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PREFACE

By the Grace and Blessings of Allah the Almighty, we would like to present, with great pleasure, the Volume 03 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 2021

Editorial Team

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MICROBIOLOGICAL ANALYSIS OF KUNUN-ZAKI: A FERMENTED MILLET DRINK IN BENIN CITY, EDO STATE, NIGERIA

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ABSTRACT

Microbiological and physicochemical analyses were carried out on samples of fermented millet drink Kunun Zaki. Fifteen samples were obtained from Ikpoba Hill Market and Aduwawa Quarters. The microbiological analysis was carried out using the standard plate count technique to determine the total microbial population. The mean count of bacteria and fungi was 2.57×10^7 cfu/ml and 0.98×10^7 cfu/ml respectively. Microorganisms identified were *Lactobacillus* sp, *Bacillus* sp, *Staphylococcus aureus*, *Streptococcus* sp, *Escherichia coli*, *Pseudomonas* sp, *Mucor* sp and *Fusarium* sp. The mean value of the pH and titrable acidity was 4.26 ± 0.09 and 2.73 ± 0.08 ml 0.1M NaOH respectively. The moisture content was high with a mean of 85.90 ± 0.95 and mean solid content of 14.1 ± 0.95 . This study has shown that kunun-zaki sold in Ikpoba Hill Market and Aduwawa Quarters is highly contaminated with microorganisms. Practices of good hygiene are therefore necessary in an environment where kunun-zaki is produced, stored, prepared and packaged.

Keywords: Benin City, fermented millet, kunun-zaki, microbiological, physicochemical

INTRODUCTION

Food borne intoxication and poisoning is common in man and local beverage and drinks may be common sources of infection (WHO, 1996). The consumption of these local drinks is of public health significance. Hence, local drinks may serve as vehicles for food-borne pathogens such as *Staphylococcus*, *Salmonella*, *Shigella*, *Listeria*, *Escherichia* etc. Millet is one of the main cereal staples of West Africa. There are various species of millets found and they include Bulrush millet (Nigeria millet), *Pennisetum typhoides* in America, popularly known as Pearl millet, *Pennisetum americanum*, foxtail millet (*Setaria italica*) and finger millet (*Eleusine coracana*). The grain had its origin in Central America and West

Asia (Efiuvwevwe and Akoma, 1995). They are widely grown in Ghana, Cameroon and throughout the Savannah zone of Nigeria such as Bauchi, Sokoto, Katsina and Kano States (Ònuorah *et al.*, 1987). The quality of millet determines its use, if the grain is to be used as seed for planting, it should be pure, have a good yield and be free from disease and insect pest. If the grains are to be eaten by man or livestock, a high protein content is desirable and it must taste good (Oranusi *et al.*, 2003). Kunun-zaki is an indigenous fermented non-alcoholic beverage that is widely consumed for its thirst quenching properties. Though consumed throughout the year, it is extensively consumed during the dry season. The drink can also be produced

from fermented sorghum, guinea-corn and maize (Amusa and Odunbaku, 2008).

It is a popular drink with characteristic sweet-sour taste and fermented cereal drink, it is consumed both in rural and urban areas of Northern Nigeria and enhances lactation in nursing mothers, increase libido, sustain erection and increase sperm count (Amusa and Ashaye, 2009). Other food products derived from these cereals include; malted alcohol known as 'Oyokpo' 'pito' or 'burukutu' (Ekanem *et al.*, 2018; Innocent *et al.*, 2011). Like other grains, maize and millet contain essential nutrients such as vitamins A, B and C, minerals like potassium, zinc, anti-diabetic, anti-diuretic and anti-cancerous compound which are useful in treatment of diseases like diabetics, cancer and urogenital tract infections (Amusa and Odunbaku, 2008).

Organisms usually associated with millets grains include *Aspergillus* sp, *Penicillium* sp., as well bacteria like *Bacillus* sp, *Staphylococcus aureus* and *Lactobacillus* sp. (Elmahmood and Doughari, 2007) which predispose consumers to food borne infection and diseases. In a study on safety and quality evaluation of street foods sold in Zaria, Nigeria, Umoh and Odo (1999) found the mean aerobic counts for kunu ranged from 3.67 ± 0.67 to $4.29 \pm 1.14 \log_{10}/g$ with those sold by mobile food sellers having a significantly higher mean ($4.29 \pm 1.14 \log_{10}/g$) than that sold by stationary food sellers ($3.67 \pm 0.4 \log_{10}/g$) with *Bacillus cereus* and *Staphylococcus aureus* being the major bacteria isolated. Oranusi *et al.* (2003) also worked on the hazards and critical control points of kunu-zaki, in Northern Nigeria and discovered that *S. aureus* contamination in all the samples after pitching increased to $2.90 \log_{10} \text{cfu ml}^{-1}$ while *Bacillus* counts increased from 1.69 to $4.36 \log_{10} \text{cfu ml}^{-1}$.

