *Coriandrum sativum* smoke on physico–chemical properties of Mudaffara cheese

The milk coposition from which Mudaffara cheese was made had 9.33%, 4.71%, 3.64%, 4.97% and 1.033 g/cm3 14.14%.for solids not fat, fat, protein, lactose and densiy, and total solids respectively.

Three kilograms of Mudaffara cheese were produced from 16 L of milk. Thus, the cheese yield of 18.75%. The obtained values were higher that the net weights of Mudaffara cheese estimated by Harun et al. (2015) who reported 11% and 12.5% for cheeses produced by *Solanum dubium* and chymosin, respectively.

Table 1 summarizes the composition of the cheese. There were more (p<0.01) total solids (61.46% vs. 56.32%), fat (28.92% vs. 22.85%) and protein (31.62% vs. 27.41%) in the smoked Mudaffara cheese compared to the control. However, the ash content was 10.99% and 10.57% (Table 1). The acidity (0.32%) of smoked Mudaffara cheese was significantly (p<0.01) lower than the control Mudaffara cheese (0.38%).

The higher composition of the smoked Mudaffara cheese proved the efficiency of Coriander in preserving the cheese. Coriander has been used for nutrition, medicine, flavoring, smoking and other industrial uses as well (Nadeem et al., 2013). Moreover, coriander seed essential oils can be used as natural antimicrobial and antioxidant in industrial food and drugs (Hassanen et al., 2015). Ahmed (1995) found similar values for total solids and crude protein, higher crude fat and lower ash content. With the exception of ash, higher values of crude fat, crude protein and total solids were observed in this study (Table 1) than those reported for Mudaffara cheese made using chymosin and Solanum dubium coat extract (Harun et al., 2015) and Mudaffara cheese using 0.3 or 0.5% Syrian thyme (El gabali et al., 2023). Riha et al. (1992) reported that the cheese processors limited the use of natural vaporous smoke only to cold smoking treatments in order to avoid free fat on the surface of the finished smoked cheese due to the high concentration of milk fat in cheese and its low melting point. The acidity of the cheese in this study was within the range reported by Ahmed (1995). However, Harun et al. (2015) reported a higher titratable acidity of 0.48±0.06%. Also, El gabali et al. (2023)reported higher acidity content of Mudaffara cheese flavored with Syrian thyme at 0.3% (0.74±0.11%) and 0.5% (0.71±0.9%) and black cumin (0.6±0.12%). The variation could be due to the antifungal properties in smoked cheese because the wood smoke components contain the primary antifungal properties (Wendorff et al., 1993).

The significant (P<0.01) increase in the total solids and crude protein of Mudaffara cheese till day 22 of storage period was obseved, then a reduction was found as the storage was progressed (Table 2). Similarly, the total solids and crude protein of Mudaffara cheese were increased gradually at week 2 and then decreased at the end of the storage period (El gabali et al., 2023). Slight reduction was observed in the total solids, while storing of Mudaffara cheese by Farah & El Zubeir (2020). Also, the coriander was found to affect significantly (P<0.05) the pH

and dry matter during the storage. Moreover, sensory scores declined during the storage period; but even on day 60, the samples were favourably scored (Turgut & Diler, 2020). Simlarly, Harun et al. (2015) found that the total solids were increased at week 2 (53.61%) before decreasing at week 3, and then increased to 58.45% at the end of the storage. However, a gradual decrease was reported in the protein of Mudaffara cheese till week 3 (19.29%) before it was increased to 21.61% by the end of the storage period.

The expulsion of moisture content from the cheese curd was possibly the reason for the increase of its total solids (Harun et al., 2015). The texture of cheese was influenced by both compositional and processing parameters (Wium et al., 2003). A significant ($P<0.01$) increase in acidity was observed till day 29 of storage. The titratable acidity was maximum at day 21 (0.57%) followed by a decrease to 0.50% at the end of the period of storage (Harun et al., 2015). The increase in the titratable acidity of Mudaffara cheese could be attributed to the growth of lactic acid bacteria leading to increase the lactic acid content of the cheese (Harun et al., 2015). In a similar study, the acidity of Mudaffara cheese showed significantly ($P<0.001$) lower values at day 0 and 7, then a sharp increase at day 21 (1.30±0.29%) towards the rest of storage period (Farah & El Zubeir, 2020).

This study reported flucationation in the fat and ash contents ($P>0.01$) during the staoge of Mudaffara cheese till day 21. to high moisture loss during the storage. Reduction in the fat content was also reported in Mudaffara cheese during the storage period (Altahir et al., 2014; Farah & El Zubeir, 2020). The high moisture loss during the storage was the reason for the apparent increase in crude fat, while the decrease in the fat content at end of storage period might be because of the breakdown of fat by microorganisms (Harun et al., 2015). However, El gabali et al. (2023) found significant ($P<0.001$) increase in the crude fat and non significant effect for the ash content of Mudaffara cheese during the storage period.of Mudaffara cheese during the storage period.

Non significant variation was found for the ash (Table 1 and 2). However, significantly ($P<0.001$) higher ash was found at day 0 (4.17±0.06%) and day 7 (4.08±0.06%) of Mudaffara cheese. Similarly, significantly ($P<0.01$) lower ash was found at day 35 (Farah & El Zubeir, 2020). An increase in the ash content at day 14 (15.59%) and day 28 (17.18%) was also reported (Harun et al., 2015). Abdalla & Mohamed (2009) reported that the total solids, fat and protein contents of cooked and vacuum packaged of white soft cheese was found to decrease with the progress of the storage period, however, the ash and titratable acidity showed continuous increase throughout storage period.

Effect of smoking on the sensory evaluation of Mudaffara cheese

The sensory acceptability of the Mudaffara cheeses are shown in Figure 1. The flavour, acid taste, saltiness and the overall acceptability showed increasing scores till day 15, then reduced at day 22 and 29 and increased again at day 35. According to Altahir et al. (2015), saltiness continuously increased ($P\leq0.05$) with the advancement in storage of Mudaffara cheese. Moreover, during the storage period, significant ($P<0.05$) variations were found in the texture, acidity, flavor, taste and general acceptability scores of Mudaffara cheese (El gabali et al., 2023).

The appearance, flavour, taste, texture and saltiness of Mudaffara cheese were reported to vary during storage (Farah & El Zubeir, 2019). Similarly, increasing acceptability scores for flavour and the overall acceptability of cheeses were during their ripening period (Nour El Diam & El

Zubier, 2007; Tarakci & Kucukoner, 2006). Also Abdalla & Mohamed (2009) reported that the flavour, taste, saltiness and the overall acceptability of cooked and vacuum packaged cheeses were gradually improved throughout storage. However, the best score for colour was reported in the non–smoked cheese (control) as shown in Figure 1.

The maximum smoking temperature of Mudaffara cheese should be 30 °C for 3 hours (Shaiban et al., 2006) or until light brown (Michalski & Germuska, 2003). This because the concentration of B(a)P strongly depends on the time and temperature of processing as the optimal temperature should be 25 °C–30 °C and that time processing should not exceed 2 hours (Michalski & Germuska, 2003). Riha et al. (1992) reported that the use of commercial smoking in the cheese products has a positive effect to control the deposition of 3, 4–benzo(a) pyrene; which is potential carcinogen; resulting from the smoking process. B(a)P is one of polycyclic aromatic hydrocarbons (PAHs), which is carcinogenic and mutagenic that are formed by the incomplete combustion of the organic matter and are widely believed to contribute in the occurrence of human cancer (Michalski & Germuska, 2003). However, when cold smoked with natural vaporous smoke, cheese was reported almost free of the potential carcinogen; 3, 4– benzo(a) pyrene level of detection at 0.1 ppb) (Riha et al., 1992). Thus, the use of coriander for smoking cheese could reduce the harmful potential carcinogen. The health benefits of coriander include antibacterial and anticancer activities (Bhat et al., 2014; Yildiz, 2015). Additionally, the essential oil and other various extracts of coriander possess some medicinal properties such as antibacterial, antioxidant, antidiabetic, anticancerous, antimutagenic and free radical scavenging activities (Sreelatha et al., 2009; Zoubiri & Baaliouamer, 2010).

The colour of the cheese revealed the best scores at the beginning (day 1) of the storage, then it decreased from day 8 until day 29 (Figure 1). Similarly, Farah & El Zubeir (2019) reported higher scores for appearance for the fresh Mudaffara cheese during day 0 and day 7 and lower scores duration storage. Caleja et al. (2015) found the incorporation of fennel–based ingredients to keep the yellowness color after 7 days of storage. Furthermore, the addition of a fennel phenolic–enriched extract improved the antioxidant properties of the cottage cheese for up to 14 days of storage. However better scores were reported at end of the storage period (Figure 1). El Owni & Hamid (2008) reported similar findings the Sudanese white cheese. In contrast, Abdalla & Mohamed (2009) reported that the colour and body of cooked and vacuum packaged cheese was constant throughout the duration of the storage.

CONCLUSION

Using *Coriandrum sativum* seed smoking was found to improve the keeping quality and acceptability of Mudaffara cheese.

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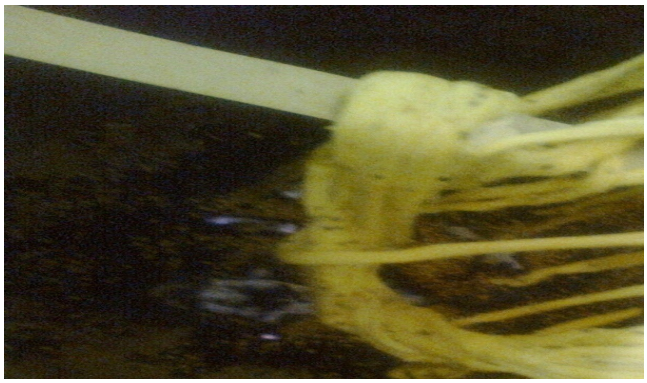

Figure 1. Mudaffara cheese hanging on stick inside the smoking chamber

Figure 2. Smoked Mudaffara cheese

Table 1. Effect of *Coriandrum sativum* smoke on physico–chemical properties of Mudaffara cheese

Treatment	Total solids (%)	Fat (%)	Protein(%)	Ash (%)	Acidity (%)
Control cheese	56.32	22.85	27.41	10.57	0.381
Smoked cheese	61.46	28.92	31.62	10.99	0.320
Means	58.89	25.33	29.52	10.87	0.351
Standard error	0.599	0.45	0.319	0.85	0.008
Level of significant	**	**	**	NS	**

** = P<0.01

NS = Not significant

Table 2: Effect of storage period on physico–chemical properties of smoked and Non–smoked Mudaffara cheese

Storage period	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	Acidity (%)
Day 1	54.32	25.5	21.05	10.82	0.300
Day 8	56.95	24.00	27.35	11.200	0.290
Day 15	56.100	26.40	30.32	10.77	0.325
Day 22	64.400	26.75	33.95	10.72	0.362
Day 29	61.87	24.87	31.10	10.87	0.415
Day 36	59.73	24.49	33.34	10.93	0.411

** = P<0.01
NS = Not significant.

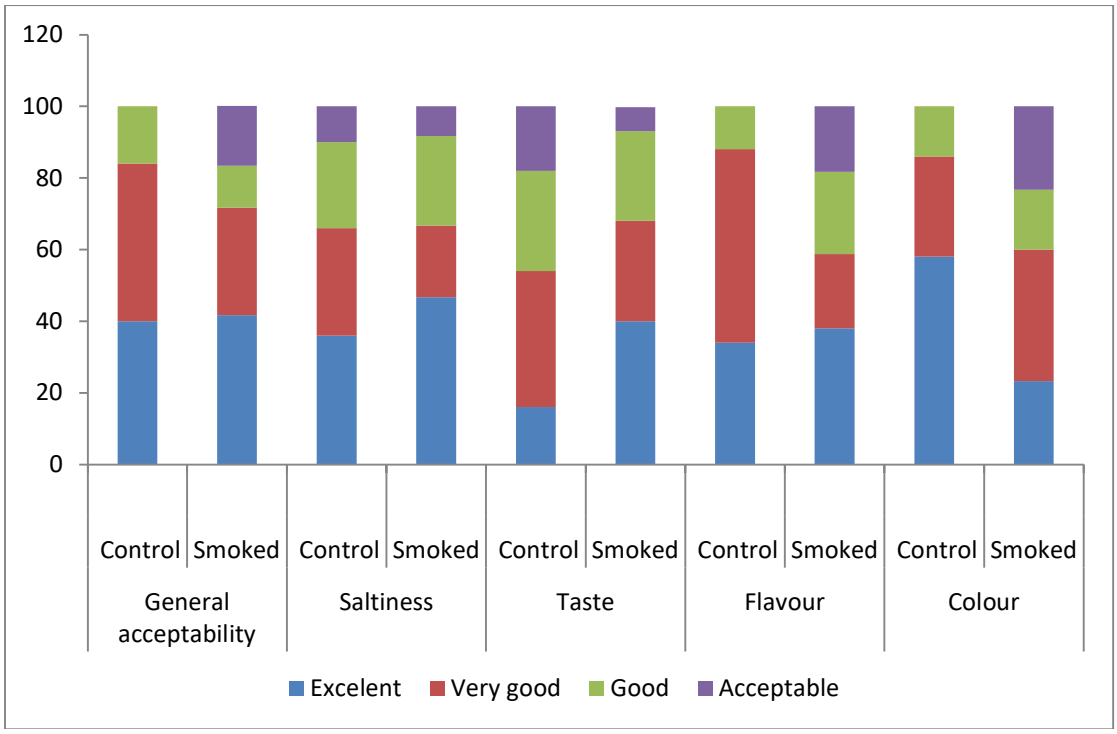

Figure 3. Variations of sensory evaluation of smoked and Non–smoked Mudaffara cheese

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Physicochemical, Mineral, and Sensory Properties of Masuku (*Uapaca kirkiana*) Beverages

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ABSTRACT

Masuku (*Uapaca kirkiana*) are wild fruits that are in abundance from December to March. Masuku are highly perishable fruits hence they have to be processed into other products to extend their shelf life. The main objective of this study was to determine the physiochemical, nutritional, and sensory properties of the masuku juices. Two formulations of masuku juice were prepared, one batch involved peeling the masuku and obtaining the pulp, while in the other batch, they were not peeled. The masuku were just washed and squeezed to obtain the pulp. The resulting pulp was used for juice processing. The juices were analyzed for physiochemical and mineral properties. Themasuku fruit had high pH content than that recommended for juice making and juice from unpeeled masuku was more acidic than juice from peeled masuku. Juice from unpeeled Masuku had high magnesium content as compared to juice from peeled masuku. While on paired preference test, the results showed that there was a significant difference in preference between the two batches ($p<0.05$) where the juice from peeled Masuku was most preferred over the juice from unpeeled masuku. In conclusion, masuku raw pulp acid content should be balanced with additional acids when making juices. Masuku juice can be used as a rich source of magnesium and zinc. There are traces of magnesium in the skin of masuku fruits hence squeezing the whole fruit when pulping can help to utilize the fruit fully.

Keywords: Masuku beverages, Physiochemical properties, Mineral, Sensory properties

INTRODUCTION

Masuku (*Uapaca kirkiana*), also known as wild loquat, is one of the indigenous fruits in Malawi that grow wildly throughout the country. For example, they are available in all three regions of Malawi (Southern region e.g. Blantyre, Central region e.g. Lilongwe, and Northern region e.g. Mzuzu). Other examples of local fruits include malambe (Adansonia Digitate), mpoza (Annona Senegalensis), matowo (Azanza Garckeana), bwemba (Tamaringus Indica), and masawu (Zizyphus Mauritiana) (World Agroforestry Center, 2020). Masuku tree is mainly distributed in semi-dry and dry areas. It produces fruits that repine from October to February (Kadzere et al., 2001). Masuku is important fruit because they are rich in minerals such as iron, calcium, magnesium, phosphorus, potassium, sodium, and zinc(Ngulube et al.,1996) and contribute to snack foods when in season.

Masuku is also important because they become available when other fruits are not in season. During this period, they contribute to the diet and nutrient intake since fruits are part of the six food groups in Malawi. Masuku are naturally available for the poor

when they are in abundance and are consumed as raw fruits. Some of the fruits are sold along the roads, contributing to the rural areas' economy.

Masuku can be considered as one of the underutilized fruits. No company processes them to add value and as a result, a large quantity of them get wasted when plentiful, thereby depriving the majority of the poor from vitamins and minerals they need to remain healthy. Masuku are in abundance when they are in season (from December to March) and wastage is due to a lack of processing techniques and knowledge on the part of rural communities and households. Like any other fruits, masuku are perishable and lack of processing and value-addition techniques means that they are not available when they are not in season e.g. from March to September. Since most rural households cannot manage to buy other fruits regularly, which denies them a readily available source of vitamins and minerals that they get from natural fruits like masuku.

Despite its abundance, not much research has been done on masuku in Malawi but elsewhere like in Asia (Hughes and Haq, 2003), Zambia (Moombe et al., 2014), and Tanzania (Ndabikunze et al., 2010). In Malawi, Saka et al (2007) have documented a study on physicochemical and organoleptic characteristics of Uapaca Kirkiana, Strychnos Cocculoides, Adensonia Digitate, and Mangifera Indica fruit products).

As it has been revealed by other researchers, masuku are rich in nutrients such as vitamins and minerals such as magnesium and zinc. Magnesium is becoming a mineral of concern in Malawi hence there is a need to start searching for it in our locally available foods such as masuku fruits and amounts of these nutrients in juices can be affected by processing procedures hence there is a need to do a nutritional assessment (mineral analysis) of the processed products from masuku such as the juice.

Until up to date, there is a lack of processed products from masuku and this means that processors do not understand the fruit due to lack of research and this leaves several questions unanswered.

Processing masuku into juice and other products can be one way of commercializing the fruit and making it available for a longer period so that families can continue to diversify their diets. Therefore, this research aims at determining the physiochemical, nutritional, and sensory properties of masuku beverages which will promote dietary diversity and commercialization of the fruit in Malawi.

The objectives of this study were to determine the physiochemical, minerals, and sensory properties of masuku beverages.

MATERIALS AND METHODS

Sample Collection

Thirty (30) kg of Masuku fruits were purchased from the Waka-waka market in Lilongwe.

Juice Processing

Ripen masuku fruits were sorted and graded to have good quality fruits. This was followed by washing the fruits to remove the dust and any adhering to the masuku fruits. In the first batch, masuku fruits were peeled and then the seeds were removed to obtain the pulp. In the second batch, the masuku fruits were placed in a perforated basket and squeezed to release the pulp through the basket without peeling the masuku first. 4 cups of water were added to one cup of pulp and 200g of sugar was added to 1 cup of pulp to maintain the original brix level. The juice was then boiled to 90^{o}C for 15 minutes to pasteurize the juice and then 0.05% of sodium benzoate was added as a preservative. The juices were cooled to 25-32^{o}C and

then they were bottled and refrigerated.

Physiochemical Analysis

pH determination

A pH meter was used to determine the pH of the juices. pH meter was standardized using pH 7.0 and 4.0 buffers. 10 ml of the sample juice was measured using a pipette and then transferred into a beaker. Then the pH meter was immersed into the sample juice until a steady reading was reached and the reading was recorded. The procedure was repeated 3 times.

Titratable acidity determination

Three drops of phenolphthalein indicator were added into a 50 ml Erlenmeyer flask containing 10 ml of the sample juice or raw fruit pulp. This mixture was titrated with Sodium Hydroxide (NaOH) until pink color appears. The volume used to titrate the juice was recorded and the procedure was repeated 3 times.

Calculations of %titratable acidity

$$\%acid\ \left(\frac{wt}{vol}\right) = \frac{N \times V1 \times EqWt}{V2 \times 1000} \times 100$$

Where:

N is Normality

Eqwt is the Equivalent weight of the predominant acid

V1 is the volume of the titrant

V2 is the volume of the sample

1000 is the factor relating mg to grams (mg/g)

Nutritional Analysis (Mineral Analysis)

Determination of Ash by gravimetric method

Five grams (W2) of the sample was weighed by difference into a pre-dried, pre-weighed crucible (W1). Then the sample was incinerated in a furnace at 525°C. The temperature of the furnace was decreased to 180°C and the crucibles were transferred into a desiccator and cooled for 15-30 minutes and weighed (W3). The ash content was calculated by the following method.