The current food safety challenges rises slowly over the years and requires strategic

efforts to be controlled (USFDA, 2008). These products are being produced on daily basis for sales in markets, offices, schools, motor parks and as drinks during festivities, weddings and naming ceremonies. The production procedures and sales of these products are carried out under unhygienic conditions which may predisposed them to many pathogens of public health importance. This study seeks to give an in-depth focus into the sources of microorganisms that could contaminate Kunun-zaki and also identify practices that would aim at reducing the microbial load of the beverage.

MATERIALS AND METHODS

Sample Collection

Fifteen samples of kunun zaki drinks were purchased from different sales outlets in Ikpoba hill market and Aduwawa quarters in Benin City, Edo State during May- June 2011. These samples were placed in sterile bags and transported in a cold pack to the laboratory for analysis within one hour of collection. Statistical analysis of the sample results were carried out using descriptive statistics (mean and standard deviation).

Preparation of Media and Samples

1 ml of every Kunun zaki beverage was placed into 9 ml of distilled water and serial dilution was carried out up to 10^{-10} dilution. The culture media used for microbiological analyses which include nutrient agar, MacConkey agar, potato dextrose agar, eosin methylene blue agar and mannitol salt agar, were prepared according to manufacturers' instruction (Gadage *et al.*, 2004).

Isolation of Microorganisms

Using pour plate method, 1 ml from the dilutions were inoculated on Nutrient agar, MacConkey agar, potato dextrose agar for the enumeration of total microorganism, coliform count and enumeration of fungi respectively. After inoculation, Petri dishes

containing Nutrient agar, MacConkey agar and Mannitol salt agar were incubated at 37°C for 24 h (Mannitol salt agar was used for the isolation of *Staphylococcus*), while inoculated plates containing Potato dextrose agar was incubated at 28°C for 5-7 days (Gadage *et al.*, 2004).

Identification and Characterization of isolates

The isolated organisms were characterized and identified based on their cultural, morphological, and biochemical tests (Buchanan and Gibbson, 1974; Gadage *et al.*, 2004). The colonies were sub-cultured, Gram stained and subjected to biochemical tests such as oxidase, catalase, coagulase, urease, citrate, indole and sugar fermentation tests. These results were also checked on PIBWIN (Probable identification of microorganisms).

Physicochemical analysis

pH of the Kunun zaki drinks was measured by dipping the pH electrode into 10 ml of the beverage placed in a beaker, and the reading were recorded. Total titrable acidity was measured as percentage lactic acid by adding 3 drops of phenolphthalein indicator into 10ml of the drink placed in a conical flask and thoroughly shaken. The mixture was then titrated against 0.1 M NaOH (Sodiun hydroxide) to a pink color end point and the titre value was calculated. Moisture and solid content were determined by methods described by Ceese (1995).

RESULTS AND DISCUSSION

The results of this study showed that the mean total viable bacterial and fungal count was 2.51×10^7 cfu/ml and 0.98×10^7 cfu/ml respectively as shown in Table 1.

These results revealed that samples gotten from Aduwawa market (B₂) had highest bacterial count while Sample from Ikpoba hill market (C₁) has the lowest bacterial count. It also showed that samples gotten from Aduwawa market (A₁) had highest fungal count while Sample from Ikpoba hill market (C₃) has the lowest fungal count. This showed that kunun drinks sold in Aduwawa are heavily contaminated.

In many earlier reports, the pH was 4.3 (Ekanem *et al.*, 2018); 3.80 and 3.99 reported by Innocent *et al.* (2011), 2.42 to 3.83 recorded by Otaru *et al.* (2013), 5.25 to 5.65 reported by Amusa and Ashaye, (2009). The acidity of the kunu drinks may be due to the presence of some bacteria which help in acid fermentation of the kunu products (Ekanem *et al.*, 2018). The results of the investigation showed that the samples of Kunun-zaki contained a fairly large microbial population. The high microbial densities could be related to the fact that usually a heterogeneous population of microorganism are usually involved in the fermentation process and also that foodstuffs are also susceptible to microbial contamination during the processing and storage. The microorganisms isolated from the samples include *Lactobacillus sp*, *Staphylococcus aureus*, *Bacillus sp*, *Streptococcus sp*, *Escherichia coli* and *Pseudomonas sp*, *Aspergillus*, *Mucor*, *Rhizopus*, *Pencillium* and *Fusarium*. The results correlate with that of Amusa and Odunbaku (2008); Oyenuga *et al.*, (2003); Elmahomood and Doughari (2007). Sources of the organisms may be traced to the cereals as had been reported by

Efiuvwevwere and Akoma (1995). The presence of *Lactobacillus* indicates that

contracted by sneezing or by picking of the nostrils by food handlers. *Staphylococcus*

Table 1. Mean Total Viable Counts of Both Bacterial and Fungal Isolates (CFU/ml)

Sample	Bacteria count	Fungal count	Location
A ₁	1.8x10 ⁷	6.00x10 ¹¹	Aduwawa
A ₂	2.56x10 ⁷	12.0x10 ⁹	Aduwawa
A ₃	2.00x10 ⁹	7.00x10 ⁷	Aduwawa
A ₄	1.15x10 ⁹	6.50x10 ⁹	Aduwawa
A ₅	2.08x10 ⁷	12.0x10 ⁷	Aduwawa
B ₁	1.96x10 ⁷	5.50x10 ⁹	Aduwawa
B ₂	5.60x10 ¹¹	20.0x10 ⁷	Aduwawa
B ₃	2.36x10 ⁹	10.5x10 ⁹	Aduwawa
B ₄	1.34x10 ⁹	7.5x10 ⁷	Ikpoba Hill
B ₅	8.70x10 ⁹	15.0x10 ⁷	Ikpoba Hill
C ₁	1.72x10 ⁷	4x10 ¹¹	Ikpoba Hill
C ₂	1.28x10 ⁹	11.0x10 ⁷	Ikpoba Hill
C ₃	1.05x10 ⁹	6.00x10 ⁷	Ikpoba Hill
C ₄	2.51x10 ⁹	9.61x10 ⁷	Ikpoba Hill
C ₅	1.47x10 ⁹	14.0x10 ⁹	Ikpoba Hill
Total Mean count	2.51x10⁷	0.98x10⁷	

kunun-zaki is a lactic acid bacteria fermented beverage and it is not unexpected because they help in fermentation process. *Lactobacillus* in food samples tend to dominate by preventing other pathogenic microorganisms from surviving in the beverage, and their ability to produce lactic acid reduces the pH of food medium. Most food poisoning organisms cannot tolerate low pH, therefore the isolation of *Lactobacillus* favors this fact. Efiuvwevwere and Akoma (1995) reported the presence of some bacteria including *Lactobacillus spp* in kunun drink. The presence of *Staphylococcus aureus* indicate contamination from handlers as *Staphylococcus aureus* is a normal flora of the skin, nose, throat, palms, hairs and mucus membrane and a common etiological agent of septic arthritis (Charles et al., 2005; Emmanuel-Akerele and Uchendu, 2021). The contamination could have been

aureus can also produce Staphylococcal bacteraemia and abscesses in cell during food infection. *Escherichia coli* in food is an indication of faecal contamination of product. However, *E. coli* is a normal flora of the intestinal tract of man, presence of it in excess could lead to gastroenteritis and bacterial diarrhea disease (Emmanuel-Akerele and Uchendu, 2021). This fail to agree with the WHO standards which suggested that water that contain >10coliforms/100ml or one *E. coli*/100ml with or without other coliforms is unsatisfactory for human consumption. *Streptococcus sp.* may also have been enumerated from the beverage as a result of the handlers, since it is also normal flora of the throat and the buccal activity. The presence of *Bacillus* could render a beverage unsuitable for human consumption (Innocent et al., 2011). It is possible that the

contamination by this pathogen may have occurred during sieving and packaging, as most of the people involved in the production, packaging and hawking do not take necessary precautions, and so such

environmental contamination. The pathogenic microorganisms isolated exceed permissible limit (Efiuvwevwere and Akoma, 1995; USFDA, 2008). Production of kunun drinks should be done under hygienic

Table 2. Characteristics of Bacterial Isolated from the Kunun-Zaki Sample

Characteristics	Description					
Cultural						
Color	Cream	Cream	Cream	Yellow	Cream	Florescent green
Surface appearance	Mucoid	Rough	Dry	Mucoid	Smooth	Rough
Elevation opacity	Umbonated translucent	Slightly convex opaque	Convex opaque	Flat opaque	Convex semi-transparent	Convex opaque
Morphological						
Gram stain	+	+	-	+	+	-
Cell type	Rods	Rods	Rods	Cocci	Cocci	Rods
Cell arrangement	Single	Chains	Single	Cluster	Single	Chains
Motility	-	+		-	+	+
Biochemical						
Catalase	-	+	+	+	-	-
Coagulase	-	-	+	+	-	-
Oxidase	-	-	-	-	-	+
Urease	-	-	-	-	-	-
Citrate	+	-	-	+	-	+
Indole	-	-	+	-	-	-
Sugar fermentation						
Glucose	AG	A	AG	A	A	-
Lactose	A	-	AG	A	A	-
Manitol	A	A	A	A	A	-
Bacteria isolated	<i>Lactobacillus sp</i>	<i>Bacillus sp</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus sp</i>	<i>Pseudomonas sp</i>

Key: AG- Acid and gas production; A- Acid production

Table 2 shows the cultural, morphological and biochemical characteristics of the isolates. The bacteria isolated were *Lactobacillus*, *Bacillus sp*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp* and *Pseudomonas sp*

contamination could be very prominent. *Bacillus* is a spore former and as such the spores were easily distributed and was able to withstand high temperature and pH to fully germinate (Otaru *et al.*, 2013). The organism has the potential of causing an array of infections. The presence of *Pseudomonas* is not of great significance due to their low population. They could have occurred due to

conditions to avoid proliferation and spread of these pathogenic organisms.