Calculations of ash content

$$Ash, (g\ per\ 100g = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

Sample Preparation for mineral analysis

One milliliter of water and 5 ml of concentrated HCL was added to the ash and then the mixture was boiled to dryness. The dried sample was moistened with 3 ml of 6N HCL and then 6 drops of concentrated Nitric acid was added. Five milliliters of warm water was added and covered with a watch glass and then the sample was boiled for 2 minutes. The sample was cooled and quantitatively transferred into a 100ml volumetric flask and filled to the mark with distilled water then mixed well. The mixture was kept in polyethylene bottles pending analysis.

Magnesium analysis

Sample preparation for Magnesium

Lanthanum chloride weighing 13.369g was transferred into a beaker. This was followed by adding concentrated HCl solution slowly until the material dissolved. The resulting solution was transferred into a 100 ml volumetric flask and distilled water was added to fill up to the mark. 6 ml of this solution was pipetted into a 100 ml volumetric flask containing 10 ml of the previously prepared sample from the ash. Distilled water was then added to fill up to the mark.

Determination of Magnesium

The prepared sample was transferred into polyethylene bottles. To determine the amount of magnesium in the sample, an AS100 spectrophotometer was used. A sucking tube of the spectroscopy was immersed into the sample and then the machine reads the absorbance of the sample which was then displayed on a computer. The standard curve for the standard solution was plotted. From the standard curve, the concentration of the sample was determined.

Calculation of Magnesium

$$Mg\ content\ gMg\ per\ 100g\ sample = \frac{C \times D \times 10}{W}$$

Where:
C is the concentration of the sample solution in ppm read off the standard curve
DF is the dilution factor
W is the Sample weight in grams

Determination of Zinc

The prepared sample was transferred into polyethylene bottles. To determine the amount of zinc in the sample, a n AS100 spectrophotometer was used. A sucking tube of the spectroscopy was immersed into the sample and then the machine reads the absorbance of the sample which was then displayed on a computer. The standard curve for the standard solution was plotted. From the standard curve, the concentration of the sample was determined.

Calculation of Zinc

$$Zn\ content\ gZn\ per\ 100g\ sample = \frac{C \times D \times 10}{W}$$

Where:
C is the concentration of the sample solution in ppm read off the standard curve
DF is the dilution factor
W is the Sample weight in grams

Sensory evaluation

Paired preference test

A panel of 40 untrained assessors was used, two coded samples of Masuku juice were presented to the panelist and they were requested to indicate the sample which they have preferred amongst the two samples and they were also requested to comment on why they have preferred the particular sample.

Statistical analysis

Data collected was entered through the Microsoft Excel version of 2016 and SPSS. The results were analyzed using Statistical Package for Social Science (SPSS) version 20. One-way ANOVA was used to compare the means.

RESULTS AND DISCUSSION

Physiochemical properties

pH and titratable acidity of masuku fruit and masuku juices

Table 1 shows the pH content of masuku raw fruit and masuku juice samples. Raw masuku pulp has an average pH of 4.5 ± 0.14^{a}, juice from unpeeled masuku has a pH of 3.47 ± 0.27^{b} and juice from peeled masuku has a pH of 3.8 ± 0.01^{c}. The results agree with the range reported by Ndabikunze et al. (2010) who reported that the pH of masuku juice ranges from 3.23 to 4.22. There was a significant difference in the pH of the samples ($p<0.05$) when run under the LSD test. The difference in pH of the raw pulp to masuku juices might be due to the addition of preservatives (Sodium benzoate) (Makina, 2018).

The pH of the raw pulp is higher than those recommended for juice making (3.0-3.5) hence there is a need to balance the pH with additional acids such as citric acid (Chawafambira *et al.,*2020). The acid content of the ripened fruit pulp affects the biotransformation of nutrients during processing and product stability in juice (FAO, 1999). Table 1.0 also shows the Titratable acidity of masuku fruit and masuku juice. There is a significant difference in titratable acidity between the samples ($P<0.05$). The TTA value of the raw fruit agrees with the results which were noted by Ndabikunze et al., (2010) (0.59%). Juice from unpeeled masuku had a TTA of 1.1 ± 0.11^{b}% while juice from peeled masuku had TA of 0.88 ± 0.01^{c}%. The TTA of juices agrees with the range which was reported by Saka *et al.,* 2007 (0.85-1.32%). Unpeeled masuku juice had higher TTA as compared to peeled masuku juice. This is due to differences in processing procedures. The masuku skin has tannins that led to an increase in the acidity of the masuku juice and TTA varies directly to acidity (Muchuweti *et al.,*2006). TTA measures the total hydrogen in the juice and it is important because it affects the taste of the juice.

Nutritional Properties of Masuku Fruits and Masuku Juice

Ash content of masuku fruit and masuku juices content

Table 2 shows the ash content of masuku raw fruit and masuku juices. Ash content ranged from $2.7g/100g \pm 0.05^{a}$, $2.2g/100g \pm 0.25^{b,}$ and $2.03g/100g \pm 0.06^{b}$ for raw masuku pulp, juice from unpeeled masuku and juice from peeled masuku respectively. There is a significant difference in the mean ash content of raw fruits and juice samples ($P<0.05$). The Ash content of the raw pulp is higher than what was noted by Moombe in 2009. Moombe (2009) noted that ash content was 2.2g/100g but is lower than 3.2g/100g which was reported by Stadlymar *et al.* (2013). The variations might be attributed to differences in varieties and stages of maturity (Nielson, 2014). The raw pulp ash content (2.7g/100g) is higher than those of the juices because no water was added to it and hence minerals were concentrated unlike in the juices where the concentration of minerals was diluted (Dietz, 1999). Ash content varies directly from the mineral content of the sample.

Magnesium and Zinc content of masuku fruit and masuku juice

Table 3 shows results of magnesium content for raw masuku fruit and masuku juice which was made from peeled masuku and juice which was made from unpeeled masuku. Magnesium content ranged from 32.65 ± 1.16^{a} to 17.20 ± 0.04^{c} mg/100g. The magnesium content of the raw fruit was significantly different from the juices ($P<0.05$). This is because in the fruit no water was added and there was a high concentration of minerals unlike in the juices. There is a significant difference in magnesium content among the juices ($P<0.05$). Juice from unpeeled masuku had higher Magnesium content as compared to juice which was made from peeled masuku. This shows that there are traces of magnesium in the masuku skin which are transferred into the pulp when the whole fruit is squeezed. The value of magnesium for the masuku pulp (raw fruit) is higher

compared to the mean value reported by Ndabikunze, Masambu, and Tiisekwa (2010) but agrees with the value noted in the *Uapaca Kirkiana* fruit by Stadlmayr et al. (2013). Saka et al. (2007) also noted that Masuku juice had a high amount of magnesium. The concentration of magnesium in masuku fruit and masuku juice can contribute 30% to recommended dietary allowances for children aged 1 to 9 years (Food and Nutrition Board, 2005). From Table 3.0, the Zinc content of masuku raw fruit is significantly different from the zinc content of the juices ($P<0.05$). Masuku fruit has 0.937 ± 0.03^{a} mg/100g Zn, this is in agreement with values reported by Chawafambira (2020) but its lower than the values reported by Saka et al. (1992). In this study, it was found that there are no significant differences among the juice samples ($P>0.05$). The Zinc content of the juice samples agrees with Saka et al. (2007). Masuku is a good source of zinc compared to other indigenous fruits (e.g. *A. digitata*; 0.14 mg/100g zinc; *V. infausta*; 0.02 mg/100g zinc) (Amarteifio and Mosase, 2006). Zinc deficiency is a major problem in sub-Saharan Africa (Gadaga et al., 2009) hence the consumption of these juices will help in fighting against this challenge.

Sensory Properties

Paired preference test for Juice from peeled masuku and juice from unpeeled masuku

The results showed that 30 out of 40 assessors preferred Juice from peeled masuku representing 75%. There is a significant difference in preference between the two samples of masuku juices. Juice from peeled masuku is significantly preferred over juice from unpeeled masuku ($p<0.05$). Most of the assessors did not prefer juice from unpeeled masuku because of its bitter/sour aftertaste.

CONCLUSION

From this study, it was noted that the processing procedure affected the nutritional, sensory, and physiochemical properties. There are nutrients in masuku skin (e.g. Magnesium) hence squeezing the whole fruit when pulping helps to utilize the fruit fully. Juice from unpeeled masuku had high magnesium content than juice from peeled masuku fruits. Masuku juice can be used as a rich source of zinc and magnesium. Masuku pulp should be treated with additional acids when processed into juice. Juice from unpeeled masuku was more acidic compared to juice from peeled masuku fruits. Juice from peeled masuku is preferred compared to juice from unpeeled masuku because the juice from unpeeled masuku had a bitter aftertaste.

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Table 1 pH content of masuku raw fruit and masuku juice samples and the titratable acidy of masuku juices

Sample ID	pH content	% Titratable acidity
Raw fruit	4.50 ± 0.14^{a}	0.50 ± 0.02^{a}
Juice from unpeeled fruits	3.47 ± 0.27^{b}	1.10 ± 0.11^{b}
Juice from peeled fruits	3.80 ± 0.01^{c}	0.88 ± 0.01^{c}

Means with different superscripts in the same column are significantly different ($P<0.05$). (Values represent mean ± standard deviation)

Table 2 Results for ash content of masuku raw fruit and masuku juice samples

Sample ID	Ash content g per 100g
Raw fruit	2.70 ± 0.05^{a}
Juice from unpeeled fruits	2.20 ± 0.25^{b}
Juice from peeled fruits	2.03 ± 0.06^{b}

Means with different superscripts in the same column are significantly different ($P<0.05$).

Table 3 Magnesium and zinc content of the juices and the raw fruit

Sample ID	Magnesium content (mg/100g)	Zinc content (mg/100g)
Raw fruit	32.65 ± 1.16^{a}	0.937 ± 0.03^{a}
Juice from unpeeled fruits	17.85 ± 0.03^{b}	0.250 ± 0.01^{b}
Juice from peeled fruits	17.20 ± 0.04^{c}	0.210 ± 0.01^{b}

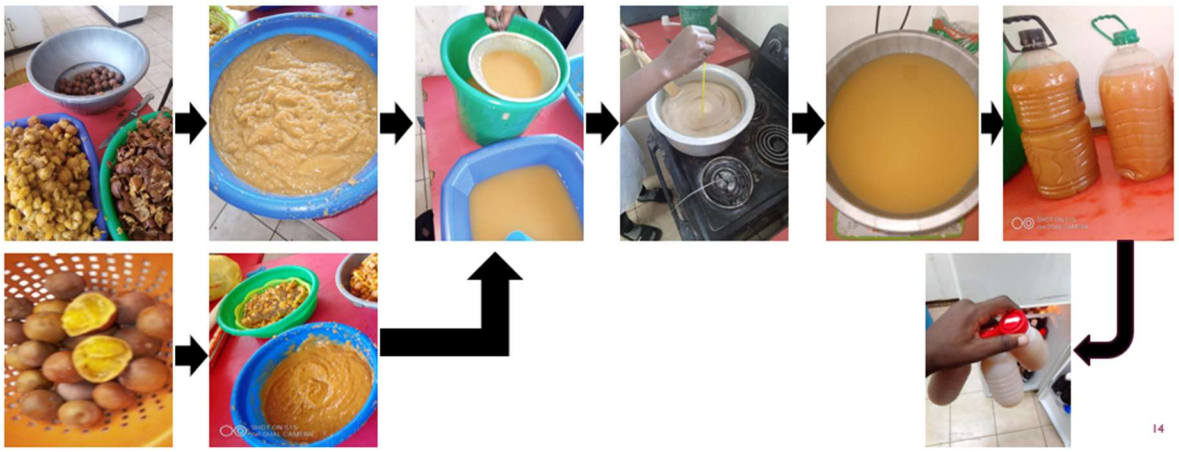
Means with different superscripts in the same column are significantly different ($P<0.05$). (Values represent mean ± standard deviation)

Table 4 Preference scores for masuku juice samples

Sample ID	Number of assessors
Juice from unpeeled masuku fruits	10^{a}
Juice from peeled masuku fruits	30^{b}

Key: The value is the number of assessors and different superscript in the same column shows that there was a significant difference ($p<0.05$)

Figure 1. The process for juice processing



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Kombucha Production in Uganda: Quality Aspects and Compliance with Standards

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ABSTRACT

Kombucha is a mildly sweet and acidic fermented tea beverage. Its production and consumption in Uganda have expanded dramatically as a result of its purported nutritional and health benefits. However, there has been little research into the quality and safety of commercially produced Kombucha in Uganda. This study evaluated the quality and safety of certified (n = 27) and uncertified (n = 16) Kombucha on the market. It also assessed the knowledge and practices of Kombucha processors with certified (n = 4) and uncertified (n = 4) products in Uganda. A HACCP plan for Kombucha processing was also developed and validated with one processor. All products passed the Kombucha requirements for Staphylococcus aureus, Escherichia coli, Salmonella spp., and heavy metals (lead, arsenic, mercury, and cadmium). However, 60.47% of the products did not meet the quality and safety specifications for Kombucha failing to meet the acidity (n = 3), alcohol content (n = 14), and yeasts and molds (n = 15). The majority of the processors (n = 6) had very good scores (> 75%) for knowledge and practices related to food safety but did not know the importance of sanitizing equipment. Half of the processors did not know about HACCP, its prerequisites, and the Kombucha specification. Four processors did not use objective methods to test product readiness. Half of the processors did not follow the Kombucha specification and had no HACCP plan. A HACCP plan with three CCPs and five CPs was developed and validated. This study, therefore, informs Kombucha processors and regulators on the safety and quality of Kombucha on the market and the importance of HACCP plan development and implementation in achieving product quality.

Keywords: Kombucha, Standards, HACCP, Food Safety, Quality

INTRODUCTION

Kombucha is a slightly sweet and acidic refreshing beverage obtained by fermenting sugared black or green tea made from *Camellia sinensis* (L.) Kuntze leaves, with a consortium of yeast and predominantly Acetic Acid Bacteria (Jayabalan et al., 2014: Coelho et al., 2020: Leonarski, et al., 2022). Kombucha production involves the use of a symbiotic culture of bacteria and yeast (SCOBY) following a fairly standard protocol (Jayabalan et al 2014). The yeast component in the SCOBY comprises *Saccharomyces cerevisiae*, along with other species such as Candida, *Saccharomyces*, *Saccharomycoides*, *Schizosaccharomyces*, and *Kluyveromyces* (Jayabalan et al., 2014). The yeasts catalyse the production of ethanol and some flavor compounds such as D-

glucuronic acid, citric acid, L-lactate, Benzeneacetaldehyde, and acetic acid (Villarreal-Soto et al., 2018). The bacterial component usually includes *Gluconacetobacter xylinus*, *Komagataeibacter xylinus*, *Acetobacter xylinum*, which oxidizes ethanol to acetic acid and other organic acids, thus increasing the product acidity and limits ethanol content (Greenwalt et al., 2000). *Komagataeibacter xylinus* is also responsible for producing cellulose (from sugars and ethanol) resulting in the formation of a pellicle in which the Acetic Acid Bacteria and yeasts are embedded (Villarreal-Soto et al., 2018).

Fermented beverages are becoming increasingly popular due to their nutritional and health benefits. Kombucha consumption has been linked to a variety of health advantages (Ernst, 2003; Jayabalan et al., 2014). Kombucha health claims include cleansing the blood, lowering blood cholesterol levels, preventing atherosclerosis, lowering blood pressure, and treating inflammatory issues among others (Gharib, 2014; Ernst, 2003). Most of these health benefits have not been proven in human trials, however, some have been proven in animal studies. Kombucha has been shown to have antibacterial, antioxidant, hepatoprotective, and anticancer effects *in vitro* (Gharib, 2014; Ernst, 2003).

Although Kombucha use is growing in Uganda, there is still limited data on sales and consumption. Kombucha is produced on a small, medium, and large scale by several 'known' and 'unknown' enterprises. In this context, 'known' companies are those that are registered and have their products verified by the Uganda National Bureau of Standards (UNBS). The total number of 'known' Kombucha-certified products in Uganda as of 17 August 2020 was twenty-five (25) (UNBS, 2020).

To regulate the manufacture of Kombucha, UNBS developed a specification (Kombucha drink - Specification US2037: 2019). This standard specifies (i) microorganisms of concern such as yeasts and molds, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., (ii) heavy metals (Lead, Cadmium, Arsenic, and Mercury), (iii) alcohol content and (iv) acidity as acetic acid (UNBS, 2019). UNBS frequently analyses the quality and safety aspects of Kombucha to ensure that products on the market conform to the standard.

Most of the kombucha on the Ugandan market is produced by small and medium-Enterprises (SMEs). However, most SME's are slow at adopting advanced processing technologies, have underdeveloped food safety control systems, and often do not comply with recommended good manufacturing practices. This is exacerbated by the fact that the majority of local SMEs are run by unqualified employees with limited knowledge of food processing, quality, safety, and hygiene. As a result, most SMEs' products frequently fail to meet product specifications and other relevant standards. This could inadvertently contribute to the burden of food-borne illnesses arising from pathogens like *Staphylococcus aureus* and *Escherichia coli*. Before this investigation, there was little information available on the quality and safety of Kombucha in the Ugandan market.

The goals of this study were to (i) determine the quality of Kombucha in the Uganda market, (ii) evaluate Kombucha processors' knowledge and practices, and (iii) develop and test a basic HACCP plan for Kombucha production. The findings will be used to inform processors and regulators about the quality of Kombucha on the market. They will also be used to recommend quality assurance mechanisms that will enable the consumers to enjoy safe and quality Kombucha.

MATERIALS AND METHODS

Study area and design

The study area included central and western districts of Uganda; Kampala, Wakiso, Mityana, Mbarara, Ntungamo, Kibale, and Kasese. These districts were chosen because they house the majority of Kombucha-producing SMEs. A mixed methods research approach with three study designs was used. Firstly, secondary data on the quality and safety aspects of certified and uncertified Kombucha products was obtained from the UNBS database for samples analysed between 2019 and 2020. Secondly, a descriptive cross-sectional design with a survey questionnaire was used to evaluate the knowledge and practices of Kombucha processors. Lastly, a longitudinal and observational study design was used to develop and validate a HACCP plan for Kombucha for one willing Kombucha producer.

Sample size and participants

Secondary data including parameters specified in the Kombucha specification; US 2037: 2019 was obtained from the Uganda National Bureau of Standards (UNBS) database for samples analysed between 2019 and 2020. The database contained results for 200 samples but only 43 samples (27 certified products and 16 uncertified products) had complete data for all parameters and were included in the study. According to UNBS (2020), there were 25 companies with certified products at the time of the study. Since the number of companies with uncertified products was not known it was assumed to be equal to that of those with certified products (thus giving a total of n = 50). Using an online sample calculator (Raosoft, 2004), a margin of error of 5%, a confidence level of 95%, an estimated population size of 50, and a non-response rate of 10%, the sample size was estimated as 50 (with 25 having certified products). It was planned to interview 50 processors (1 processor per company × 50 companies = 50) to ascertain their knowledge and practices concerning product safety and quality. The participants targeted were those in positions of either quality assurance manager, quality supervisor, quality controller or anyone directly concerned with production and quality management. However, a number of companies declined to participate in the study while some were out of production at the time of the study due to challenges associated with the COVID-19 pandemic. In the end, only eight (8) companies agreed to participate in the study.

Analysis of quality and safety parameters of Kombucha

Data on the quality of Kombucha was obtained from the UNBS. The data set comprised the sample identifier, company name, product name, and results of analyses based on the Kombucha specification (US 2037:2019). The parameters tested included; alcohol content, acidity (as acetic acid), microbial counts (yeast and molds, *Escherichia coli*, *Staphylococcus* spp, *Salmonella* spp), and heavy metals namely; lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (Ar). The alcohol content was determined by the specific gravimetric method (UNBS, 2014), and acidity was determined by UNBS (1998). *E. coli*, yeasts and molds, and *Staphylococcus* spp counts were determined following UNBS (2012), UNBS (2008), and UNBS (2014) standard methods, respectively. Detection of *Salmonella* spp. was done according to UNBS (2017). The determination of heavy metals was based on the analyses of the ash obtained by dry ashing at 400 °C (UNBS, 2007). Lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (Ar) were determined using inductively coupled plasma optical emission spectrometry.