The fungal isolates present could be traced right to when the grains were either being harvested or stored. The presence of *Aspergillus*, *Penicillium*, and *Fusarium* in the kunun-zaki samples might not be too surprising as they are known as common spoilage organism of carbohydrate foods as

Table 3. Cultural and Morphological Characteristics of the Fungal Isolate

Isolates	Physical Appearance	Microscopic Observation	Fungi isolated
F ₁	Black powdery threads	Septated mycelium conidiophore septate and arising from foot cell. Bear sterigmata conidia in chains and black coloration	<i>Aspergillus sp</i>
F ₂	Dirty blue powdery growth, hair-line	Septated branched mycelium, septated aerial conidiophores with brush-like spore bearing head with sterigmata bore in clusters	<i>Penicillium sp</i>
F ₃	White thread with surface colored black	Non-septated with aerial sporangiophore. Round columella smooth spores. No stolons and rhizoids	<i>Mucor sp</i>
F ₄	Pure white thick and abundant cotton mycelium	Non-septated with stolons and rhizoids. Sporangiophores arising at the nodes. Sporangia are usually black	<i>Rhizopus sp</i>
F ₅	Cotton-like growth with white coloration	Separated with large canoe-shaped microconida on branched conidiophores	<i>Fusarium sp</i>

well as storage microflora of many cereals including sorghum (Ekanem et al., 2018;

preparation and finally germinate in the finished product. The presence of these fungi

Table 4. pH and Titratable Acidity

Sample	pH	Titratable acidity (ML 0.1M NaOH)	LOCATION
A ₁	4.22	2.90	Aduwawa
A ₂	4.21	2.80	Aduwawa
A ₃	4.22	2.84	Aduwawa
A ₄	4.31	2.80	Aduwawa
A ₅	4.20	2.80	Aduwawa
Mean value	4.23	2.83	
B ₁	4.30	2.75	Aduwawa
B ₂	4.27	2.65	Aduwawa
B ₃	4.21	2.70	Aduwawa
B ₄	4.98	2.66	Ikpoba Hill
B ₅	4.27	2.60	Ikpoba Hill
Mean value	4.21	2.67	
C ₁	4.33	2.68	Ikpoba Hill
C ₂	4.31	2.68	Ikpoba Hill
C ₃	4.33	2.66	Ikpoba Hill
C ₄	4.27	2.72	Ikpoba Hill
C ₅	4.30	2.70	Ikpoba Hill
Mean value	4.33	2.70	
Total mean	4.26±0.09	2.73±0.08	

Omonigho and Osubor, 2002). The fungal may produce spores attached to the grains and overcome adverse condition during the

such as *Aspergillus*, *Mucor*, *Fusarium* and *Rhizopus* is associated with spoilage of the beverage (Oyenuga et al., 2003). Some of

these fungal species elicit some toxins which are very hazardous. One of such is *Aspergillus* which produce aflatoxins that are quite harmful and as such their occurrence is undesirable. Table 3 shows the cultural and morphological characteristics of the fungi isolated and they are *Aspergillus sp*, *Penicillium sp*, *Rhizopus sp*, *Mucor sp* and *Fusarium sp*. The pH value of the samples ranged from of 4.21 to 4.30, while the titrable acidity shows the presence of organic acids and it ranged from 2.67 to 2.82ml.

The acidity level of kunun-zaki drinks have been described by several researchers including Efiuwewwere and Akoma (1995) and Amusa and Ashaye (2009), who attributed these to the presence of lactic acid bacteria. The acidity tends to increase with increase in fermentation period resulting into spoilage. Consequently, the low pH value may have encouraged the growth of fungi and

this could be responsible for the species of microorganism isolated. The pH brought about a corresponding increase in the titratable acidity and sour taste flavour of the kunun-zaki drink. The moisture and solid content of the analyzed Kunun-Zaki had overall mean of 85.90±0.95 and 14.1± 0.95 respectively. The low moisture contents could be due to the ease of moisture loss during production (drying resulting from hydrolytic enzyme activities during malting and incubation). The relatively high solid content indicates that the kunun can develop off flavor and colour if stored at room temperature for few days. The presence of all these organisms indicated in this report are of great public health concern as it has passed the permissible limit by WHO (WHO, 1996; Ekanem *et al.*, 2018), and in situations where the beverage is contaminated, quick medical

Table 5. Percentage of Moisture and Solid content present in the Sample

Sample	Moisture content (%)	Solid content (%)	Location
A ₁	86.47	13.53	Aduwawa
A ₂	85.80	14.20	Aduwawa
A ₃	85.85	14.15	Aduwawa
A ₄	85.83	14.17	Aduwawa
A ₅	85.89	14.11	Aduwawa
Mean value	85.97	14.03	
B ₁	87.22	12.78	Aduwawa
B ₂	86.83	13.17	Aduwawa
B ₃	86.70	13.30	Aduwawa
B ₄	86.86	13.14	Ikpoba Hill
B ₅	87.08	12.92	Ikpoba Hill
Mean value	86.94	13.06	
C ₁	84.99	15.01	Ikpoba Hill
C ₂	83.90	16.10	Ikpoba Hill
C ₃	85.25	14.75	Ikpoba Hill
C ₄	85.27	14.73	Ikpoba Hill
C ₅	84.51	15.49	Ikpoba Hill
Mean value	84.78	15.22	
Total mean	85.90±0.95	14.10±0.95	

care should be sought to avoid food poisoning.