Evaluating knowledge and practices of Kombucha processors

Face-to-face interviews with eight processors (4 from companies with certified products and 4 from companies with uncertified products) were carried out using a researcher-administered questionnaire. The questionnaire used earlier for *Obushera* processors was adopted and modified (Byakika et al., 2019). The questionnaire was composed of sections to capture information on; the company profile, processing of Kombucha, knowledge of basic food safety and hygiene aspects, knowledge of relevant standards/specifications essential for beverage production, and execution of appropriate or recommended practices which were verified by the researcher, HACCP system, Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP) and product certification among others. The questionnaire had provisions for ''YES'' or ''NO'' responses concerning knowledge and practice questions.

Developing and validating a Hazard Analysis and Critical Control Point plan for Kombucha processing

One willing company with uncertified Kombucha was selected to develop and validate a HACCP plan for Kombucha. A detailed recommended HACCP plan was developed following UNBS (2017) and UNBS ISO (2015). The HACCP plan was developed and given to the company for adoption and implementation. The company employees were trained in HACCP system implementation using the Uganda standard for HACCP requirements (US 130:2017). Monitoring of HACCP plan implementation was done through evaluating record keeping and documentation, onsite observations, and product testing for compliance with product standards. HACCP plan validation was done practically by in-plant observation of production processes as stipulated in UNBS (2017) and UNBS ISO (2015). A baseline to assess product quality and documentation processes was carried out before the adoption of the proposed HACCP plan. Post-adoption tests were done for one month at intervals of one week to analyse for microbial counts, alcohol and acetic acid content, and heavy metals as described in section 2.3. Two (2) samples per week were picked for analysis for one month.

Statistical analysis

Results of analysis of Kombucha (acidity, alcohol content, microbial counts, and heavy metal analysis) were checked for conformation with the Kombucha specification (pass or fail). Descriptive statistics were used to compile data on the knowledge and practices of Kombucha processors. A mark/point was scored for each correct response while no point was given for a wrong response for data on the knowledge and practices of processors. Total points per processor per section were computed as a percentage. Final percentage scores per section were categorized as; 0–25% (very poor), 25–50% (fairly poor), 50–75 (fairly good), and 75–100% (very good). Means of data on samples tested before and after HACCP plan development and implementation were compared using a t-test. The significance level was set at < 0.05. All data were analyzed using Statistical Package for Social Science (SPSS), version 19.0.

RESULTS AND DISCUSSION

Quality and safety of Kombucha on the market in Uganda

Table 1 summarizes the conformity assessment of Kombucha from different products and their conformance with specifications. All the samples (n = 43) passed the specifications for acetic acid, heavy metals, *Staphylococcus aureus*, *E.coli*, and *Salmonella* spp. However, only 28 and 29 of the samples passed fungal counts and

alcohol content, respectively. Some samples passed yeast and molds but failed the alcohol content and vice versa. This resulted in only 17 of the samples having overall conformance with the Kombucha specification. It is a requirement by UNBS that for a product to be certified as safe for final consumption it must comply with all the requirements in the product specification (UNBS, 2019). Therefore, some samples conforming partly to the requirements in the standard did not guarantee total compliance with the specification. Failure on the yeast and molds parameter for both certified and uncertified Kombucha products could be because Kombucha is fermented by a SCOBY containing yeast and most of the products are not pasteurized post-fermentation. Kombucha thus contains leftover yeast from the SCOBY which can continue growing during storage, especially at room temperature thus continuing to catalyse the production of alcohol from fermentable sugars in non-alcoholic Kombucha (Varzakas, 2020), which contributed to the failure of both parameters. Filtration process could be used and considering high temperature short pastuerisation process to eliminate the residual yeast. Continued growth of the SCOBY can also lead to overproduction of acetic acid causing undesirable souring of Kombucha. It is, therefore, important to control microbial growth and this can be done by pasteurizing the final product, the addition of 0.1% of sodium benzoate and 0.1% of potassium sorbate as food preservatives, and finally, keeping it refrigerated (Watawana et al., 2015).

Knowledge and practices of Kombucha processors in Uganda

Characteristics of the Kombucha processors

Table 2 summarizes the major characteristics of the Kombucha processors (n = 8) interviewed. There was an equal proportion of processors with certified and uncertified products. Most of the processors (n = 6) had 2-4 years of experience in Kombucha production. This can be explained by the fact that commercial Kombucha processing is relatively new in Uganda having started in July 2019 with the first sample certified by UNBS (cims.unbs.go.ug as of October 2021). Most of the processors (n = 6) had very good knowledge and practices related to food safety. Several studies on the knowledge and practices of processors of fermented products have reported a high proportion of processors with very good knowledge and self-reported practices related to food safety (Mukisa et al., 2020; Byakika et al., 2019; Muwanguzi, 2018; Kiberinka, 2018; Akabanda et al., 2017). High scores on the knowledge and practices of processors may translate into improved product quality and safety (UNBS, 2017). However, some studies have reported that high scores on knowledge and practices may not necessarily translate into products conforming to standards (Byakika et al., 2019; Akabanda et al., 2017). This is because the operators may know what the standard requires but may opt not to implement the requirements due to a poor attitude, or lack of appreciation of the importance of the specification among other things.

Knowledge of Kombucha processors

Table 3 summarizes the food safety knowledge of the Kombucha processors interviewed. Although the processors had fairly good to very good knowledge scores (Table 2), some were ignorant about key food safety issues. All processors (n = 8) were knowledgeable about the importance of product certification, hand washing practices, use of clean raw materials, and that eating and drinking in the processing area can lead to product contamination. Food product

certification is a reflection of standards implementation and uptake thus correlates with food safety improvement (Teixeira and Sampaio, 2013). Food safety certification does not only provide proof that the product itself is safe to use but also warrants that the business holding the certification has met both the professional and ethical standards to run a business selling food to the public (Kaczorowska et al., 2021). Additionally, personnel hygiene through hand washing and cleanliness is important in the prevention of food product contamination (Djekic et al., 2014; Margas and Holah, 2014).

Most processors (n = 6) did not know the importance of sanitizing utensils while half of the (n = 4) had no knowledge of the prerequisites of HACCP (i.e. GMP/GHP) and a HACCP system as from their responses subtmited. In earlier studies by Rossoni and Gaylarde (2000) sodium hypochlorite was reportedly used to sanitize equipment. Its application during cleaning is hence relevant in sanitizing equipment before Kombucha production thus ensuring product safety (Rossoni and Gaylarde, 2000). HACCP system implementation is key in the identification of food safety hazards and preventing them before they can cause significant food safety risks to end-product consumers (Liu et al., 2021). Furthermore, the HACCP system and other food safety systems facilitate trade at national, regional, and international levels as a number of countries adopt similar standard practices in ensuring control of food safety hazards that lead to foodborne illnesses (Caswell and Hooker, 1996). However, HACCP system implementation is guided by initially complying with the HACCP prerequisites for example GMP and GHP (UNBS, 2017). These prerequisites ensure that food handlers and the environment are safe for hygienic and safe food production (Roberts and Sneed, 2003). Conversely, failure to observe the prerequisite programs may lead to retained challenges in HACCP plan implementation (Baş et al., 2006). HACCP plan implementation should be applied in all stages of food chain production to ensure that the safety of the final product is not compromised (Pierson, 2012).

Food safety practices of Kombucha processors

Table 4 summarizes the self-reported food safety practices of the Kombucha processors interviewed. All the processors (n = 8) claimed to have good hygiene practices, sanitized utensils, had vermin-proof storage facilities, or used treated water for processing. Food processors are expected to observe proper hygiene and sanitation as the chances of food contamination largely depend on their health status and hygiene practices. All of the processors (n = 8) indicated that they usually washed their hands before handling food and after handling money or any contaminated surfaces. Effective hand washing is an essential control measure for the prevention of pathogens (Ifeadike et al., 2014). Food industries must use portable water that meets microbiological, physicochemical, and organoleptic characteristics as indicated by national standards (UNBS, 2014). Water when used as a processing aid has a direct quality impact on final product quality. Therefore, clean and safe water must be used in Kombucha production (Brennan and Grandison, 2012). Water management is critical in the food sector, both in terms of water quality and quantity. This is because if not adequately treated, reused water might contaminate the finished product compromising its safety (Kirby et al., 2003). About half of the processors (n = 4) did not use: (i) objective methods for testing product quality, (ii) running water for washing bottles and their caps, and (iii) did not have the Kombucha specification and a HACCP plan. These results were similar to those in earlier

studies on fermented traditional foods like *Obushera* and food from serving points such as rice, beans, and beef (Jeffer et al., 2021; Byakika et al., 2019; Baluka et al., 2015). All of these findings indicate that the food chain's food safety performance was poor, owing to poor sanitation, hygiene, and handling standards, as well as inadequate HACCP plan implementation. Therefore, HACCP-based training coupled with robust preventive, intervention, and monitoring systems should be strengthened in food production with the SMEs in Uganda.

Hazard Analysis and Critical Control Point plan for Kombucha processing

Technical staff members (n = 4) of one company with uncertified Kombucha were trained in developing and managing a HACCP system. After the training, a process flow diagram (Figure 1) was developed. Each step on the flow diagram was assessed for potential hazards and used to identify critical control points on the HACCP plan. This was important in eliminating significant food safety hazards (UNBS, 2017; UNBS ISO, 2015; Corlett, 1998).

A HACCP plan for the company product was developed (Table 5). Three CCPs and five CPs were identified. The CCPs included: (i) boiling of sugar, tea, and water mixture, (ii) sieving, and (iii) pasteurization of the fermented Kombucha. The CPs included: (i) reception of raw materials and other materials, (ii) storage of raw materials, (iii) fermentation process, (iv) packaging, and (v) storage of finished product. CCPs are important for the complete elimination of significant food safety hazards or for reducing them to acceptable levels that do not compromise consumer safety and health (UNBS, 2017; UNBS ISO, 2015; Corlett, 1998). At these CCPs, critical limits were established as a criterion for separating acceptability from unacceptability for example maximum limits for pathogenic microorganisms as detailed in the Kombucha specification (UNBS, 2019). At each CCP particular control measures like time-temperature regimes during boiling were established and monitored to prevent any deviations from the critical limits. This is because the loss of control at a CCP would lead to failure in eliminating specified food safety hazards hence affecting the safety of the final product (UNBS ISO, 2015; Corlett, 1998). The HACCP plan was thereafter, given to the company for implementation and validation.

The results of the validation of the developed HACCP plan are shown in Table 6. Before the HACCP plan adoption, the products did not meet the yeasts and molds requirement. This might have been due to the continued growth of residual yeasts and molds from the added SCOBY during the Kombucha production. The yeasts and molds metabolized sugars to produce alcohol and carbon dioxide (Mukisa et al., 2017). Adoption of the HACCP plan resulted in the products meeting the important microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, Salmonella spp. and yeast and molds requirements. This was due to the introduction of the pasteurization step as a new CCP during process improvement thus resulting in the removal of yeasts and molds (Byaruhanga and Ndifuna, 2002). After the HACCP plan adoption, the alcohol content was reduced and the acidity increased. The alcohol content after the HACCP plan adoption was reduced due to the post-process elimination of yeasts and mold that were responsible for its synthesis. There was no continued fermentation to produce alcohol after yeasts removal during pasteurization (Byaruhanga, and Ndifuna, 2002). Termination of fermentation led to a moderation of the alcohol and acid content, which would ultimately result in improved product shelf stability and shelf life (Gimbi et al., 1997). Other added benefits of

moderation of alcohol and acid content in the product included; improved product sensory acceptability, product safety, and quality (Farag et al., 2020; Mukisa et al., 2012; Byaruhanga, and Ndifuna, 2002) and reduced incidence of acidosis upon consumption of Kombucha (Farag et al., 2020).

Results for post-HACCP adoption showed improved compliance with the Kombucha specification, implying that the HACCP plan had a significant and positive effect on the quality and safety of Kombucha as in earlier reports (Liu, 2021; Bai et al., 2007). The overall non-compliance of the products before the HACCP plan adoption and overall compliance of the products after the HACCP plan adoption might have been due to acquired knowledge and skills imparted by participants during the HACCP training (Ghafar et al., 2015; Chang et al., 2003). This was envidenced from the improved microbiological quality after HACCP plan implementation. The HACCP plan training and adoption might have improved the industry's food safety system hence leading to products complying with the specification.

CONCLUSION

Despite the good knowledge and practices, only a few 39.53% products met quality and safety specifications for Kombucha. This was due to failure in complying with the requirements for yeasts and molds as well as alcohol content. Although the Uganda specification for Kombucha has a limit for yeasts and molds, yeasts such as *Saccharomyces cerevisiae* are part of the normal flora of the SCOBY and remain after fermentation provided no post-process treatments are carried out. Therefore, the presence of yeasts in Kombucha may not necessarily amount to a microbial hazard. Their presence is only likely to lead to the production of high amounts of ethanol and early product spoilage. Therefore, this needs to be taken into consideration when revising the maximum limits for yeasts and molds requirement. A company may have to introduce a pasteurization step post-fermentation or use antifungal preservatives to inactivate the remaining flora from the SCOBY just to ensure product stability and enhanced safety and quality.

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Table 1. Conformity assessment of Kombucha on the Uganda market and its conformance with specifications

Product	Specification requirements (US 2037:2019)										
	***Staph* (cfu/ml)**	***E.coli* (MPN/ml)**	***Salmonella* Spp. (25ml)**	**Yeast (cfu/ml)**	**Alcohol (%v/v)**	**Acidity (g/L)**	**Lead (mg/l)**	**Cadmium (mg/l)**	**Arsenic (mg/l)**	**Mercury (mg/l)**	**Overall compliance**
*C1+	<1	0	ND	<1	<0.07	0.6	0.05	0.002	<0.05	<0.1	P
C2+	<1	<1	ND	<1	10	0.5	0.05	0.002	<0.05	<0.1	P
*C3	<1	<1	ND	TNTC	2.5	0.1	0.05	0.002	<0.05	<0.1	F
C4+	<1	0	ND	<1	4.3	0.3	0.05	0.002	<0.05	<0.1	P
C5+	<1	0	ND	<1	11	0.2	0.05	0.002	<0.05	<0.1	P
C6	<1	0	ND	<1	<0.07	0.2	0.05	0.002	<0.05	<0.1	F
*C7+	<1	<1	ND	<1	<0.07	0.2	0.05	0.002	<0.05	<0.1	P
*C8+	<1	<1	ND	21	<0.07	0.4	0.05	0.002	<0.05	<0.1	P
*C9+	<1	<1	ND	19	<0.07	0.4	0.05	0.002	<0.05	<0.1	P
*C10	<1	0	ND	<1	6	0.8	0.05	0.002	<0.05	<0.1	F
*C11	<1	0	ND	500	0.07	0.4	0.05	0.001	<0.05	<0.1	F
*C12	<1	0	ND	TNTC	0.07	0.4	0.05	0.001	<0.05	<0.1	F
*C13	<1	0	ND	TNTC	0.07	0.4	0.05	0.001	<0.05	<0.1	F
C14	<1	0	ND	TNTC	5.7	0.2	0.05	0.002	<0.05	<0.1	F
*C15+	<1	0	ND	20	3.7	0.02	0.05	0.002	<0.05	<0.1	P
C16	<1	<1	ND	TNTC	6.9	4	0.05	0.002	<0.05	<0.1	F
C17	<1	<1	ND	TNTC	8.8	5	0.05	0.002	<0.05	<0.1	F
C18	<1	0	ND	<1	0.07	1	0.05	0.002	<0.05	<0.1	F
*C19	<1	0	ND	<1	5.6	1	0.05	0.002	<0.05	<0.1	F
C20	<1	0	ND	TNTC	5	0.2	0.05	0.002	<0.05	<0.1	F
C21+	<1	<1	ND	<1	<0.07	0.4	0.05	0.002	<0.05	<0.1	P
*C22	<1	<1	ND	<1	1.8	16	0.5	0.05	<0.002	<0.05	F
C23+	<1	<1	ND	35	5.9	0.9	0.05	0.002	<0.05	<0.1	P
C24	<1	<1	ND	TNTC	2	0.5	0.05	0.002	<0.05	<0.1	F
C25	<1	<1	ND	TNTC	7.2	0.2	0.05	0.002	<0.05	<0.1	F
C26	<1	<1	ND	TNTC	5.3	1	0.05	0.002	<0.05	<0.1	F
C27+	<1	0	ND	<1	4	0.6	0.05	0.002	<0.05	<0.1	P

*UC1	<1	0	ND	TNTC	3.1	0.2	0.05	0.002	<0.05	<0.1	F
*UC2	<1	0	ND	<1	10.9	1	0.05	0.002	<0.05	<0.1	F
*UC3	<1	<1	ND	<1	2.6	0.4	0.05	0.002	<0.05	<0.1	F
UC4^{+}	<1	<1	ND	1	3.9	0.2	0.05	0.002	<0.05	<0.1	P
*UC5	<1	<1	ND	TNTC	4.5	0.3	0.05	0.002	<0.05	<0.1	F
*UC6	<1	0	ND	<1	4.2	2	0.05	0.002	<0.05	<0.1	F
UC7^{+}	<1	0	ND	<1	6	0.4	0.05	0.002	<0.05	<0.1	P
UC8^{+}	<1	0	ND	<1	2.3	0.2	0.05	0.002	<0.05	<0.1	P
*UC9	<1	<1	ND	<1	5.6	0.1	0.05	0.002	<0.05	<0.1	F
*UC10	<1	0	ND	TNTC	2.1	5	0.05	0.002	<0.05	<0.1	F
*UC11^{+}	<1	0	ND	<1	0.07	0.7	0.05	0.002	<0.05	<0.1	P
*UC12	<1	0	ND	TNTC	<0.07	0.3	0.05	0.002	<0.05	<0.1	F
*UC13	<1	<1	ND	58	4.2	0.4	0.05	0.002	<0.05	<0.1	F
UC14^{+}	<1	<1	ND	<1	2.1	0.2	0.05	0.002	<0.05	<0.1	P
UC15	<1	0	ND	TNTC	1.9	1	0.05	0.002	<0.05	<0.1	F
UC16^{+}	<1	0	ND	<1	2	1	0.05	0.002	<0.05	<0.1	P
STD	Absent	Absent	Absent in 25 ml	100 Max. (cfu/ml)	0.5 (Max non-alcoholic) 0.6-15 (Alcoholic)	2 Max.	0.05 Max.	0.003 Max.	0.05 Max.	0.001 Max.	