CONCLUSION

Since there are no routine hygiene standard techniques for preparation of kunun-zaki such food will always contain an unusual large population of fermentative beneficial organisms and some pathogenic microorganisms. This study has shown that the preparation procedure for kunun-zaki does not completely eliminate microorganisms from the finished products. Storage of the product at room temperature allowed for proliferation of microorganisms and this tends to utilize the kunun-zaki constituents resulting in significant changes in the physicochemical composition (pH and titrable acidity) of the product. The isolation of pathogens as *Staphylococcus*, *Streptococci* and *Aspergillus* could be indicative of health hazards, even when their population has been inhibited to an extent by acid produced by the lactic acid bacteria. In order to reduce the rate of contamination and gently enhance the microbiology qualities of the product the following measures should be adhered to; educate producers and hawkers of the product on good sanitary practice during the preparing and sale of the product; advocate the use of boiled water in washing utensils; treated municipal water should be used during processing and dilution of the processed drinks to avoid contamination with entero-pathogenic bacteria; the processing environment should be hygienic and the packaging materials should be sterilized.

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THE USE OF STEVIA AND MONK FRUIT SWEETENERS FOR SUGAR REPLACEMENT IN GREEN TEA AGAR JELLIES

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ABSTRACT

This study aimed to develop healthy agar jellies with low-sugar content, linking to a decreased risk for heart attack, diabetes, and other chronic diseases. Two natural non-nutritive sweeteners, stevia and monk fruit were studied at 25%, 50%, 75%, and 100% sugar replacement based on the equivalent sucrose sweetness. The physical properties assessed on the total soluble solids, firmness, color, and syneresis. Still, the sensory attributes on color, taste, flavor, texture, and overall acceptability was evaluated by a 9-point hedonic scale. The more sugar replacement lowered the total soluble solids but increased the gel firmness ($p < 0.05$) and the lightness (L^*). The syneresis declined in reduced-sugar jellies with stevia and monk fruit against the whole sugar jellies. The stevia and monk fruit replacement affected agar jellies' taste and flavor attributes ($p < 0.05$). Jellies with 50% and 75% sugar replacement, either stevia or monk fruit, were preferred over 25% and 100% replacement. The jelly with 50% stevia was the optimal formulation.

Keywords: Agar jelly, healthy dessert, monk fruit, stevia, sugar replacement

INTRODUCTION

The global growth of the sugar-free food and beverage market has risen to a 9.36% CAGR during the forecast period (2021-2026) (Mordor intelligence, 2021). Increasing concerns regarding chronic diseases, such as hypertension, heart disease, diabetes, and obesity, have correlated with new free or lower sugar foods introduced to the food market. The sugar level limit by the American Heart Association (2021) recommended most men and women should consume no more than 36 g and 25 g of added sugar per day. Replacing sugary foods and drinks with non-nutritive sweeteners, a substance that provides a sweet taste and few or no calories but offers no nutritional benefits such as vitamins and minerals can help people gain weight, tooth decay, and

better health. Artificial non-nutritive sweeteners widely available for the food industry are saccharine, acesulfame-K, aspartame, and sucralose; these are considered safe under an acceptable daily intake (ADI) level of each sweetener approved by the FDA. However, two plant-based sweeteners, including stevia and monk fruit extracts, have been increasingly applied in food products because they are natural and Generally Recognized as Safe (GRAS) for use (Tey *et al.*, 2017).

Stevia, or *Stevia rebaudiana*, is an intensely sweet-tasting, zero-calorie plant extract providing 120-200 times sweeter than sucrose. It has a negligible effect on tooth decay, blood glucose, and insulin levels; it is attractive to people with health concerns (Gao *et al.*, 2016). Stevia is commonly heat

and pH stable, maintaining its sweetening power during processing and does not caramelize; thus, it has the potential to be widely used. Sometimes it tastes bitter, has a licorice-like or metallic aftertaste, mainly used in high concentrations (Goyal *et al.*, 2010). In the meantime, monk fruit (*Siraitia grosvenorii*), commonly known worldwide as Luo Han Guo, is a gourd native to South East Asia. It is a zero-calorie sweetener with compounds called mogrosides, having high biological effects and sweet taste (Li *et al.*, 2014). In 2010, the extract of monk fruit was approved in the USA as GRAS for non-nutritive sweetening and flavor-enhancing purposes. It is around 150-250 times the sweetness of sucrose, pH and heat stable, and has no tooth decay and side effects but may cause rare digestive issues if consuming too much of it, such as nausea and upset stomach. Some people notice an unpleasant aftertaste at its high concentrations. In recent years, pharmacological studies reveal the monk fruit has several health-protective properties such as liver protection, anti-oxidative, anti-inflammatory, anti-hyperglycemic, and anti-cancer effects (Pandey and Chauhan, 2020). Monk fruit is becoming more popular in the natural health sector, but it is more expensive than other sweeteners, especially it costs more than twice that of stevia.