*STD = Standard, C = certified products, UC = Uncertified product, Staph= Staphylococcus aureus, Max. = Maximum, ND = Not detected in 25 ml, P = Passed, F = Failed. *product was labelled as non-alcoholic. $^{+}$Sample which passed all the parameters in the specification.*

Table 2. Characteristics of the Kombucha processors interviewed in the study

Characteristic	Frequency (n = 8)
Certification status	
Uncertified	4
Certified	4
Location (district)	
Kampala	2
Wakiso	1
Mityana	1
Mbarara	1
Ntungamo	1
Kibale	1
Kasese	1
Kombucha processing experience (years)	
< 2	2
2-4	6
Food safety knowledge Category	
Very Good	6
Fairly Good	2
Food Safety Practices Category	
Very Good	6
Fairly Good	1
Fairly Poor	1

Table 3. Food safety knowledge of Kombucha processors

Food safety knowledge questions/statements	**Response (Frequency)**	
	Correct	**Wrong**
Necessary to have your product certified by UNBS	8	0
Hand washing prior to processing can affect Kombucha safety	8	0
Hand washing after touching money can affect Kombucha safety	8	0
Hand washing after using washrooms can affect Kombucha safety	8	0
Hand washing after touching the body can affect Kombucha safety	7	1
Hand washing after using the phone can affect Kombucha safety	6	2
Hand washing after each break can affect Kombucha safety	7	1
Hand washing after handling garbage can affect Kombucha safety	8	0
Sanitizing utensils increases the risk of Kombucha contamination	2	6
Washing utensils with detergent makes them sterile	8	0
Eating and drinking during processing increases the risk of Kombucha contamination	8	0
Diarrhea, vomiting, and stomach pain arise from drinking Kombucha made unhygienically	5	3
Microorganisms are found on the skin, hair, and hands of processors and they are potential pathogens	6	2
The use of clean and well stored raw materials is vital for Kombucha safety	8	0
Pathogens change the sensory properties of Kombucha	7	1
Monitoring of water quality is important in ensuring Kombucha safety	7	1
What is GMP/GHP program?	4	4
What is a HACCP plan?	4	4
What do you understand by a product standard/ specification?	5	3
Does Uganda have a product specification for Kombucha?	8	0
Name the standard/specification for Kombucha?	4	4

Table 4. Self-reported food safety practices of Kombucha processors

Food safety practice questions/statements	Response (Frequency)	
	Correct	Wrong
Have a foot bath at the entry to the facility	7	1
Check the length and cleanliness of the nails of the processors	8	0
Ensure workers wear proper head gear during processing	8	0
Ensure workers wear closed shoes during processing	8	0
Processors remove the jewelry and other accessories before processing	8	0
Ensure workers wear separate clothes specific for processing	8	0
Processors are examined for contagious diseases	7	1
Workers wash and sanitize their hands before and during work	8	0
Sanitize utensils before processing	7	1
Sanitize utensils after processing	8	0
Sanitize packaging material before use	5	3
The facility is vermin proof storage	8	0
Use treated water for Kombucha processing	8	0
Use of objective methods to test the readiness of Kombucha	4	4
Adequately clean packaging materials (use soap, clean water, and sanitizer)	7	1
Use running water/regularly change water for washing used bottles and cups	4	4
Wash utensils after Kombucha processing	8	0
Store utensils in a clean area separate from raw materials	5	3
Use Kombucha preparation utensils for other purposes	5	3
Dispose garbage in a covered garbage receptacle	5	3
Have /follow a Kombucha specification, if yes, state it	4	4
Follow a Hazard Analytical Critical Control Point (HACCP) plan	3	5

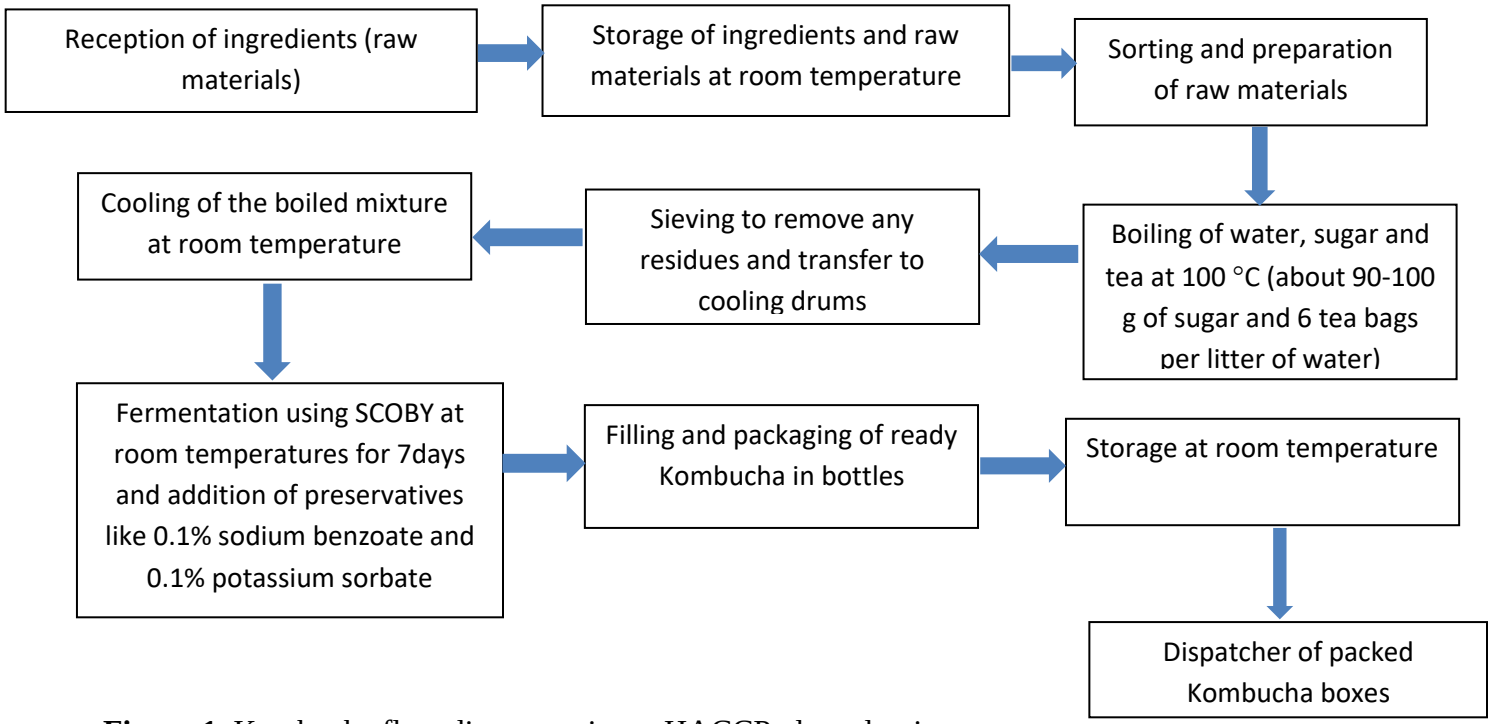

Figure 1. Kombucha flow diagram prior to HACCP plan adoption

Table 5. The HACCP control chart for Kombucha developed in this study

Process Step: CP or CCP#	**Hazard**	**Critical Limits**	**Monitoring Procedure**	**Frequency**	**Preventative Measure**	**Corrective Action**	**Record**	**Responsible Person**
Reception of raw materials (sugar, tea leaves) and packaging materials **CP# 1**	• Biological hazards such as insects from tea leaves • Chemical hazards such as migratory materials from plastic packaging materials • Physical hazards (small stones and dust from sugar or tea leaves)	No unqualified product to be used	Apply supply quality assurance by use of standards	Each supply	• Qualified raw materials and ingredients • Checked Material Safety Data sheet • Approved suppliers list • Use of specifications	• Reject defective batches of supplies • Change of suppliers or brand • Employees training	Material receiving report	Assigned Quality assurance Officer
Storage of Sugar and tea leaves at room temperature **CP# 2**	Microorganisms (Yeast and molds)	Not more than 100 CFU/ml	• Proper safety data sheets for raw material quality and storage conditions as per standards • Constant monitoring and regular microbial counts checks to ensure safety of raw materials	Routinely monitoring of the humidity in the stores to prevent wetting of sugar and other raw materials to prevent microbial growth	Proper storage in dry places and humidity checks	Reject the raw material	Humidity log sheets and microbial counts	Assigned Quality assurance Officer
Boiling water and added raw materials such as sugar and tea leaves **CCP# 1**	Microorganisms (Yeast and molds, *E. coli*, *Staphylococcus aureus* and *Salmonella* spp)	• Not more than 100 CFU/ml for yeasts and molds • Absent in 25ml for *Salmonella* spp • Absent for *Staphylococcus aureus* • Absent for *E.coli*	Check the Core Temperature and time	Each batch	• Heating to boiling point of 100 ◦C • Check the core-temperature (CT) of the product keep records	Adjust the temperature and time by setting the equipment well; Call the engineer to repair	Time and Core Temperature log: Maintenance register	Assigned Quality Assurance Officer
Sieving **CCP# 2**	Physical contaminants	No physical foreign matter	Check the sieve clothes for the right	Each Batch	Prior check of sieve clothes for hygiene and right sieve sizes	Changing the sieve clothes to	Inspection report	Production Manager

	(insects, small stones and dust		sizes prior to sieving of the product			replace right ones,		
Fermentation using SCOBY **CP# 3**	Pathogenic microorganisms	• No pathogenic microorganism s: *Salmonella* spp (Absent in 25 ml), *Staphylococcus aureus* (absent in what volume?) *E.coli* (absent in what volume?)	Monitoring the fermentation conditions like temperature and final pH	Each batch	Route checking of the Time-temperature and pH	Adjustment of the temperatures and pH	Time, pH meter and CT log: Maintenance register	Assigned Quality Assurance Officer
Pasteurization of Kombucha **CCP# 3**	Residual Pathogenic microorganisms, or utensils and handlers during the fermentation process	Absent for pathogenic molds • No pathogenic microorganism (Absent in 25ml for *Salmonella* spp • Absent for *Staphylococcus aureus* Absent for *E.coli*	Check the core temperature (CT) of the product and holding time	Each batch	• Heating to 85 ◦C and holding for 10 minutes (Leonarski, et al., 2022) • Check the CT and time • Keep records	Adjust the temperature and time by setting the equipment well; Call the engineer to repair	Time and CT log: Maintenance register	Assigned Quality Assurance Officer
Packaging **CP# 4**	Chemical hazards such as migratory materials from plastic packaging materials	No unqualified product is to be used	Visual inspection for foreign materials, hygiene, leaking, and following of packaging specifications	Each Pack	• Disinfection of packaging bottles • Personal hygiene and physical inspection • Use of specifications for packaging materials	Retain, rework, or discard based on foreign material identified	Inspection report	Packaging operator and Quality Assurance Manager
Storage and distribution of Kombucha **CP# 5**	Microorganisms from packaging materials)	Absent	Check the time and temperature regime	Routinely	• Keeping the products at < 4◦C for a shelf life 14 days • Check storage temperature, shelf life	Retain or reject based on the product testing panelist Record keeping	Temperature log: Delivery report	Quality assurance Manger

Table 6. Comparison of microbial and physicochemical parameters of Kombucha products pre and post-HACCP implementation

Parameter	Before HACCP implementation		After HACCP implementation	
	Sample	Values	Samples (week)	Values
Microbial				
Escherichia coli (CFU/ml)	1	<1	1	<1
	2	<1	2	<1
			3	<1
			4	<1
Yeast and molds (CFU/ml)	1	TNTC	1	<1
	2	TNTC	2	<1
			3	<1
			4	<1
Staphylococcus aureus (CFU/ml)	1	<1	1	<1
	2	<1	2	<1
			3	<1
			4	<1
Salmonella spp. (/25ml)	1	ND	1	ND
	2	ND	2	ND
			3	ND
			4	ND
Physicochemical				
Alcohol content (%v/v)	1	2.1	1	1.1
	2	2	2	1.0
			3	1.1
			4	1.0
	Mean	2.0250[a]		1.0750[b]
Acidity as (acetic acid, g/L)	1	0.5	1	0.9
	2	0.5	2	0.9
			3	0.9
			4	0.9
	Mean	0.5000[a]		0.9000[b]
Lead (mg/L)	1	<0.05	1	<0.05
	2	<0.05	2	<0.05
			3	<0.05
			4	<0.05
Cadmium (mg/L)	1	<0.002	1	<0.002
	2	<0.002	2	<0.002
			3	<0.002
			4	<0.002
Arsenic (mg/L)	1	<0.05	1	<0.05
	2	<0.05	2	<0.05
			3	<0.05
			4	<0.05
Mercury (mg/L)	1	<0.001	1	<0.001
	2	<0.001	2	<0.001
			3	<0.001
			4	<0.001

N = 2 (2 samples per week before HACCP plan adoption). N = 4 (2 samples per week after HACCP plan adoption). TNTC= Too numerous to count, (Dilution factor for E.coli and yeast and molds were 1×10^{0} and 1×10^{1}, respectively). ND = Not detected. Means with different superscripts (a,b) in a row are significantly different (P < 0.05).

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Improvement of Bread Nutrition with The Addition of Coffee Silverskin as a Source Of Dietary Fiber And Antioxidants

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ABSTRACT

Obesity can be prevented by reducing the intake of high carbohydrates and fats, then replacing with high dietary fiber. Coffee silverskin is a by-product of coffee beans with high dietary fiber (54-74%) and antioxidants, which can potentially treat obesity. Indonesia has a traditional bread named "Gandjel Rel" from Semarang, which has a box-shaped, brown color with a sprinkling of sesame on the surface, and cinnamon flavor. The aims of this study are to increase its functional properties. In the bread making process, silverskin from Robusta coffee (Coffea canephora) flour is added with a variation of 0; 1; 2,5; and 5% (dw). The dough was baking at a temperature of 120˚C for 30 minutes. The bread was analyzed in terms of its dietary fiber, antioxidant activity by DPPH method, physical (hardness and color intensity), chemical properties (proximate), and sensory analysis. Bread with robusta coffee silverskin has higher dietary fiber (3,33-7,18% (w/w) insoluble fiber and 0,25-0,77% (w/w) soluble fiber) and antioxidant activity (26,23-31,36 g trolox eq./ 100 gr). Additional of coffee silverskin in the bread dough did not change the texture significantly, however, it did alter its physical appearance due to coffee silverskin's brown color obtained from the coffee bean roasting process, so reducing brightness level and the yellowness of the bread. The panelists preferred bread by adding 1% robusta coffee silverskin. This bread has higher protein (11,33%, w/w), than conventional bread's protein (8%). In addition, this bread contains dietary fiber and antioxidants, so it has the potential as a functional food.

Keywords: Antioxidant, Bread, , Dietary Fiber, Obesity, Robusta Coffee Silverskin

INTRODUCTION

Based on the 2018 Basic Health Research, the prevalence of overweight in children aged 5-12 years and adults aged > 18 years was 18.8% and 13.5%, respectively. Meanwhile, the prevalence of obesity in children aged 5-12 years and adults aged > 18, respectively, amounted to 10.8 and 28.7%. Based on WHO guidelines for Asian adults, the category is overweight if BMI is between 23–24.9 kg/m2, and the obese category is if BMI is ≥ 25 kg/m2. Body mass index (BMI) is determined by comparing body weight and height. One of the causes of overweight and obesity is the accumulation of body fat due to energy intake exceed the energy used (Saraswati et al., 2021). Sedentary behavior, lower physical activity,

and higher intake of fried foods, sweet foods and beverages, also food high in refined carbohydrates and fatty also significant contributors to overweight and obesity in general (Nurwanti et al., 2018).

Prevention of obesity could be done by reducing the intake of high in carbohydrates and fats, then replacing them with foods high in fiber. Dietary fiber consumption is reported to be able to reduce the risk of obesity because of its role in regulating energy balance and increasing satiety. Dietary fiber reduces food energy density or calories, so consuming it has less energy than other foods in the same portion. High dietary fiber can reduce digested energy intake to reduce the risk of obesity (Waddel and Orfila, 2022).

Coffee consumption in Indonesia increased by 8.22% per year, indirectly increasing the number of coffee by-products. Dry, semi-dry or wet coffee processing produces by-products reaching 45-50% of the whole coffee fruit, namely skin, husk, pulp, parchment, and silverskin (Santos et al., 2021). Coffee silverskin is a by-product obtained from the roasting process, which has a thin layer like the epidermis attached to coffee beans.

The use of coffee silverskin is still limited to making compost and biogas (Santos et al., 2021). Coffee silverskin is rich in nutrients, such as dietary fiber (60%) and minerals (8%), which are dominated by potassium, magnesium and, calcium, and protein (20%) (Santos et al., 2021). In addition, several bioactive compounds in coffee silverskin have anti-obesity properties, namely caffeine (1,37 g/100 g) and polyphenols such as chlorogenic acid (56,5 mg CGA eq./g) and 5-caffeoylquinic acid (21,3 mg/g) (Martinez-Saez et al., 2014). Therefore, coffee silverskin can potentially be processed and utilized as a functional food for anti-obesity. Coffee silverskin has been used in food products, such as beverages, yogurt, bread, cookies, biscuits, and cakes (Klingel et al., 2020; Martinez-Saez and del Castillo, 2018; Bertolino et al., 2019; Pourfarzad et al., 2013; Gocmen et al., 2019; Ateş and Elmacı, 2018; 2019).

One of Indonesian's traditional breads, named "Gandjel Rel" bread from Semarang, which has a box-shaped, brown color with a sprinkling of sesame on the surface and a cinnamon flavor. The brown color is obtained using palm sugar with a low glycemic index (35.56) (Ismail et al., 2020). Consumption of foods with a low glycemic index can improve insulin sensitivity, delay hunger, and reduce the rate of glucose absorption and it is useful in treating and preventing obesity (Pereira et al., 2014; Schwingshackl and Hoffman, 2013). The purpose of this study was to increase the functional properties of "Gandjel Rel" bread through the addition of robusta coffee silverskin in the bread dough in order to enrich the content of dietary fiber and antioxidant activity by DPPH method so that it is hoped that it can be used to treat obesity.

MATERIALS AND METHODS

Tools and Materials

The tools in this study were analytical balance (Excellent HZK, Indonesia), dehydrator (16 Tray FDH-16 Wirastar, Indonesia), a grinder (4MFJ - Huang Cheng-800g, China), texture analyzer (TVT-300XP, Sweden), chromameter (Konica Minolta CR-400, Japan), oven (Mito Fantasy Mo-888, Indonesia), and spectrophotometer (Genesys 150 UV-Vis, USA).

The main ingredient in this study was coffee silverskin from Robusta coffee obtained from coffee farmers in Ungaran, Central Java, Indonesia. The ingredients used in making the bread were wheat flour, bread flour, palm sugar, egg yolks, margarine, full cream milk, baking soda, baking powder, cinnamon powder, vanilla, salt, and white sesame obtained from a shop in Semarang.

Coffee Silverskin Extraction

Coffee silverskin extraction was carried out based on patent WO 2013/004873 (del Castillo, 2013) with several modifications in dried process. A total of 50 g of silverskin was extracted with 1 liter of boiling water for 10 minutes. The extract was filtered and dried using a Food Dehydrator (16 Tray FDH-16 Wirastar, Indonesia) at 50˚C until a constant weight was obtained. The dried extracts were ground using a grinder (4MFJ - Huang Cheng - 800g, China) to form flour. The resulting silverskin (S) extract flour is filtered using a sieve with a size of 30 mesh and stored in a closed container.

Bread Making

The bread making is based on the recipe for making "Gandjel Rel" bread which was obtained from a native person from Semarang with some modifications. The first step is to weigh the wheat flour with the addition of robusta coffee silverskin flour with different concentrations (0, 1, 2.5, and 5%) with a total weight of 50 g. The composition of other ingredients is made the same (Table 1), such as bread flour, egg yolks, margarine, full cream milk, baking soda, baking powder, cinnamon powder, and vanilla. All ingredients are mixed until everything is evenly mixed. Palm sugar that has been dissolved is added to the dough. Leave the bread dough for 30 minutes. The dough is molded in rectangular molds measuring 5 x 2 cm with a thickness of ±0.5 cm. After that, the dough was put into the oven (Mito Fantasy Mo-888, Indonesia) at a temperature of 120˚C for 30 minutes and then cooled to room temperature (±32 ˚C).

Research design

This study used an experimental method with a completely randomized design (CRD) with a single factor, namely the addition of robusta coffee silverskin flour with different concentrations (0%, 1%, 2,5%, and 5%) (dw). The experiment was carried out with 3 repetitions. Each analysis was performed 2 times a repetition.

Dietary Fiber Analysis

Insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF) were determined using a gravimetric test based on the AOAC-991.43 and AACC-32.07.01 methods (McCleary et al., 2012). Dry samples were gelatinized with α-amylase enzymes (Sigma-Aldrich Ireland Ltd). After gelatinization, the samples were digested with protease (Sigma-Aldrich Ireland Ltd) and amyloglucosidase (Sigma-Aldrich Ireland Ltd) to remove protein and starch from the samples. The IDF is filtered and washed. The filtrate and washing water were combined with four volumes of 95% ethanol (Merck, Germany) at 60˚C to precipitate the SDF. The mixture is filtered, and the residue (soluble fiber) is dried and weighed. The total value of dietary fiber is calculated as the sum of insoluble dietary fiber and soluble dietary fiber. The analysis was repeated twice for each sample, and the results were expressed as a percentage by weight (%).