The sugar reduction frequently has caused changes in product characteristics and sensory acceptability against full-sugar counterparts. Several non-nutritive sweeteners are permitted for use in foods with sugar reduction on a sweetness equivalency basis. However, the sweeteners are only successful if they denote a similar sensory profile as sugar. To date, information comparing the quality of low-calorie products with stevia against monk fruit is little. Therefore, this study assessed the physical properties and sensory profile of agar jellies with sugar replacement by stevia and monk fruit. The optimal formulation with

palatable and acceptable was also investigated.

MATERIALS AND METHODS

Materials

Agar powder (Platapiantong Seng Huad Co., Ltd., Thailand), green tea powder (Cha Tramue, Siam F.B. Product Co., Ltd., Thailand), and sugar were purchased from a supermarket. Stevia (BetterStevia™, Now Foods, IL, USA) and monk fruit extract (NuNaturals, Inc, OR, USA) were used.

Experimental Design

Eight green tea agar jellies with sugar replacement by stevia and monk fruit were prepared. The sugar replacement was calculated as equivalent sweetness basis; 45 mg of stevia is equivalent to 6 g of sugar, and 35 mg of monk fruit is equivalent to 4 g of sugar. Thus, the stevia amounts used for replacing 25%, 50%, 75%, and 100% sugar content (16 g in recipe) was 0.03, 0.06, 0.09, and 0.12 g, respectively. At the same time, the monk fruit used was 0.035, 0.07, 0.105, and 0.14 g, respectively.

Green Tea Agar Jelly Production

A regular agar jelly recipe included 1 g agar powder and 16 g sugar in 100 mL green tea water. Initially, a green tea bag (1 g) was soaked in a 100 mL hot water (80 ± 2 °C) for 20 min. Added agar powder, followed by sugar, and thoroughly stirred for 5 min. Poured the hot mixture (60 ± 2 °C) into cups (2×4 cm²) and refrigerated at 4 °C for 20 min.

Physical Analysis

Total soluble solid was measured by a hand refractometer (Atago®, Fukaya factory, Saitama, Japan). A texture analyzer (LRX Plus, Lloyd Instruments, Hampshire, UK) was performed for the firmness measurement. The instrument was equipped with a cutting test cell and operated at a

crosshead speed 200 mm/min. The peak force (N) was recorded and reported as the firmness.

Color analysis was determined using a colorimeter (MiniScan EZ, Hunter Associates Laboratory, Reston, VA). L^* (lightness) (100 = white, 0 = black), a^* (+ = red, - = green) and b^* (+ = yellow, - = blue) were recorded.

A gel sample packed in a polyethylene bag was stored in a refrigerator (10 ± 2 °C) for 2, 4, and 6 days. The syneresis was determined by the difference of sample weight before and after storage to sample weight, as according to Banerjee and Bhattacharya (2011) with some modification.

Table 1. Physical properties of green tea agar jellies with different sweeteners

Treatment	Sugar replacement (%)	Total soluble solid ($^{\circ}$ brix)	Firmness (N)	L^* (Lightness)	a^* (Redness)	b^* (Yellowness)
Control	-	18.1 \pm 0.30 ^a	5.05 \pm 0.17 ^d	2.16 \pm 0.13 ^d	7.28 \pm 0.01 ^a	3.57 \pm 0.14 ^c
Stevia extract	25%	6.9 \pm 0.48 ^b	5.29 \pm 0.09 ^c	2.85 \pm 0.12 ^b	5.53 \pm 0.02 ^c	4.25 \pm 0.05 ^b
	50%	5.2 \pm 0.25 ^c	5.65 \pm 0.15 ^c	2.44 \pm 0.01 ^c	5.75 \pm 0.18 ^c	4.36 \pm 0.14 ^b
	75%	3.2 \pm 0.22 ^d	5.80 \pm 0.66 ^c	2.36 \pm 0.03 ^c	5.56 \pm 0.06 ^c	4.65 \pm 0.05 ^b
	100%	1.8 \pm 0.18 ^d	6.21 \pm 0.13 ^b	2.51 \pm 0.05 ^c	5.86 \pm 0.04 ^c	4.36 \pm 0.03 ^b
Monk fruit	25%	7.1 \pm 0.35 ^b	6.47 \pm 0.03 ^b	3.43 \pm 0.04 ^a	6.44 \pm 0.03 ^b	5.20 \pm 0.06 ^a
	50%	5.0 \pm 0.38 ^c	6.86 \pm 0.25 ^b	3.09 \pm 0.09 ^b	6.83 \pm 0.08 ^b	5.23 \pm 0.17 ^a
	75%	2.8 \pm 0.12 ^d	7.19 \pm 0.10 ^a	3.08 \pm 0.21 ^b	6.45 \pm 0.05 ^b	5.74 \pm 0.05 ^a
	100%	1.5 \pm 0.06 ^d	7.28 \pm 0.34 ^a	3.22 \pm 0.14 ^a	6.33 \pm 0.02 ^b	5.69 \pm 0.06 ^a

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

Sensory Evaluation

The sensory test was performed in an individual booth, and served with samples coded with random three-digit numbers. Sixty panelists, who regularly consume jellies, were instructed to assess the color, taste, flavor, texture, and overall acceptability. Sensory evaluation was assessed using a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like). All panelists were invited to clean and rinse their palates between samples with drinking water (Lawless and Heymann, 1998).

Data Analysis

The statistical analysis was conducted on analysis of variance (ANOVA) and Duncan's new multiple range test using the SPSS version 17.0 (Cochran and Cox, 1992). Principal component analysis was performed

by the R-program for the external preference mapping (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Agar jelly characteristics

The impact of sugar replacement with stevia and monk fruit on the characteristics of agar jellies are presented in Table 1.

a. Soluble solids in agar gel

The control jelly was made from the whole sugar, showing the highest soluble solid content. Sugar provides a soluble solid contributing to a significant relation to $^{\circ}$ brix. With increased sugar replacement by both stevia or monk fruit, the samples significantly declined in soluble sugar. A small amount of these sweeteners was used instead of sugar because of their high sweeter than sucrose.

Thus, it negligibly raised a soluble solid in jellies. The finding was in line with the study of Bajwa and Mittal (2015) for the reduced-sugar milk drink supplemented with mango pulp and sucralose. It was agreed by

Rocha and Bolini (2015), who studied the sweetness perception of passion fruit juices with sucrose and high-intensity sweeteners, including aspartame, stevia extract, sucralose, and neotame.

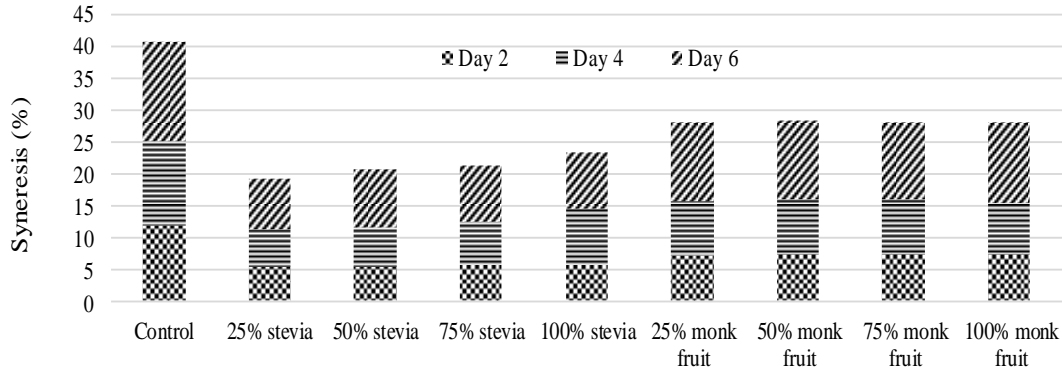


Figure 1. Syneresis of agar jellies with different levels of sugar reduction for 6 days at refrigerated storage.

b. Gel texture

Higher gel firmness in agar jellies was associated with increasing sugar replacement (Table 1). The control sugar jelly was rigid and brittle and had easily broken down, showing the lowest firmness. The more decreasing sugar content has caused the jellies with stevia and monk fruit less brittle and elastic but more cohesive, presenting a higher firmness. This observation denotes that sugar has a considerable effect on the gel. The high sugar content can reorganize the agarose bonds, contributing to the formed agar gel (more elastic but less cohesive) (Dorohovich *et al.*, 2018). The sugar itself might tighten the cross-linking of the agar molecules by its high water-binding capacity for the hydration of free water in the gel, greatly structuring the gel network (Saha and Bhattacharya, 2010). Vilela (2015) showed lower gel strength of pectin jellies with one-third sugar reduction with sorbitol and xylitol against the sugar control. Lau *et al.* (2000)

suggested that the strength of the gel structure is associated with the type and concentration of hydrocolloids levels and the amount of total solids content in the gel system.

c. Color

According to Table 1, increases in L^* and b^* values were observed in agar jellies with stevia and monk fruit, but a^* values were decreased ($p < 0.05$). The control had the lowest L^* or darker than the jellies with both sweeteners. An explanation might involve the jelly chromaticity derived from the mixed color between green tea and brown pigment by the sugar-amine reaction through the Maillard browning formation (Alais and Linden, 1991). In other words, stevia and monk fruit jellies were lighter or more transparent with high intensely yellow. This is related to their lower sugar participating in the brown formation reaction; the less sugar content, the less the intensity of darker color. According to Gao (2016), the lower a^* might be due to lowering the availability of sugar amounts to form brown pigments through the non-enzymatic browning reaction. When considering the h° value, the control had the lowest ($h^\circ = 26.1$), relating to a reddish-

orange shade, while the jellies with stevia ($h^\circ = \sim 37.8$) and monk fruit ($h^\circ = \sim 40.2$), which became more yellowish-orange tonality due

to higher h° (data not shown). The monk fruit extract has light yellow, making jelly with higher h° than the stevia, a white powder.