Antioxidant Activity Analysis

Determination of antioxidant activity was carried out based on the DPPH method (Molyneux, 2004). Bread samples were crushed using a porcelain cup, then added methanol solution (Merck, Germany) and proceeded with filtering. Samples were made at a concentration of 1,000 ppm in methanol solution, then diluted to obtain 200, 400, 600, 800, and 1,000 ppm concentrations. Each 4 mL was reacted with 1 mL of 0.2 mM DPPH solution (Sigma-Aldrich Ireland Ltd). Sample absorbance was measured using a spectrophotometer (Genesys 150 UV-Vis, USA) at a wavelength of 517 nm. A calibration curve was prepared using trolox

as standard in the range of 5.62 to 75.87 mg/L (r = 0.9896). The results were expressed as g of trolox equivalent (TE)/100 g of dry weight.

Physical Properties Analysis

Analysis of physical properties includes the level of hardness and color intensity. The hardness level of the bread was measured using a texture analyzer (TVT-300XP, Sweden). Color intensity analysis was measured using a Chromameter (Konica Minolta CR-400, Japan), which is expressed as brightness values, L* (black-and-white), a* (green-red), and b* (blue-yellow).

Sensory Analysis (Damat et al., 2019)

Sensory analysis was carried out to determine the acceptability of the bread. A total of 20 untrained panelists were randomly selected to assess the acceptability of the 4 bread formulas using a structured quantitative acceptance assessment test. Each panelist was given a drink after assessing each sample to eliminate taste disturbances. The parameters assessed were texture, taste, aroma, and level of preference with a rating scale, namely dislike very much (1), dislike (2), like (3) and like very much (4).

Chemical Properties Analysis

Analysis of chemical properties included moisture, ash, carbohydrates, fats, and proteins according to standard AOAC procedures (2016). Each analysis was performed 2 times a repetition.

Data analysis

The data (dietary fiber, antioxidant activity, physical properties, and sensory) obtained was tested statistically with Analysis of Variance (ANOVA) analysis using IBM SPSS Statistics 23 (2015, United States) and continued with the Duncan's Multiple Range Test (DMRT) with a significance level of 95%. The data of chemical properties was tested statistically with T test.

RESULTS AND DISCUSSION

Total Dietary Fiber and Antioxidants

The total dietary fiber content in bread increased with robusta coffee silverskin flour concentration (Table 2). It was dominated by insoluble fiber content than the soluble fiber content in bread in all treatments. Coffee silverskin contains total dietary fiber ranging from 60-70%, most of which is insoluble fiber (53-64%), while soluble fiber is 7.6-8.8% (Pourfarzad et al., 2013; Behrouzian et al., 2016). Therefore, bread with robusta coffee silverskin flour at all concentrations contained more insoluble fiber than soluble fiber.

The addition of coffee silverskin to the production of Barbari bread, Iranian flatbread and gluten-free bread showed similar results in this study. Barbari bread and gluten-free bread with the addition of coffee silverskin had higher total dietary fiber with higher insoluble and soluble dietary fiber content compared to bread without the addition of coffee silverskin (Pourfarzad et al., 2013; Guglielmetti et al., 2019). These results indicate that coffee silverskin can be used for nutrification of dietary fiber in bread making.

Consumption of dietary fiber has a positive impact on the physiological functions of the body related to the management of obesity, which can occur through a direct mechanism by affecting intestinal function in the process of digestion, absorption, and appetite or through an indirect mechanism by affecting the composition and metabolism of the microbiota in the intestine (Waddell and Orfila, 2022). High-fiber foods can reduce calorie intake. Low-calorie intake is known to help the body regulate energy balance for weight maintenance (Church and Martin, 2017). Consumption of dietary fiber can also

trigger a satiety effect by stimulating the release of hormones such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). The CCK hormone regulates satiety and the absorption and metabolism of nutrients (Burton-Freeman et al, 2002) while GLP-1 controls insulin and glucagon secretion, which is related to response and blood glucose levels (Ye et al, 2015).

The antioxidant activity of bread increased with the increased concentration of robusta coffee silverskin flour added (Table 2). Research by Guglielmetti et al. (2019) showed that adding coffee silverskin to gluten-free bread had an antioxidant activity that was 3.78 times higher when compared to control. Coffee silverskin has a high antioxidant component (169.5 mg/g) consisting of caffeine (53.3 mg/g), chlorogenic acid (56.5 mg/g), and flavonoids (6.3%), which contribute to its high antioxidant activity. (Iriondo-DeHond et al., 2017).

Gocmen et al. (2019) also reported that all cookies with coffee silverskin had significantly higher antioxidant capacities than control. The antioxidant properties of coffee silverskin are related to the content of dietary fiber in it, which is categorized as antioxidant dietary fiber (AODF). The dietary fiber of coffee silverskin has the highest antioxidant activity compared to the dietary fiber of other coffee by-products. The antioxidant activity of dietary fiber in silverskin is due to the binding of chlorogenic acid or other antioxidant compounds with fiber as a macromolecular matrix to form a complex carbohydrate structure (Quiros-Sauceda et al., 2015).

Martinez-Saez's research (2014) showed that antioxidant drinks made from coffee silverskin Arabica and Robusta could reduce body fat in experimental animals by up to 30% and 29%. Total cholesterol and blood plasma triglyceride levels also decreased after 45 days of treatment, followed by a decrease in lipase activity as much as in vitro at a 36 mg/mL concentration. This shows that processed food from coffee silverskin can improve the functional properties of food products with the presence of antioxidant activity and dietary fiber content, which has the potential to be used as an anti-obesity agent.

Bread Physical Properties

The addition of robusta coffee silverskin flour did not affect the hardness of the bread because the addition was not too much (1-5%) (Table 3). Robusta coffee silverskin has a low water content of 4-7% (Ates and Elmacı, 2019) because it is processed through roasting, which can reduce its moisture, so it does not affect the hardness of the bread. This also happened to cookies that were added coffee silverskin (0; 2.5; and 7.5%) which had no different level of hardness (Gocmen, 2019). This shows that robusta coffee silverskin can be used as a food additive such as bread products, cakes, and cookies.

The brightness level of the four bread formulas (Table 3) shows that the more robusta coffee silverskin flour added, the lower the brightness of the bread. Robusta coffee silverskin has a dark brown color (Figure 1) obtained from the roasting process, which reduces the bread's brightness level. Adding coffee silverskin flour also reduced the yellowness of the bread, but not the redness. The addition of robusta coffee silverskin resulted in the bread being darker and yellower. Cake formulations with the addition of coffee silverskin also show a decrease in brightness and yellowness, resulting in cake being darker and yellower with an increase of coffee silverskin added. (Ates and Elmaci, 2018).

Sensory Bread

The addition of robusta coffee silverskin flour (1-5%) did not make a

difference in the panelists' assessment of the texture of the bread. Robusta coffee silverskin has a low water content of 4-7% (Ates and Elmacı 2019), because it is processed through roasting, which can reduce its moisture, so it doesn't change the texture of the bread.

The panelist's assessment of aroma and taste showed a scale of dislike as the concentration of robusta coffee silverskin added to the bread increased. Robusta coffee silverskin has a roasting aroma produced from the roasting process of coffee beans, so the more robusta coffee silverskin added, the stronger the aroma will be. Adding silverskin flour with a concentration of > 1% gives a bitter taste from the roasting process. Robusta coffee silverskin has a high caffeine and chlorogenic acid content, 53.3 mg/g and 56.5 (Iriondo-DeHond et al., 2019). Caffeine and chlorogenic acid result in a bitter taste (Santosa et al., 2020), so give a bitter taste in bread with more coffee silverskin.

The panelist's assessment based on the preference level showed that they liked all four samples. However, based on the aroma and taste assessment, they preferred the S1 formula, namely bread with the addition of 1% robusta coffee silverskin flour. Therefore, the best bread of the four formulas is bread with the addition of 1% robusta coffee silverskin flour.

Bread Chemical Properties

Bread with the addition of 1% robusta coffee silverskin, which is the best assessment of sensory analysis, was analyzed for its chemical composition. Adding robusta coffee silverskin flour increased the bread's protein and carbohydrate content (Table 5). Bread with robusta coffee silverskin has a higher protein (11-19%) and carbohydrate content (62-67%) than bread without adding coffee silverskin (Gemechu, 2020). Robusta coffee silverskin's water and fat content are low, as much as 4-7% and 2.6-5.2 (Gemechu, 2020), so it does not significantly affect bread.

CONCLUSION

Adding robusta coffee silverskin to bread can increase insoluble dietary fiber from 3.33-7.18% and soluble dietary fiber 0.25-0.77%. Robusta coffee silverskin also increases bread's antioxidant activity, which can increase functional properties that have the potential as an anti-obesity agent. Robusta coffee silverskin has a high antioxidant component (169.5 mg/g) consisting of caffeine (53.3 mg/g), chlorogenic acid (56.5 mg/g), and flavonoids (6.3%), which contribute to its high antioxidant activity of coffee silverskin. Robusta coffee silverskin did not affect the hardness of the bread but made it darker and yellower. But, panelists preferred bread with the addition of 1% robusta coffee silverskin (S1) with chemical properties of 11.33% protein, 17.40% fat, and 66.42% carbohydrates. Therefore, further research should improve the formulation by adding more robusta coffee silverskin flour because it can increase the content of dietary fiber and antioxidants activity.

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Table 1. Bread Formulation Composition

Material (gram)	S0	S1	S2	S3
Parchment Flour	-	1	2.5	5
Wheat Flour	50	49	47.5	45
Bread Flour	33	33	33	33
Full Cream Milk	4	4	4	4
Baking Soda	0.3	0.3	0.3	0.3
Baking Powder	1.2	1.2	1.2	1.2
Vanila	0.3	0.3	0.3	0.3
Egg Yolk	5	5	5	5
Cinnamon Powder	1.2	1.2	1.2	1.2
Margarine	20	20	20	20
Palm Sugar	30	30	30	30

Noted: S0 = without the addition of silverskin flour, S1 = the addition of 1% parchment flour, S2 = the addition of 2.5% silverskin flour, and S3 = the addition of 5% silverskin flour).

Table 2. Total Dietary Fibre (% w/w))and Antioxidants Activity (DPPH methods) of Breads (g Trolox eq./ 100 g)

Treatment	Insoluble Diet Fiber	Soluble Diet Fiber	Total Diet Fiber	Antioxidant Activity
S0	$3.83\pm0,09^{b}$	$0.28\pm0,01^{a}$	$4.10\pm0,02^{b}$	$18.23\pm0,23^{a}$
S1	$3.33\pm0,07^{a}$	$0.25\pm0,00^{ab}$	$3.52\pm0,07^{a}$	$26.23\pm0,23^{b}$
S2	$4.37\pm0,07^{c}$	$0.29\pm0,00^{b}$	$4.66\pm0,07^{c}$	$28.11\pm0,12^{c}$
S3	$7.18\pm0,14^{d}$	$0.77\pm0,00^{d}$	$7.95\pm0,13^{d}$	$31.36\pm0,23^{d}$

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with $p<0.05$.

Figure 1. A = Coffee SIlverskin, B = Silverskin Flour, and C = Bread with adding of Silverskin (copyright 2023 by author).

Table 3. Hardness Level using texture Analyzer and Color Intensity Bread using Chromameter

Treatment	Hardness Level	Color Intensity		
		Brightness Level (L)	Redness Level (a)	Yellowness Level (b)
S0	56.92±7,04[a]	46.70±0,04[d]	6.46±0,55[a]	28.43±1,65[d]
S1	51.96±1,67[a]	38.38±0,94[c]	6.62±0,25[a]	24.98±0,70[c]
S2	51.68±3,16[a]	35.47±0,27[b]	5.60±0,02[a]	19.87±0,15[b]
S3	55.46±2,50[a]	28.00±0,77[a]	5.65±0,17[a]	15.46±0,45[a]

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with $p<0.05$.

Table 4. Sensory Evaluation of Breads Formula (Damat, et al., 2019)

Treatment	Parameter			
	Flavor	Texture	Taste	Taste of Preference
S0	3.50[b]	2.15[a]	3.65[b]	2.70[a]
S1	3.65[b]	2.50[b]	3.60[b]	2.85[a]
S2	3.43[ab]	2.65[b]	3.05[ab]	2.35[a]
S3	2.25[a]	2.70[b]	3.20[a]	2.65[a]

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with $p<0.05$

Table 5. Bread Chemical Properties (% w/w) (AOAC Procedure)

Treatment	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
S0	7.36±0,10[a]	1.58±0,10[a]	7.42±0,14[a]	16.86±0,09[a]	46.77±0,15[a]
S1	6.02±0,16[a]	1.82±0,04[a]	11.33±0,18[b]	17.40±0,04[a]	66.42±0,10[b]

Noted: All data is the average of 2 repetitions. The different letters in the same column show a significant difference with $p<0.05$

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Quality of Gluten Free Bread with the Addition of Xanthan Gum and Different Kneading Methods

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ABSTRACT

White bread generally uses wheat as the main ingredient due to its high viscosity and elasticity in the presence of gluten. However, because gluten is an allergen, not everyone can eat white bread. The aim of this study is to determine the quality of gluten-free white bread with the addition of xanthan gum and different stirring methods by testing the physical, chemical, and organoleptic properties of the product. This research was conducted using experimental research methods. The design uses a fully randomized factorial design (RALF) with two factors, namely xanthan gum concentration and different mixing methods with three replicates. This study uses the statistical ANOVA test with a significance of $\alpha<0.05$. The result: the higher the xanthan gum concentration in gluten-free white bread, the higher the texture value and the lower the swelling and moisture content. The result is that the longer the fermentation time when using the dough-free method with gluten-free white bread, the higher the value of swelling power and water content, as well as the textural value decreases. The results also showed that the best treatment was the addition of a xanthan gum concentration of 1.25% and the direct dough method with a texture value of 1562.9, swelling 430.269%, moisture content 35.18%, ash content 0.945%, fat content 6.675%, protein content Was 6.71%, carbohydrate content 50.45%, Hedonic test results ranged from 3-4 (rather similar) and Hedonic quality test results for crust color attributes 3.11-3.78 (brownish-yellow), crumb color (beige-brownish), aroma (slightly acidic).), pore uniformity 3.33–3.55 (rather uniform), texture (slightly soft), and flavor (slightly sour). The gluten-free test did not reveal any detectable gluten content in this study.

Keywords: hydrocolloid, gluten-free white bread, straight dough, no-time dough

INTRODUCTION

In general, white bread still uses wheat flour because of its high viscosity and elasticity in the presence of gluten (Herawati et al., 2017). When making white bread, gluten is used to bind the dough and make it elastic so it can be easily shaped. However, due to the allergenic gluten, white bread cannot be consumed by all groups. Especially those with celiac disease. This encourages the development of processed foods made from gluten-free flour (Herawati and Sunarmani 2016). One of them is gluten-free baked goods. Based on research (Sanchez et al., 2002), the use of corn starch, rice flour, and tapioca flour was found to have a ratio of 74.2; 17.2; 8.6 can produce an acceptable white bread, but the taste and final appearance are unacceptable.

Cornstarch is used as a starch source because it contains 88.11% starch (Nur, 2008). Cornstarch has a larger granule size of

around 15–20 µm, so it can improve the texture of white bread. Tapioca flour is used as a source of starch due to its high amylopectin content, which means it has good water-holding capacity and forms a dough mass that is thick, sticky, and slightly elastic (Kuswardani et al., 2008). Rice flour is used as a protein source because it has a protein content almost equal to that of rice, namely 7.9 grams per 100 grams (Dep. Kes RI, 1992). Gluten-free flour generally lacks the potential for viscosity and elasticity like wheat (Herawati, et al. 2017). One way to overcome this is to add hydrocolloids (Herawati and Sunarmani 2016). Xanthan gum can form a thin film with starch, allowing it to act like gluten (Kuswardani et al., 2008). In the production of gluten-free white bread, xanthan gum is able to interact with other components present, such as starch and protein, and to bind water during dough formation, so that the water required for starch gelatinization is available during baking and gelatinization occurs more quickly.

Regardless of the presence of gluten in the raw materials, different mixing methods can affect the quality of the resulting white bread. According to Syarbini (2013), there are three main factors that can affect the quality of the bread produced, namely raw materials, recipe balance, and manufacturing process. According to Mudjajanto and Lilik (2013), there are three different mixing methods, namely the indirect method (sponge and dough), the direct method (straight dough), and the quick method (no time dough). Only two dough preparation methods were used in this study, namely the direct straight dough and the no-time dough method since the addition of xanthan gum is expected to play a role in replacing gluten by forming a thin film layer with starch (Kuswardani et al., 2008) and optimizing the rapid fermentation time with the presence of xanthan gum to allow the end result to stretch gluten-free bread as much as possible. Based on this, this research was carried out to obtain the best quality gluten-free white bread with the addition of xanthan gum and a different stirring method.

MATERIALS AND METHODS

The study was conducted with an experimental method using a completely randomized factorial design (CRFD) with two factors, namely the factor A xanthan gum concentration consisting of 3 levels (1.25% b/w, 1.5% b/w, and 1 .75% v/w) and the Factor B method with a different mixture of 2 stages (straight dough and timeless dough) with 3 repetitions so that the treatment combinations became 18 experimental units. Data analysis was performed using analysis of variance (ANOVA) at a significance level of 5% to determine if there were any factors that differed significantly. If there is a significant difference in any of the factors, the analysis continues with Duncan's test.

The tools used to make gluten-free white bread consist of a gas oven, proofer, planetary mixer, baking sheet, scale, stainless bowl, brush, parchment paper, stainless knife, and stainless spoon. The tools used for testing in this study included hedonic testing and hedonic quality, chinaware, oven binders, frames, analytical balances, texture analyzer spectrophotometers, soxhlets, desiccators, and ovens. The ingredients used in making gluten-free white bread include cornstarch, rice flour, tapioca flour, xanthan gum, yeast, sucrose, chicken eggs, powdered milk, margarine, and salt. The materials used for the analysis are distilled water, NaOH, H_2SO_4, Hexane, H_3BO_3, and HCl.

The process of making gluten-free white bread using the straight dough method refers to research by Nur'utami et al. (2020), modified for the quick dough method (no time dough) and based on research by Mudjajanto et al. (2013), which has been amended. The first process was making a

yeast solution by mixing warm water, sugar, and yeast and then letting it sit for 15 minutes until foam forms. Next is mixing with a planetary mixer. The first mix mixes cornstarch, rice flour, tapioca flour, and milk powder at low speed for one minute. Then proceed to the second mix by mixing the finished egg and yeast solution on medium speed for 4 minutes. Then proceed to the third mix by mixing xanthan gum (1.25%, 1.5%, and 1.75%) on medium speed for 4 minutes. After that, proceed to the fourth mix by mixing the salt and melted margarine on medium speed for 3 minutes. Then, in the no-time dough method, the dough is placed on a baking tray and fermented in a proofer for 45 minutes at 35°C and 80% relative humidity. In addition, roasting is done with a gas oven at a temperature of 170 °C for 35 minutes in an open pan. In the straight dough method, after mixing, the four batters are added to the pan, where they are first left to ferment for 15 minutes with the pan closed. The air is then released from the dough. The dough is then left to rest in a closed mold for 15 minutes, after which the dough is deflated. The final fermentation then took place in a fermentation cabinet for 60 minutes at a temperature of 35 °C and a relative humidity of 80 %. This is followed by roasting in a gas oven at a temperature of 170 °C for 35 minutes in an open pan.

RESULTS AND DISCUSSION

Physical Test

Texture (hardness)

The texture test carried out is the hardness test. Hardness or hardness is the presence of a compressive force which indicates the resistance of the food product being tested to change shape due to the force exerted in the form of pressure (Astuti and Andarwulan, 2014). Testing the texture value (hardness or hardness) is an indicator that is quite important for the analysis of the texture of food products such as bread and biscuits (Wenzhao et al., 2013). The value of the texture test results can be seen in Table 1.

Table 1. shows the results of texture testing ranging from 1562.9 gf to 1862.9 gf. The highest hardness value, namely 1862.9 gf, was found in gluten-free white bread with the addition of 1.75% xanthan gum; method no time dough. The lowest hardness value, namely 1562.9 gf, was found in gluten-free white bread with the addition of 1.25% xanthan gum; straight dough method. Based on the results of the analysis of variance (ANOVA) on the texture test, it showed that the significance value of the xanthan gum concentration was 0.008 or ($p < 0.05$), which means that H0 was rejected and H1 was accepted, so the addition of xanthan gum concentration affected the texture. The significance value of the different kneading methods is 0.001 or ($p < 0.05$) which means H0 is rejected and H1 is accepted, so the different kneading methods affect texture. The interaction between the two has a significance value of 0.004 or ($p < 0.05$), so the interaction between the two also affects texture.

Based on the results of Duncan's test, the texture test treatment showed that the concentration of xanthan gum had a significantly different effect at the 0.05 significance level. The higher the concentration of xanthan gum added, the higher the hardness value, which means the texture of the gluten-free white bread is getting harder. Xanthan gum is a heteropolysaccharide of β-D-glucose via β1-4 glycosidic bonds. So, increasing the concentration of xanthan gum means increasing the amount of starch in the flour mixture so that the texture of the bread is hard because the starch granules increase and the water needed by the starch is taken from the gluten structure. According to Lineback and Inglett (1982), the texture of bread will become hard when xanthan gum is added at high concentrations due to gluten functional

damage. The results also showed that different kneading methods had significantly different effects at the 0.05 significance level. The no-time dough method with a 45-minute fermentation time showed the highest results compared to the straight dough method with a 90-minute fermentation time. The longer the fermentation time used, the lower the hardness texture value, which means the longer the fermentation time, the softer the texture of the gluten-free white bread produced. According to Hendrasty (2013), in the fermentation process (proofing) dough development occurs because yeast breaks down sugar to form carbon dioxide gas (CO2) then carbon dioxide gas is trapped in the gluten network which causes the dough to expand and produce soft bread. Meanwhile, according to Adiluhung and Sutrisno (2018), due to the metabolic activity of yeast, it produces CO2 gas, where the longer the proofing time, the more gas is produced to increase the volume and the bread becomes softer (Adiluhung and Sutrisno, 2018).

Swelling

Swelling is the ability of a dough to form and retain gas due to processing (Saputra, 2014). According to Cauvin (2012), during the fermentation process, the added yeast will produce carbon dioxide gas as a result of glucose metabolism, and then carbon dioxide gas will increase air bubbles in the bread. Carbon dioxide gas is retained in the dough and cannot be released into the air so that it can expand the dough and during the baking process with the oven at high temperatures the carbon dioxide gas expands so that the dough expands more and more (Shabrina, 2017). The value of the swelling power test results can be seen in Table 2.

Table 2 shows the value of the expandability test results ranging from 242.5% to 430.2%. The highest swelling value, namely 430.2%, was found in gluten-free white bread with the addition of 1.25% xanthan gum; the straight dough method, while the lowest value, namely 242.5%, was found in gluten-free plain bread with the addition of 1.75% xanthan gum; method no time dough. Based on the results of the analysis of variance (ANOVA) the swelling power test showed a significant value of xanthan gum concentration was 0.00 or ($p < 0.05$) which means H0 was rejected and H1 was accepted, so the addition of xanthan gum concentration affected swelling power. The significance value of different kneading methods is 0.001 or ($p < 0.05$) which means H0 is rejected and H1 is accepted, so different kneading methods affect swelling power. The interaction between the two has a significance value of 0.00 or ($p < 0.05$), so the interaction between the two affects developmental power.

Based on the results of Duncan's test, the treatment of developmental power scores had a significantly different effect at a significance level of 0.05. The higher the concentration of xanthan gum added, the lower the swelling value, which means that the swelling power of gluten-free white bread decreases. Xanthan gum is a heteropolysaccharide containing 1-4 β-D glucose (2 glucose) bonds which causes xanthan gum to have a high water-holding capacity (Glicksman, 1983). According to Parwiyanti et al., (2016), the water-holding capacity of xanthan gum can influence the degree of development. The degree of swelling value reflects the ability of the bread dough to hold the gas that is formed during the baking process so that it affects the ability of starch to produce a hollow matrix in the bread. According to Lineback and Inglett (1982), the texture of bread will become hard when xanthan gum is added at high concentrations due to gluten functional damage. Based on this, the results obtained for the swelling power value are inversely proportional to the texture value (hardness), as evidenced by the lower the texture value

(hardness), the higher the swelling power value obtained along with the lower concentration of xanthan gum which is added to gluten-free bread, the softer it is. The results also showed that different kneading methods had significantly different effects at the 0.05 significance level. The no-time dough method with a fermentation time of 45 minutes showed the lowest results compared to the straight dough method with a fermentation time of 90 minutes. The longer the fermentation time used, the higher the swelling power value, which means the longer the fermentation time, the higher the swelling power produced. This can be caused by the metabolic activity of yeast producing CO2 gas, where the longer the proofing time, the more gas is produced to increase the volume of bread (Adiluhung and Sutrisno, 2018). The resulting carbon dioxide gas is retained in the dough and cannot be released into the air so that it can expand the dough and during the baking process with the oven at high temperatures the carbon dioxide gas will expand so that the dough expands more and more (Shabrina, 2017).

Chemical Test

Water content analysis

Water content is one of the substantial compounds in food products because it can affect shelf life (Rochmawati N, 2019). The moisture content in food also determines freshness, durability, and acceptability. The more the addition of xanthan gum, the water content of non-gluten white bread will increase. The increase in water content is due to xanthan gum having a molecular weight of 2-11 million, with the greater the molecular weight, the more hydroxyl groups, and water trapping (Hui, 1992). According to SNI-8371-2018 the maximum limit for moisture content contained in white bread is 40%. The observed value of water content can be seen in Table 3.

Table 3 shows the results of the water content test ranging from 37.15% to 38.12%. The highest water content value, namely 38.12%, was found in gluten free white bread with the addition of 1.75% xanthan gum; the no-time dough method, while the lowest value, namely 37.15%, was found in gluten-free plain bread with the addition of 1.5% xanthan gum; method no time dough. Based on the results of the analysis of variance (ANOVA) the water content test showed that the significant values of xanthan gum concentration and different kneading methods were 0.392 and 0.306 or ($p > 0.05$). Thus H0 is accepted and H1 is rejected, which means that the xanthan gum concentration and the different kneading methods do not affect the water content of the gluten-free white bread. The significance value ($p > 0.05$) means that H0 is accepted and H1 is rejected, so Duncan's further test was not carried out on the moisture content of gluten-free bread. This is similar to the results of research by Kuswardani et al (2008), in the manufacture of gluten-free white bread made from cornstarch, tapioca flour, and rice flour with the addition of 0.5% to 2.5% xanthan gum concentration, with no significant effect on water content. the resulting white bread. The results of Sasaki's research (2017) stated that bread made from rice flour and wheat flour with the addition of xanthan gum concentrations of 0.5%, 1.0%, and 2.0% also had no significant effect on water content. According to the results of Sika's research (2006), stated that the use of xanthan gum with a concentration of 0.5% - 2.5% in donut products without gluten from tapioca flour also did not affect the water content of the resulting product. Xanthan gum is a hydrocolloid that binds water, but the water content that can be measured by the gravimetric method is not bound to water but free water contained in the product so that the binding of water by xanthan gum does not

affect the free water content in bread products (Kuswardani et al, 2008).

The water content in food ingredients can affect several characteristics such as taste, color, solubility, and food shelf life. Moisture content is an important parameter for dry products due to the tendency of damage to a food product. White bread is a type of food with wet bread with a high enough water content, causing a low shelf life. High water content can also cause mold and yeast to multiply rapidly because water is a medium for mold, yeast, and other microbes to grow so there will be changes in the quality of food ingredients. (Fadillah et al., 2020). The water content in this study refers to SNI-8371-2018, namely the maximum water content requirement for white bread is 40%. Gluten-free white bread with the addition of xanthan gum and a different kneading method for each treatment was still within the maximum limit, ranging from 37.15% to 38.12%.

Organoleptic Test

Organoleptic tests on gluten-free white bread with the addition of xanthan gum and different kneading methods were carried out based on hedonic and hedonic quality assessments by 30 semi-trained panelists. Assessment using sensory tools includes quality specifications for crust color, crumb color, aroma, pore uniformity, texture, and taste. The results of the organoleptic test will provide information on consumer acceptance of the product.

Color

Hedonic quality test

Crust color

Color is the first sensory that can be seen directly by the panelists using the senses, namely the eyes (Negara et al., 2016). Crust color is the color found on the outer skin. Crust color is an important aspect of sensory testing because the color of the crust can indicate that the product is cooked (Sachriani, S. and Yulianti, Y., 2021). The ideal crust color for making white bread is brown evenly. The results of the hedonic quality test can be seen in Figure 1.

Figure 1 shows the average score of the hedonic quality test for the preference level of panelists for crust color ranging from 3.44 to 3.96 (fawn). Based on the results of the analysis of variance (ANOVA) it showed that the significant values of xanthan gum concentration and different kneading methods were 0.059 and 0.342 or ($p > 0.05$). Thus H0 is accepted and H1 is rejected, which means that the concentration of xanthan gum and the different kneading methods do not affect the color of the crust on gluten-free bread. The significance value ($p > 0.05$) which means that H0 is accepted and H1 is rejected, so Duncan's further test was not carried out on the hedonic quality of crust color on gluten-free bread. The treatment of xanthan gum concentration and the best kneading method which had the highest crust color hedonic quality test was at 1.25% xanthan gum concentration using the straight dough method with a value of 3.96 (yellowish brown).

The brown color of the bread is caused by the Maillard reaction and caramelization of the sugar during baking (Sitepu, 2019). Caramelization of sugar is the degradation of sugar due to heating above its melting point resulting in a brown color change. This shows that the higher the simple sugar content in the bread, the browning that occurs during baking is higher (Sitepu, 2019).

Crumb color

Color is the first sensory that can be seen directly by the panelists using the senses, namely the eyes (Negara et al., 2016). Color is an important component in determining the acceptance of a product by consumers because it is the first visual appearance in addition to several other factors such as taste, aroma, and nutritional value (Winarno,

2004). The results of the hedonic quality test can be seen in Figure 2.

Figure 2 shows the average score of the hedonic quality test for the panelist's preference for crumb color ranging from 3.11 to 3.78 (brownish beige). Based on the results of the analysis of variance (ANOVA) it showed that the significance value of the xanthan gum concentration was 0.000 or ($p < 0.05$), so the addition of xanthan gum concentration affected the color of the crumb on gluten-free bread. The significance value of the different kneading methods was 0.002 or ($p < 0.05$), so the different kneading methods affected the color of the crumb on gluten-free white bread. The interaction between the two has a significance value of 0.000 or ($p < 0.05$), so the interaction between the two affects the color of the crumb on gluten-free bread. The longer the fermentation time used, the higher the hedonic quality value of the crumb color. The interaction treatment between xanthan gum concentration and the best kneading method had the highest crumb color hedonic quality test value, namely at 1.5% xanthan gum concentration using the straight dough method with a value of 3.78 (brownish beige).

Figure 3 shows the hedonic test average score of the panelists' preference for color ranging from 3.73-4 (somewhat like). Based on the results of the hedonic color test analysis of variance (ANOVA) in Table 15, the significance value of xanthan gum concentration was 0.004 or ($p < 0.05$), so the addition of xanthan gum concentration affected the color of gluten free bread. The significance value of the different kneading methods was 0.000 or ($p < 0.05$), so the different kneading methods affected the color of gluten-free white bread. The interaction between the two has a significance value of 0.000 or ($p < 0.05$), so the interaction between the two affects the color of the crust on gluten-free bread. The best interaction treatment between xanthan gum concentration and the best kneading method which had the highest color hedonic test value was at 1.25% xanthan gum concentration using the straight dough method with a value of 4 (likes).

Aroma

hedonic quality test

Aroma is one of the factors that determine the acceptance of a product so that it can be accepted by consumers. Aroma can be a determining factor whether a product is acceptable or not, besides that aroma can be used as an indicator of damage to the product (Kartika et al., 1988). Aromas arise from aroma-producing substances that can evaporate such as volatile compounds (Ratri lusia, 2019). According to Syarbini (2013), a good aroma of white bread is a distinctive aroma of wheat or a distinctive smell of grains or nuts. Aroma is a parameter that can be observed with the sense of smell. The value of the aroma hedonic quality test results can be seen in Figure 4.

Figure 4 shows the average score of the hedonic quality test for the panelist preference for aromas ranging from 2.90 to 3.01 (sour-slightly sour). Based on the results of the analysis of variance (ANOVA) hedonic aroma quality test in Appendix 18, the significance values of xanthan gum concentration and different mixing methods were 0.825 and 0.884 or ($p > 0.05$). Thus H0 is accepted and H1 is rejected, which means that the xanthan gum concentration and the different kneading methods do not affect the flavor of the gluten-free white bread. The significance value ($p > 0.05$) means that H0 is accepted and H1 is rejected, so Duncan's further test was not carried out on the hedonic quality of aroma on gluten-free white bread. The best treatment of xanthan gum concentration and kneading method which has the highest aroma hedonic quality test is at 1.5% xanthan gum concentration using the

straight dough method with a value of 3.01 (slightly sour).

Figure 5 shows the average hedonic test score for the panelists' preference for aromas ranging from 3.43 to 3.6 (rather like). Based on the results of the analysis of variance (ANOVA) the significance value of xanthan gum concentration and different kneading methods was 0.815 and 0.696 or ($p > 0.05$). Thus H0 is accepted and H1 is rejected, which means that the xanthan gum concentration and the different kneading methods do not affect the flavor of the gluten-free white bread. The significance value ($p > 0.05$) which means that H0 is accepted and H1 is rejected, so Duncan's further test was not carried out on the hedonic aroma of gluten-free bread. The treatment of xanthan gum concentration and the best kneading method which had the highest aroma hedonic test value was at 1.25% xanthan gum concentration using the straight dough method with a value of 3.60 (rather like).

Texture

Hedonic quality test

Texture (hardness or hardness) is an important indicator for food products such as bread and biscuits (Wenzhao et al., 2013). The value of the texture hedonic quality test results can be seen in Figure 6.

Figure 6 shows the average score of the hedonic quality test for the panelists' preference for texture ranging from 3.35 to 3.63 (slightly soft). Based on the results of the analysis of variance (ANOVA) for the texture hedonic quality test in Appendix 19, the significance value for the xanthan gum concentration was 0.015 or ($p <0.05$), so the addition of xanthan gum concentration affected the texture of gluten-free white bread. The significance value of the different kneading methods was 0.052 or ($p > 0.05$), so the different kneading methods did not affect the texture of the gluten-free white bread. The interaction between the two has a significance value of 0.024 or ($p <0.05$), so the interaction between the two affects the texture of the gluten-free white bread. The treatment of the interaction between xanthan gum concentration and the best kneading method which had the highest textural hedonic quality test value was at 1.25% xanthan gum concentration using the straight dough method with a value of 3.63 (a bit soft).

Bread pore uniformity

Bread pores are thin layers formed on gluten which function to capture carbon dioxide gas. Pores are formed during the fermentation process, and yeast activity (Pusuma et al., 2018). The following results of the analysis of hedonic quality testing for pore uniformity of gluten-free plain bread with the addition of xanthan gum and different kneading methods can be seen in Figure 7.

Figure 7 shows the average score of gluten-free plain bread with xanthan gum concentrations and different kneading methods. The hedonic quality test score of the panelist's preference level for pore uniformity ranged from 3.33 to 3.55 (slightly uniform). Based on the results of analysis of variance (ANOVA) the hedonic quality test for pore uniformity in Appendix 20 showed a significant value of xanthan gum concentration was 0.004 or ($p <0.05$), so the addition of xanthan gum concentration affected pore uniformity in gluten-free bread. The significance value of the different kneading methods was 0.636 or ($p > 0.05$), so the different kneading methods did not affect pore uniformity on gluten-free white bread. The interaction between the two has a significance value of 0.024 or ($p <0.05$), so the interaction between the two affects pore uniformity in gluten-free white bread.

The higher the concentration of xanthan gum, the lower the hedonic quality value of the pore uniformity or the less uniform it is.

According to Sukamto (2010), xanthan gum plays a role in regulating water distribution and preventing syneresis so that the dough structure forms more even pores. The interaction treatment between xanthan gum concentration and the best kneading method which had the highest pore uniformity hedonic quality test value was at 1.25% xanthan gum concentration using the straight dough method with a value of 3.55 (rather uniform).

Figure 8 shows the average hedonic test score for the panelists' preference for texture ranging from 3.57-4 (somewhat like). Based on the results of the analysis of variance (ANOVA) hedonic texture test in Appendix 24, the significance value of the xanthan gum concentration was 0.003 or ($p < 0.05$), so the addition of xanthan gum concentration affected the texture of gluten-free white bread. The significance value of the different dough methods was 0.000 or ($p < 0.05$), so the different dough methods affected the texture of the gluten-free white bread. The interaction between the two has a significance value of 0.000 or ($p < 0.05$), so the interaction between the two affects the texture of gluten free white bread. Treatment of the hedonic value of texture on gluten free white bread with the addition of xanthan gum and different kneading methods showed that the concentration of xanthan gum had a significantly different effect at the 0.05 significance level. The best interaction treatment between xanthan gum concentration and the best kneading method which has the highest textural hedonic test value is at 1.25% xanthan gum concentration with the straight dough method with a value of 4 (likes).

Flavor

hedonic quality test

Taste is the main factor that is important in an assessment of food products using the sense of taste. Taste is very important to determine the panelist's decision to accept a food product. The taste of a food can come from the food itself or from ingredients added during the processing (Kumalaningsih et al., 2005). The value of the taste hedonic quality test results can be seen in Figure 9.

Figure 9 shows the average score of the hedonic quality test for the panelists' preference for flavors ranging from 3.75 to 3.81 (slightly sour). The sour taste that appears on gluten-free white bread is suspected when the fermentation time is too long. According to Prabowo et al., (2021) states that the occurrence of over proofing causes the taste of the bread to become sour. Based on the results of the analysis of variance (ANOVA) it showed that the significant values of xanthan gum concentration and different kneading methods were 0.686 and 0.920 or or ($p > 0.05$). Thus H0 is accepted and H1 is rejected, which means that the concentration of xanthan gum and the different kneading methods do not affect the taste of the gluten-free white bread. The significance value ($p > 0.05$) means H0 is accepted and H1 is rejected, so Duncan's further test was not carried out on the hedonic quality of taste on gluten-free white bread. The best treatment of xanthan gum concentration and kneading method which had the highest taste hedonic quality test was at xanthan gum concentrations of 1.25% and 1.5% with the straight dough method with a value of 3.81 (slightly sour).

Based on the results of analysis of variance (ANOVA) it showed that the significance value of xanthan gum concentration was 0.017 or ($p < 0.05$), so the addition of xanthan gum concentration affected the taste of gluten-free white bread. The significance value of the different kneading methods was 0.014 or ($p < 0.05$), so the different kneading methods affected the taste of gluten-free white bread. The interaction between the two has a

significance value of 0.008 or ($p < 0.05$), so the interaction between the two influences the taste of gluten-free white bread. The treatment of the interaction between xanthan gum concentration and the best kneading method which had the highest taste hedonic test value was at 1.5% xanthan gum concentration using the straight dough method with a value of 3.83 (rather like).

CONCLUSION

Based on the research results on the quality of gluten-free white bread with the addition of xanthan gum and various kneading methods, the following conclusion can be drawn:

1. Different concentrations of xanthan gum (1.25%, 1.5% and 1.75%) have a significant effect on the texture test, the swelling test, the organoleptic test (hedonic quality: crust color, crumb, pore and texture uniformity) and the organoleptic test (hedonic: color, texture and taste).
2. Different kneading methods (straight dough and no time dough) had a significant impact on the texture test, swelling test, organoleptic test (hedonic quality: crust and crumb color) and organoleptic test (hedonic: color, texture and taste).
3. The interaction between different concentrations of xanthan gum (1.25%, 1.5% and 1.75%) and different kneading methods (straight dough and no time dough) has a significant impact on texture testing, swelling testing and organoleptic testing (hedonic quality : Color). of crust, crumb, pore uniformity and texture) and organoleptic test (hedonic: colour, texture and taste).
4. The best gluten-free white bread was found when treated with 1.25% xanthan gum concentration and the straight dough method with a texture score of 1562.9, 430.269% swelling power, 35.18% moisture content, 0.945% ash content, 6.675% fat content, protein content 6 .71%, carbohydrate content 50.45%, hedonic test scores range from 3-4 (rather similar) and hedonic quality test scores for crust color attribute 3.11-3.78 (brownish-yellow), crumb color (brownish-cream), flavor (slightly acidic), pore uniformity 3.33-3.55 (rather uniform), texture (slightly soft) and taste (slightly acidic). The results of the gluten free test showed no gluten content in gluten free white bread.

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Table 1. Texture (hardness) test results (gf)

XG concentration	Kneading method		Average
	Straight dough	*No time dough*	
1,25%	$1562,9^{ab} \pm 200,32$	$1658,3^{b} \pm 55,01$	1610,7
1,5%	$1655,8^{a} \pm 150,58$	$1693,0^{b} \pm 108,83$	1674,4
1,75%	$1861,8^{b} \pm 20,80$	$1862,9^{c} \pm 195,88$	1862,4
Average	1693,5	1738,1	1715,8

Note:

- The straight dough kneading method with different xanthan gum concentrations provides a significant difference in the texture values at concentrations of 1.5% and 1.75% but does not provide a significant difference at a xanthan gum concentration of 1.25%.
- The no time dough kneading method with different xanthan gum concentrations does not provide a significant difference in texture at concentrations of 1.25% and 1.5% but provides a significant difference at a xanthan gum concentration of 1.75%

Table 2. The value of the swelling test results (%)

XG concentration	Kneading method		Average
	Straight dough	*No time dough*	
1,25%	$430,2^{c} \pm 8,78$	$357,0^{b} \pm 10,67$	393,6
1,50%	$406,6^{c} \pm 8,50$	$265,4^{a} \pm 5,74$	336,0
1,75%	$353,3^{b} \pm 23,98$	$242,5^{a} \pm 19,17$	297,9
Average	396,7	288,3	342,5

Table 3. Value of water content test results (%)

XG concentration	Kneading method		Average
	Straight dough	*No time dough*	
1,25%	37,93 ± 0,51	37,40 ± 1,26	37,7
1,50%	37,82 ± 0,04	37,15 ± 0,64	37,5
1,75%	37,86 ± 0,13	38,12 ± 0,20	37,9
Average	37,9	37,6	37,7

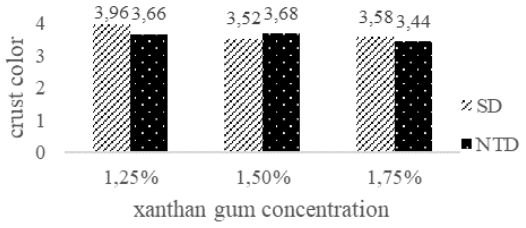

Figure 1. Diagram of the crust color hedonic quality test

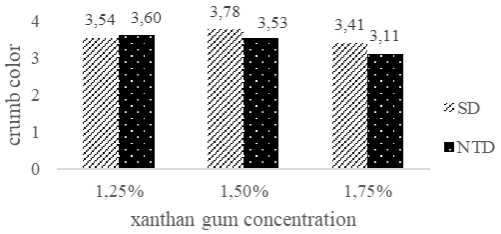

Figure 2. Diagram of crumb color hedonic quality test results

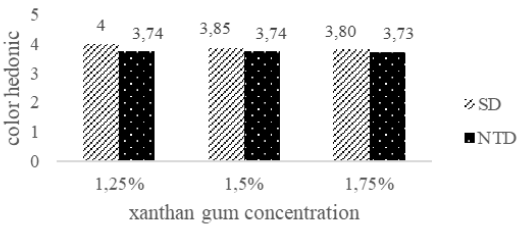

Figure 3. Color hedonic test result diagram

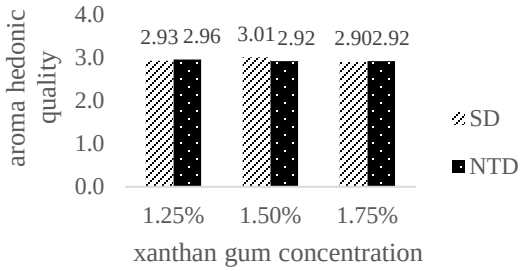

Figure 4. Diagram of aroma hedonic quality test

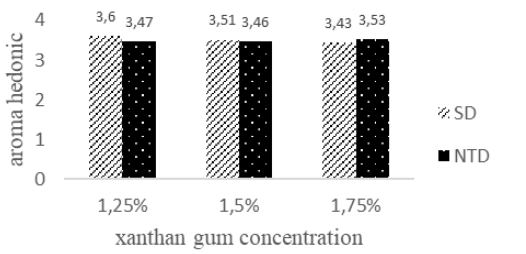

Figure 5. Aroma hedonic test result diagram

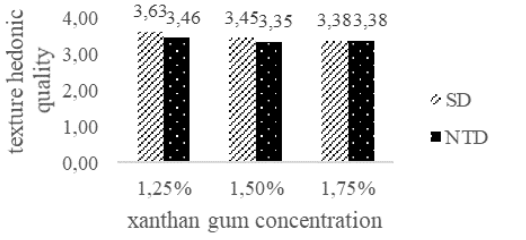

Figure 6. diagram of texture hedonic quality test

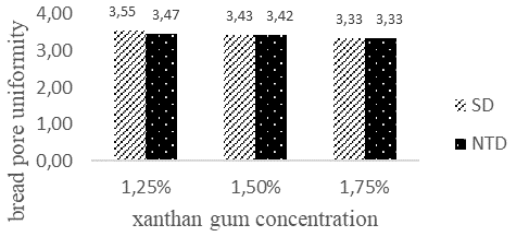

Figure 7. Diagram of pore uniformity hedonic quality test results

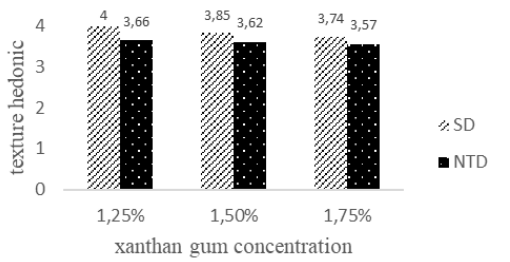

Figure 8. Diagram of the texture hedonic test results

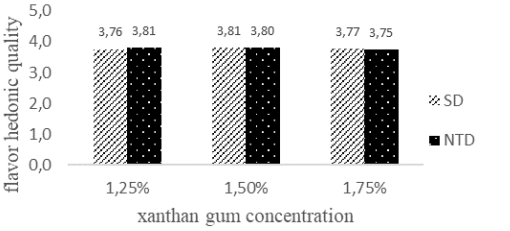

Figure 9. Diagram of the hedonic flavor test results

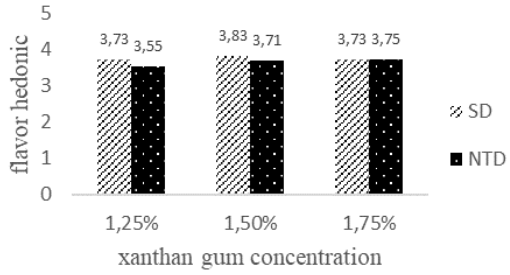

Figure 10. Diagram of the hedonic taste test results

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Assessment of Unfermented Beverages Intake by Adult Population in Herat City

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ABSTRACT

Beverages play a prominent role in regulating body activities, such as regulating body temperature, helping to absorb food, protecting tissues, disposal of waste materials, refreshing the body, and increasing body performance. Usually, the human body obtains its required water through the consumption of drinking water, tea, cola, energy drinks, milk and dairy products, coffee and other beverages. We evaluated the amount of beverage intake by the adult population of Herat city during summer 2021. In this research 583 standard questionnaires were randomly distributed among adult population of 15 regions of Herat city and the results were analyzed. The highest beverage intake was observed for water (2.81±0.92 times/day @ 349.57±190.12 ml for each time) and followed by was tea (1.84±0.89 times/day @ 426.76±228.78 ml each time). The average fluid intake by the adult population was 2390.61 ml per day, where the men drank an average of 2528.91 ml/day and women averaged 2363.88 ml/day of beverages. Most of the participants consumed beverages for the purposes of quenching thirst, health concerns, habitual and refreshment. The findings indicate that the amount of beverage intake by adult population of Herat city is lower than the WHO guidelines and the United States fluid intake recommendations.

Keywords: Adult, Beverages, Herat, Water, Tea

INTRODUCTION

Water is one of the most essential components of human diet. Commonly, water is consumed by every person for nutritional, medical and social reasons. The human body obtains a large part of its required water through beverages and water which is present in food products. Unfermented beverages include water, tea, fruit drinks, artificial drinks, and milk which contain 90 to 95 percent of water (Ashurst and Hargitt, 2009). Beverages play a vital role in human metabolism for health and physical performance, especially for the elderly, sick and pregnant women. Mild dehydration can have adverse effects on mental and physical performance (Liberman, 2001). Most of the international recommendations for beverages consumptions have taken into account the factors such as age, weight and physical activity. Human's daily water needs are different according to people's characteristics and lifestyle. In normal conditions, the recommended total daily consumption of fluids is 2.6 liters per day, this can be provided by direct consumption of beverages or by foods which are consumed daily

(Martin, 2007). According to the guidelines of the World Health organization, daily intake of 2.9 liters and 2.2 liters of fluid intake is considered necessary for men and women respectively (Howard and Bartram, 2003). The National Academy of Sciences, Engineering and Medicine of the United States of America in 2004 recommended daily beverage intake of about 3.7 liters for men and about 2.7 liters for women, which includes drinks such as plain water, beverages, and water in food. The percentage of water intake from food varies according to the type of diet. Usually, about 20 percent of daily fluid intake comes from food and the rest from drinks (Nissensohn et al., 2013).

The beverages consumption by the adult population of Tehran city was about 1.9 liters per day. Where, pure water (without considering the water used in the preparation of food or drinks) intake is about 5 percent of all liquids intakes (hot and cold) which includes: tea, coffee, juice and carbonated drinks (Abdullahi et al. (2013). Plain water, soft drinks, coffee, tea, milk, and fruit drinks are the most important liquids consumed by Americans, respectively (Randy et al., 2011). 76 percent of people, 2 years and older in the United States consume an average of 3.9 cups (one cups=238 ml) of water per day (Rhonda et al., 2011). A study conducted in Indonesia showed that sugar-sweetened beverages (artificial beverages) constitute 35 percent of the total fluid intake of youth and adults (Strippoli et al., 2011). A systematic review of total fluid consumption between 2000 and 2013 showed that the amount of total fluid consumption were between 0.6 and 3.5 liters per day and this amount is different for age groups, the difference is more among men than women (Ozen et al., 2015). The French population usually consumes liquids with the main meals, while the elderly more than 65 years consume liquids with snacks (Zizza et al., 2009). Daily water intake by U. S. men and women were reported 3.46 liters and 2.75 liters respectively (Rosinger and Herrick, 2016). Present research was conducted to analyze amount of beverages consumption by the adult population of Herat city during the summer of 2021.

MATERIALS AND METHODS

Research Type

This research was carried out to assess beverages consumption by the adult population of Herat city during the summer season of 2021. The research was designed as descriptive-survey method such that the subjects were randomly selected from the Herat city population.

The area under study (case study)

Herat is the largest province in the west of Afghanistan, which has a common border with Iran and Turkmenistan. The latitude of Herat, Afghanistan is 34.343044, and the longitude is 62.199074. Herat is located category with the GPS coordinates of 34° 20' 34.9584" N and 62° 11' 56.6664" E. According to the Afghanistan statistical yearbook 2019-20 Herat city population estimated was 632206 people distributed in 15 regions.

The statistical population under investigation

Since the population of Herat city estimated was 632206 people, Morgan's table was used to retrieve the data and for more accuracy, 583 standard questionnaires with 16 questions (for each questionnaire) were distributed to the study population. Among questionnaires, 285 were assigned to men and 298 to the women. The questionnaires included three main parts: demographic characteristics, amount of different beverages consumption (ml) and reasons for consumption of beverages. The community under investigation was randomly selected and the questions were asked to them face to face.

Data analysis

First, the obtained data was checked for their validity. After that, the questionnaires were analyzed by using SPSS-26 software and descriptive statistics methods (frequency, percentage, average, and standard deviation). At the end, the amount of beverages consumed by the adult population of Herat city was compared with the WHO and the National Research Council of the United States guidelines.

RESULTS AND DISCUSSION

The demographic characteristics of the participants of this survey are shown in Table 1. 48.88 percent of participants were males. Regarding educational qualifications, out of 568 respondents 38.4 percent were diploma holders followed by 35.0 % bachelors. With respect to economic status, most participants (74.6%) had an average income of 10,000 to 50,000AFN. Regarding the age, the 18-25 years old were maximum respondents (50.1 %). Similarly, Table 1 indicates weight and height of all participants in the survey. 50.86% population had the weight range of 60-79 Kg and 50.61% population were in the height range of 150-169 cm.

Table 2 shows the average unfermented beverages intake by the adult population of Herat city. The consumption of plain water was reported highest as compared with other listed beverages. The response for the water intake was 2.81±0.92 times per day with 349.57±190.12 ml for each time of consumption. This was followed by tea consumption with 1.84±0.89 times/day and 426.76±228.78 ml for each time of intake. Similarly, the response for the consumption of other beverages included buttermilk, fruit drinks, milk, energy drinks, cola, and coffee @ 1.28±0.86, 0.31±065, 0.28±0.54, 0.26±0.63, 0.25±0.58, 0.09±0.36 times/day with 351.98±215.60, 175.64±159.54, 150.60±142.25, 114.24±152.59, 165.01±178.99, 55.23±97.57 ml for each time of consumption respectively. The average beverage intake by the adult population of Herat city reported 2390.61 ml per day, which is lower than the WHO guidelines and the daily water consumption by American men and women as reported by Rosinger and Herrick, (2016). It was slightly more than the amount of beverages intake by the adult population of the Tehran city which was reported by Abdullahi et al. (2013).

Men drank an average of 2528.91 ml/day while women averaged 2363.88 ml/day of beverages. There was no significant difference in the consumption of water, tea, coffee, milk, fruit drinks and cola, while significant difference was recorded in the consumption of energy drinks and buttermilk between men and women participated in this survey (table 3). Men were more likely to consume energy drinks and butter milk than Women ($P<0.05$). Higher consumption of energy drinks by men might be due to traditional masculinity ideology of men, financial independency by men, sports and more daily activity. Similar finding was reported by Alrasheedi, 2016 in Jeddah, Saudi Arabia and Wimer, 2013 in United states of America. The amount of beverages intake by men was slightly higher than the women but was lower than the WHO guidelines recommended for beverages intake. The results showed that the beverages intake by the adult population of Herat city is lower than the amount of beverage intake reported in the United States by Rosinger and Herrick, (2016) while this amount is higher than the beverage intake by men and women in Tehran city reported by Abdullahi et al. (2013). Consumption of water lower than the WHO and other international guidelines could lead to dehydration and adverse effects including decrease of attention, concentration and other cognitive and motor functions, feeling of fatigue, headache, increase risk of stroke and mental fog (Krecara et al., 2014).

Table 4 shows the reasons for consuming different drinks. Tea, soft drinks and fruit drinks were mostly used to quench thirst. Most people consumed butter milk, coffee and energy for health purposes. Likewise, most respondents consumed water to quench their thirst, health, and refreshment.

CONCLUSION

Beverages intake by the adult population of Herat city was analyzed using, 583 respondents. Water, tea and butter milk were reported as the most consumed beverages among people who participated in this survey, while energy drink and coffee reported as the lowest consumed beverages. An average of 2528.91 ml beverage per day was consumed by men while, women consumed 2363.88 ml/day of beverages. The beverages intake by men showed a slightly higher than women. No significant difference was recorded in consumption of water, tea, coffee, milk, fruit drinks and cola, while significant difference was recorded in the consumption of energy drinks and buttermilk between men and women. Beverages were consumed for health benefits and to quench thirsty.

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Table 1. Demographic characteristics of adult population of Herat city

Variables		Frequency (n=583)	Percent (%)	Mean ± SD	Total (Valid)
Gender	Male	285	48.88	-	583
	Female	298	51.12		
Education	Illiterate	73	12.85	-	568
	Primary School	72	12.68		
	Diploma	218	38.38		
	Bachelors	199	35.03		
	M. Sc.	6	1.06		
	Ph. D.	0	0.00		
Economic Status (AFN/Month)	Low (<10000 AFN)	112	19.21	-	583
	Moderate (10000-50000 AFN)	435	74.61		
	High (>50000 AFN)	36	6.18		
Age (years)	18-25	292	50.09	31.28±13.10	583
	26-40	179	30.70		
	41-0	102	17.50		
	60-100	10	1.71		
BMI (Kg/m^2)	Underweight	134	22.98	-	583
	Normal weight	353	60.55		
	Overweight	96	16.47		
Weight (Kg)	40- 59	171	29.69	68.02±15.13	576
	60-79	293	50.86		
	80-99	96	16.67		
	100-120	16	2.78		
Height (cm)	120-149	20	3.49	168.20±12.39	573
	150-169	290	50.61		
	170-189	252	43.98		
	190-210	11	1.92		

Table 2. Average of unfermented beverages intake by the adult population of Herat city

Beverage Type	Mean±SD (Times/day)	Mean±SD (ml/time)
Water	2.81±0.92	349.57±190.12
Tea	1.84±0.89	426.76±228.78
Coffee	0.09±0.36	55.23±97.57
Milk	0.28±0.54	150.60±142.25
Fruit Drinks	0.31±065	175.64±159.54
Energy Drinks	0.26±0.63	114.24±152.59
Cola	0.25 ±0.58	165.01±178.99
Butter milk	1.28±0.86	351..98±215.60
Total (ml/day)		**2390.61**

Table 3. Unfermented beverages intake by men and women of Herat city population

	Male (n=285)		Female (n=297)		
Beverage Type	**Mean±SD (Times/day)**	**Mean±SD (ml/time)**	**Mean±SD (Times/day)**	**Mean±SD (ml/time)**	**Mean Compression**
Water	2.87±0.90	370.53±197.81	2.74±0.95	329.97±180.68	NS
Tea	1.77±0.86	414.04±225.99	1.91±0.91	439.06±231.526	NS
Coffee	0.10±0.41	56.84±99.84	0.07±0.32	53.87±96.18	NS
Milk	0.31±0.59	160.00±150.87	0.25±0.0.47	140.74±132.50	NS
Fruit Drinks	0.33±0.64	173.33±160.52	0.29±0.66	177.78±159.10	NS
Energy Drinks	0.32±0.66	138.25±156..02	0.20±0.59	91.58±145.99	*
Cola	0.24±0.56	164.21±181.49	0.25±0.60	166.33±176.90	NS
Butter milk	1.38±0.86	387.37±233.43	1.18±0.86	317.85±191.70	*
Total (ml/day)	**2528.91**		**2363.88**		

NS- Non Significant

*- Significant at 5 percent level

Table 4. Purpose of unfermented beverages intake

		Beverages							
		Water (%)	Tea (%)	Milk (%)	Butter Milk (%)	Cola (%)	Fruit Drinks (%)	Energy Drinks (%)	Coffee (%)
Purpose	Feeling thirsty	41.74	46.30	7.29	6.49	63.13	60.25	17.36	19.48
	Health purpose	3.13	8.15	53.80	3.82	6.19	22.96	16.60	1.95
	Habit/Enjoy	0.70	39.26	35.87	84.35	23.89	8.64	53.21	72.73
	All	54.43	6.30	3.04	5.34	6.78	8.15	12.83	5.84
	Total	100 (%)							

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Physicochemical Characteristics and Dietary Fiber of Analog Rice from Seaweed (*Sargassum* sp.) and Beneng Taro Combination

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ABSTRACT

Development of analog rice from *Sargassum* sp. and beneng taro combination is expected to be an alternative food to increase the amount of dietary fiber intake for the community. The aim of this research is to determine the best characteristics of analog rice from *Sargassum* sp. and beneng taro as a high fiber food. The concentration of added *Sargassum* sp. seaweed in this study were 0%, 5%, 10%, and 15%. The results showed that a concentration of 15% was the best treatment with a white degree value 1.23%; water absorption capacity 61% and swelling power 59.3%. The chemical characteristics of the best analog rice are 10.14% moisture content; ash content 4.14%; fat content 0.60%; protein content 5.69%; and carbohydrates content 79.44%. The dietary fiber of analog rice is 23.74%.

Keywords: analog rice, dietary fiber, physicochemical, Sargassum sp., taro beneng

INTRODUCTION

Fruit and vegetables are sources of fiber which the body really needs. It is known that the percentage of the Indonesian population that does not consume enough fruit and vegetables has reached 95.4% (Kemenkes 2019). The recommendation for good fiber intake according to WHO is 25-30 grams per day, while the average consumption of dietary fiber for the Indonesian population is 10.5 grams per day (Rahmah *et al.* 2017). Lack of fiber consumption can increase the risk of death from non-communicable diseases such as colon cancer, diabetes, hypertension, stroke, heart disease and obesity (Anggraini 2018).

One effort that can be made to overcome the above problems is to increase consumption of foods high in dietary fiber. These food ingredients can be found in seaweed *Sargassum* sp. Based on research by Matanjun *et al.* (2009) the amount of dietary fiber contained in 100 grams of dry *Sargassum* sp. is 39.67%. People don't like to consume seaweed directly as a vegetable because it smells fishy and tastes unpleasant. Therefore, it needs to be formulated into processed products so that the level of acceptance increases and at the same time we get the benefits we want.

Analog rice is a processed product that can be made using non-rice ingredients and has a shape like rice (Mishra *et al*. 2012). Analog rice has advantages over rice because its nutritional composition can be designed so that it has better functional properties

(Noviasari *et al*. 2017). The main ingredients in making analog rice usually come from local ingredients high in carbohydrates, such as sorghum (Anggraeni *et al*. 2016), corn (Noviasari *et al*. 2015), taro (Kumolontang and Edam 2020), cassava and sweet potato (Saragih *et al.* 2020). One of Banten's local foods that has the potential to be used in making analog rice is taro beneng (*Xanthosoma undipes* K.Kock). Taro beneng has a carbohydrate content of 79.80-84.10% (Putri *et al*. 2021).

The development of analog rice as a functional food is still ongoing, apart from reducing dependence on rice through food diversification, the functional properties of analog rice are very beneficial for health. Several studies have examined the addition of various ingredients to improve the functional properties of analog rice. Fauziyah *et al*. (2017) examined the addition of bean flour to increase fiber content and antioxidant activity in rice analog from sorghum. Anggraeni *et al*. (2016) examined the addition of fish bone meal and seaweed to gembili tubers to increase the fiber and calcium levels of analog rice. This research aims to evaluate of characteristics in making analog rice from a combination of seaweed (*Sargassum* sp.) and beneng taro as a high fiber food.

MATERIALS AND METHODS

Tools and Materials

The main ingredients used in this research were beneng taro flour from Pandeglang Regency, Banten and seaweed (*Sargassum* sp.) from the waters of Anyer Beach, Banten. Other materials used are carboxymethyl cellulose (CMC), (Ca(OH)2) 1% and water. The tools used in this research were baking pans, basins, winnowing pans, 80 mesh strainers, stoves, plastic packaging, pans, noodle makers (ATLAS), ovens (Memmert), analytical scales (Boeco BBI-32), choppers and glassware.

Making Seaweed (*Sargassum* sp.) Flour

Making seaweed flour refers to Hudaya (2008). Procedure for making seaweed (*Sargassum* sp.) flour begin by soaking the seaweed for 24 hours in fresh water to remove dirt and mineral salts. The seaweed that has been soaked is then washed under running water, then soaked in 1% lime water (Ca(OH)2) for 1 hour. Next, the samples were washed again until clean and soaked in fresh water for 24 hours, then rinsed until clean and reduced in size. The samples were dried using oven at 60°C for 15 hours. The dried seaweed is ground into flour then sieved and then stored in plastic packaging.

Making Analog Rice

Making analog rice refers to the research of Agusman *et al*. (2014) with modifications to the formulation and molding tools used. Seaweed (*Sargassum* sp.) flour and beneng taro flour mixed in a bowl. Percentage of seaweed flour addition based on the weight of beneng taro flour, namely 0, 5, 10, and 15%. Next, carboxymethyl cellulose (CMC) is added and mixed with water, then stirred until it forms a semi-wet dough. The next stage is formed using a noodle maker, then cut into pieces (3-5 mm) and shaped like rice grains. The granules are then steamed for 6 minutes at a temperature of 90 – 100 °C until they gelatinize. The steamed granules were cooled at room temperature for 20 minutes, then dried in an oven at 40°C for 10 hours. The formulation for making analog rice is presented in Table 1.

Characterization of Analog Rice

Analog rice is characterized physically, including the white degree (Kaemba *et al*. 2019), water absorption capacity and swelling power (Yudanti *et al*. 2015). Chemical characterization uses water, protein, fat, ash and carbohydrate content

tests (AOAC 1980). The dietary fiber content test refers to the AOAC method (1995).

Data Analysis

The data in this study were analyzed using the ANOVA test with a confidence level of 95%, if there was a significant difference (P<0.05), then continued with the Duncan test.

RESULTS AND DISCUSSION

Physical Characteristics of Analog Rice

The white degree of analog rice shown at figure 1. The addition of more seaweed flour had a significant effect (P<0.05) on reducing the whiteness value of analog rice (Table 2). The decrease in the white degree was caused by the influence of the brown color of the seaweed (*Sargassum* sp.). This is in line with the statement by Karina and Desrizal (2021), that the addition of brown seaweed makes the color of the dodol darker. Apart from that, the dark color can also be influenced by the browning reaction (maillard) during heating processes such as steaming and drying. According to Damat *et al*. (2020) the heating process can cause maillard reactions between sugars from carbohydrates and amino acids.

The whiteness degree of analog rice added to seaweed flour ranged from 1.23 - 1.65% (Table 2). This value is lower when compared to analog rice from combination of seaweed (*E. cottonii*) and mocaf flour (17.70%) (Agusman *et al*. 2014), analog rice from combination of white corn and sago flour (66.81%) (Noviasari *et al.* 2013), analog rice from combination of cassava, coconut and sago (73.08%) (Kharisma *et al.* 2014), and rice from paddy (80.23%) (Noviasari *et al*. 2017). A low degree of whiteness can result in a decreased level of likeness (Karina and Desrizal 2021).

Water absorbtion capacity was carried out to determine the ability of analog rice to absorb water after the boiling process (Lindriati *et al*. 2014). Table 2 shows that the addition of seaweed did not have a significant effect (P>0.05) on the difference in the water absorbtion capacity. The average value of the water absorbtion capacity of analog rice added with seaweed flour ranges from 61 - 63%. The water absorption capacity of various rice analogues has been studied, the water absorption capacity of rice from paddy is 24.3% (Yulviatun *et al*. 2022), analog rice combination of a composite of cassava flour, corn flour and soybean flour is 60.52% (Pudjihastuti *et al.* 2021), analog rice from combination of mocaf, corn flour, and mung bean sprout flour 105.8-118% (Yulviatun *et al.* 2022). The higher the water absorption capacity, the higher the water needs for cooking. Water absorption capacity is influenced by the amylose content of the material, the higher the amylose content will have a positive correlation with water absorption capacity. Amylose in starch has the ability to form hydrogen bonds with water and consists of glucose units linked with α-1,4-glycosidic bonds (Srihari *et al*. 2016). Beneng taro has amylose and amylopectin contents of 19.27% and 37.02% respectively (Kusumasari *et al.* 2019).

Swelling power shows the level of volume expansion of analog rice due to the cooking process (Yulviatun *et al*. 2022). Table 2 shows that the addition of seaweed did not have a significant effect (P>0.05) on the difference in the swelling power. The swelling power of analog rice added with seaweed flour ranged from 59.3 - 62.0%. The swelling power in this study was higher compared to analog rice combination of taro flour, maizena flour and sweet potatoes (8.8%) (Srihari *et al*. 2016), analog rice from a combination of mocaf flour, corn flour and green bean sprout flour (27.25 - 30.86%) and rice from paddy (50%) (Yulviatun *et al*. 2022). The research results of Andika *et al.* (2021) indicated that the greater the swelling power, the shorter the cooking time. Swelling power is influenced by the ratio of amylose

and amylopectin in the raw material. The higher the amylose content, the more difficult to form a gel because the amorphous structure formed increases the gelatinization temperature, resulting in low swelling power (Srihari *et al.* 2016). The levels of amylose and amylopectin greatly influence the texture of analog rice, the higher the amylose will produce chewy rice, while the higher the amylopectin will produce fluffier and stickier rice (Adicandra and Estiasi 2016).

Chemical Characteristics of Analog Rice

The moisture content of analog rice is an important parameter to know because it affects to shelf life. According to Miftahudin *et al.* (2015), the lower the moisture content, the longer the shelf life of the product. Based on Table 3, the moisture content of analog rice added with seaweed flour ranges from 10.14 - 13.89%. The moisture content of analog rice in this study is similar to the moisture content of analog rice from corn flour (10.37%-13.76%) (Santoso *et al.* 2013), rice analog combination of banana flour and cassava flour (10,41%-13,08%) (Yudanti *et al.* 2015), and rice from paddy (9,86%) (Yulviatun *et al*. 2022). The moisture content of analog rice can be influenced by the length of the drying process, the longer the drying process will cause the moisture content to decrease (Santoso *et al*. 2013). The water content in this study is almost similar to rice from paddy, so it can be stored for a long time like rice from paddy.

Ash content is a rough description of the mineral content found in food (Spiraliga *et al.* 2017). The addition of more seaweed flour caused a significant increase ($P<0.05$) in the ash content of analog rice (Table 3). The ash content in analog rice with addition of seaweed (*Sargassum* sp.) flour ranges from 2.75 - 4.14% (Table 3). Ash content from various analog rice has been studied, ash content from analog rice of *Eucheuma cottonii* seaweed, mocaf and sago combination is 2.24 - 3.13% (Finirsa *et al*. 2022), analog rice from cassava, corn and black soybeans combination is 1.21% (Pudjihastuti *et al.* 2021), and analog rice from white corn is 0.38% (Noviasari *et al.* 2013). The ash content in this study also showed higher results compared to the ash content of rice from paddy which was only 0.92-1.28% (Yulviatun *et al*. 2022). This is due to the mineral content in *Sargassum* sp. quite high, reaching 33.74% dry weight (Siregar *et al*. 2018). Mineral content in *Sargassum* sp. are potassium (K), sodium (Na), magnesium (Mg), and iron (Fe) (Syad *et al*. 2013). Analog rice with 15% seaweed added does not meet healthy food standards based on SNI 01-7111.1-2005, because it has an ash content above 3.5% (BSN 2005).

Addition of seaweed (*Sargassum* sp.) flour had a significant effect ($P<0.05$) on increasing the fat content of analog rice (Table 3). The fat content of analog rice with the addition of seaweed flour has a value ranging from 0.36 - 0.72%. The results of the fat content in this study were still lower than the fat content in rice from paddy, which was 1.3% (Yulviatun *et al.* 2022). The low fat content in analog rice is caused by the low fat content in the raw materials for making analog rice. The fat content of the main raw material for making analog rice is 0.17% in taro beneng flour (Kusumasari *et al.* 2019) and 0.79% in *Sargassum* sp. seaweed (Gazali *et al*. 2018). Finirsa *et al* (2022) stated that analog rice which has a low fat content will not easily become rancid, so it will last longer when stored.

Table 3 shows that the addition of seaweed did not have a significant effect ($P>0.05$) on differences in protein levels. The protein content in analog rice ranges from 5.11 - 5.69%. The protein content of analog rice in this study showed higher results than analog rice combined with seaweed *Eucheuma cottonii*, mocaf and sago (0.60 - 0.73%) (Finirsa *et al*. 2022) and analog rice

from combination of taro flour, maizena flour and sweet potatoes (1.78%) (Srihari *et al*. 2016). The protein content of analog rice in this study were also close to the protein content of rice from paddy, which was 7.51% (Yulviatun *et al*. 2022). The high levels of analog rice protein in this study were influenced by the main raw materials, namely taro beneng and seaweed *Sargassum* sp. which has a protein content of 6.10% (Budiarto and Rahayuningsih 2017) and 8.42% (Siregar *et al*. 2018).

Addition of seaweed flour had a significant effect (P<0.05) on differences in carbohydrate levels (Table 3). Carbohydrate content in analog rice with the addition of seaweed ranges from 77.63-79.43%. Carbohydrate content of analog rice has been studied, carbohydrate content of analog rice from combination of seaweed (*Eucheuma cottonii*) and mocaf flour is 69.99% (Agusman *et al*. 2014), analog rice from white corn (91.54%) (Noviasari *et al.* 2013), and analog rice from cassava, corn and black soybeans combination (73.3%) (Pudjihastuti *et al.* 2021). The carbohydrate content of analog rice in this study is similar to rice from paddy 80.14% (Rasyid *et al.* 2016). This shows that analog rice with the addition of 5-15% seaweed can be used as an alternative food source of carbohydrates that is equivalent to rice from paddy.

Dietary Fiber

Dietary fiber is part of the carbohydrates that can be consumed and it is found in many plant cell walls. This fiber cannot be hydrolyzed by human digestive enzymes because it is resistant to the digestive and absorption processes in the small intestine, and can undergo complete or partial fermentation in the large intestine (Sari *et al*. 2019; Sardi *et al.* 2021). Based on Figure 2, dietary fiber in analog rice with the addition of seaweed ranges from 18.22–23.74%. Addition of seaweed had a significant effect (P<0.05) on increasing the dietary fiber content of analog rice (Figure 2). The highest levels of dietary fiber are found with the addition of 15% seaweed flour. This result is higher than the dietary fiber in analog rice from *Gracillaria* sp. (9.44%) (Purwaningsih 2022), analog rice from white corn (5.35%) (Noviasari *et al.* 2013), analog rice from sorghum (5.50%) and rice from paddy (0.19%) (Noviasari *et al*. 2015).

The dietary fiber in analog rice can be classified as a high fiber food, because it has a fiber content more than 6% (BPOM 2016). Based on BPOM (2021), the serving size for instant rice is 50-60 grams, so by consuming 50 grams of analog rice can fulfills 30.37-39.57% of daily dietary fiber intake (30 grams per day for recommended dietary fiber intake). Dietary fiber has various benefits if consumed in sufficient quantities. Consumption of dietary fiber can prevent obesity, reduce blood sugar levels, prevent cancer, stimulate the growth of good bacteria in the intestine and reduce the risk of cardiovascular disease (He *et al.* 2022).

CONCLUSION

The analog rice in this study had a darker color compared to rice from paddy. The analog rice in this research can be used as an alternative source of carbohydrates to replace paddy rice. The best treatment in this study was the addition of 5% and 10% seaweed because the ash content complies with standards and has a high dietary fiber content.

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Table 1. Rice analog formulations

Material	Addition of seaweed flour			
	0%	5%	10%	15%
Seaweed flour (g)	0	25	50	75
Beneng taro flour (g)	500	475	450	425
CMC (g)	5	5	5	5
Water (mL)	500	500	500	500

Table 2. Physical characteristics of analog rice

Parameters (%)	Addition of seaweed flour			
	0%	5%	10%	15%
White degree	$4,42 \pm 0,14^{c}$	$1,65 \pm 0,03^{b}$	$1,38 \pm 0,06^{a}$	$1,23 \pm 0,03^{a}$
Water absorption capacity	$63 \pm 0,01^{a}$	$63 \pm 0,01^{a}$	$61 \pm 0,01^{a}$	$61 \pm 0,01^{a}$
Swelling power	$61,8 \pm 0,14^{a}$	$62,0 \pm 0,13^{a}$	$61,3 \pm 0,14^{a}$	$59,3 \pm 0,12^{a}$

Note: Value with different notation in the same row has a significant differences (P<0.05)

Tabel 3. Chemical characteristics of analog rice

Parameters (%)	Addition of seaweed flour			
	0%	5%	10%	15%
Moisture content	$11,56 \pm 0,32^{b}$	$13,89 \pm 0,06^{c}$	$12,38 \pm 0,08^{b}$	$10,14 \pm 0,23^{a}$
Ash	$2,62 \pm 0,04^{a}$	$2,75 \pm 0,04^{a}$	$3,24 \pm 0,22^{b}$	$4,14 \pm 0,08^{c}$
Fat	$0,21 \pm 0,00^{a}$	$0,36 \pm 0,06^{a}$	$0,72 \pm 0,14^{b}$	$0,60 \pm 0,25^{b}$
Protein	$5,47 \pm 0,21^{a}$	$5,37 \pm 0,16^{a}$	$5,11 \pm 0,02^{a}$	$5,69 \pm 0,14^{a}$
Carbohydrat	$80,14 \pm 0,14^{d}$	$77,63 \pm 0,12^{a}$	$78,55 \pm 0,14^{b}$	$79,43 \pm 0,25^{c}$

Note: Value with different notation in the same row has a significant differences (P<0.05)

Figure 1. Analog rice: (A) 0%, (B) 5%, (C) 10%, (D) 15%

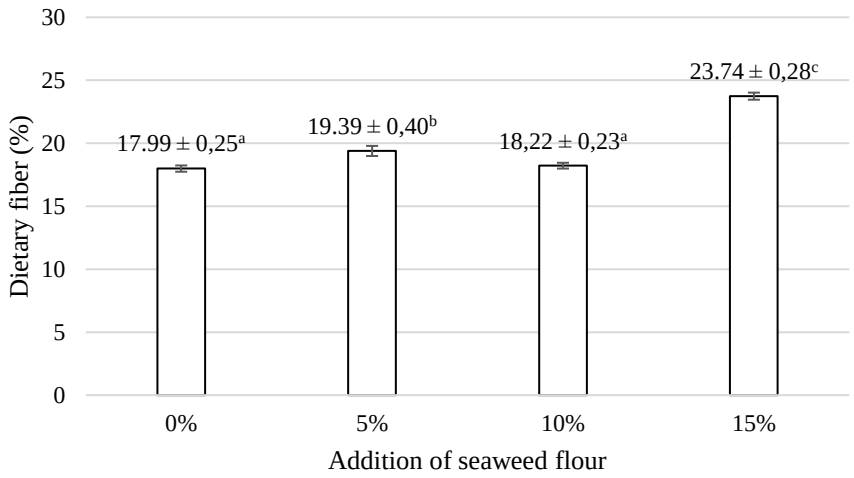

Figure 2. Dietary fiber in analog rice

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Acknowledgement (if necesary)

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b. Title of book is written with a capital letter for each initial word, except for conjunctions and forewords, while title of journal is only written in capital letter for the initial letter of the first word.
c. The name of journal/bulletin is written using standard abbreviation according to ISI's list of journal title abbreviations. http://images.webofknowledge.com/WOK46/help/WOS/C_abrvjt.html
d. Year, volume and pages should be completely written.
e. Reference from the internet is written along with the date accessed.
f. Minimum 80% of the cited references should be from the journals published within the last 10 years.
g. DOI (*Digital Object Identifier*) number should be mentioned, if applicable.

Examples:

Reference to a journal publication:

Yuliana ND, Iqbal M, Jahangir M, Wijaya CH, Korthout H, Kottenhage M, Kim HK, Verpoorte R. 2011. Screening of selected Asian spices for anti obesity-related bioactivities. Food Chem 126: 1724–1729. DOI: 10.1016/j.foodchem. 2010.12.066.

Reference to a book:

Lioe HN, Apriyantono A, Yasuda M. 2012. Soy Sauce: Typical Aspects of Japanese Shoyu and Indonesian Kecap. 93-102. CRC Press, Boca Raton, Florida.

Reference to a thesis/dissertation:

Merdiyanti A. 2008. Paket Teknologi Pembuatan Mi Kering dengan Memanfaatkan Bahan Baku Tepung Jagung [Skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.

Reference to an internet website:

Van der Sman RGM. 2012. Soft matter approaches to food structuring. http://www.sciencedirect.com/science/article/pii/S0001868612000620. [04 Juni 2012].

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