Table 2. Sensory evaluation of green tea agar jellies with different sweeteners

Treatment	Sugar replacement (%)	Color	Taste	Flavor	Texture	Overall acceptability
Control	-	6.38±1.41 ^b	6.96±1.49 ^a	6.67±1.76 ^a	6.54±1.53 ^a	6.96±1.52 ^a
Stevia extract	25%	6.57±1.10 ^b	4.33±1.55 ^d	5.33±1.43 ^c	5.65±1.36 ^b	4.83±1.37 ^b
	50%	6.71±1.30 ^{ab}	6.50±1.41 ^b	6.29±1.55 ^b	6.58±1.32 ^a	6.68±1.40 ^{ab}
	75%	6.98±1.29 ^a	6.75±1.51 ^b	6.30±1.52 ^b	6.25±1.98 ^{ab}	6.95±1.58 ^a
	100%	6.95±1.35 ^a	4.00±1.77 ^d	5.21±1.56 ^c	5.04±1.85 ^c	4.42±1.35 ^c
Monk fruit	25%	6.50±1.57 ^b	5.71±1.43 ^c	5.64±1.38 ^c	6.00±1.50 ^b	5.50±1.14 ^b
	50%	6.72±1.54 ^{ab}	6.08±1.21 ^b	6.33±1.31 ^b	6.54±0.93 ^a	6.63±1.06 ^{ab}
	75%	7.08±1.47 ^a	6.63±1.79 ^b	6.45±1.70 ^b	6.42±1.56 ^a	6.80±1.67 ^a
	100%	6.85±1.79 ^a	4.71±1.94 ^d	5.54±1.56 ^c	5.25±1.90 ^c	4.83±1.70 ^c

Means in the same column with different superscripts are different ($p < 0.05$)

d. Water weeping

Figure 1 shows the results of water expelling determined in the control and agar jellies with stevia and monk fruit during 6 days in refrigerated storage (10 ± 2 °C). Syneresis, a liquid that is expelled or extracted from a gel, is undesirable because the wetness can cause unacceptable changes in texture, smell, and appearance, sensory preference and promote the growth of microbiological spoilage. The result revealed a higher syneresis in the control jelly than those with sugar replacement at every 2-day interval storage. In general, the agar gel forms a meshwork that contains pores, which have sizes depending on the concentrations of agarose and sugar. The agar gel with sugar may have large pores in the gel structure, and it would contribute to shrinking over and releasing higher water during aging (Somboon *et al.*, 2014). The decrease in syneresis was related to lowered total solids in the samples with stevia and monk fruit inclusion. The low amount of sugar content could affect the internal hydrophobicity by incorporating the water-binding capacity of sugar. It might cause more reduced pore sizes

in the agar gel matrix, lowering the water expelling from reduced-sugar agar jellies (Divoux *et al.*, 2015). It noted that the syneresis was decreased mainly in the agar jelly with stevia compared to that with monk fruit.

Sensorial preference

a. 9-point hedonic scale

The variation of sensory attributes was observed in samples sweetened with stevia and monk fruit against the control made from the sugar, as presented in Table 2. Jellies with stevia and monk fruit were transparent than the control, particularly at 75% and 100% replacement, thus raising their color scores. The level of sugar replacement was crucial on the preference of taste, flavor, and texture, showing their higher scores ($p < 0.05$) in the jellies with 50% and 75% sugar replacement relative to 25% and 100% replacement. Most panelists disliked the aftertaste (bitter/metallic), thereby rejecting the jellies with 100% sugar replacement. They preferred the balanced sweetness from blending stevia or monk fruit with white sugar based on the equivalent sweetness. Since each sugar replacer has a

sweeter taste than cane sugar (Tey *et al.*, 2017), the panelists might find the jellies with 50% and 75% sugar replacement had taste and flavor in the right level. With stevia and monk fruit inclusion, the 75% sugar replacement samples received the highest overall acceptability but were not statistically significant ($p>0.05$) compared to 50% replacement. The products were acceptable, corresponding to the “like slightly” response.

The ANOVA result shows whether the differences between the attributes are statistically significant according to sugar replacement. It does not disclose the most appreciated sample. The preference mapping was used to depict the main attributes affecting the liking and determine the best sample.

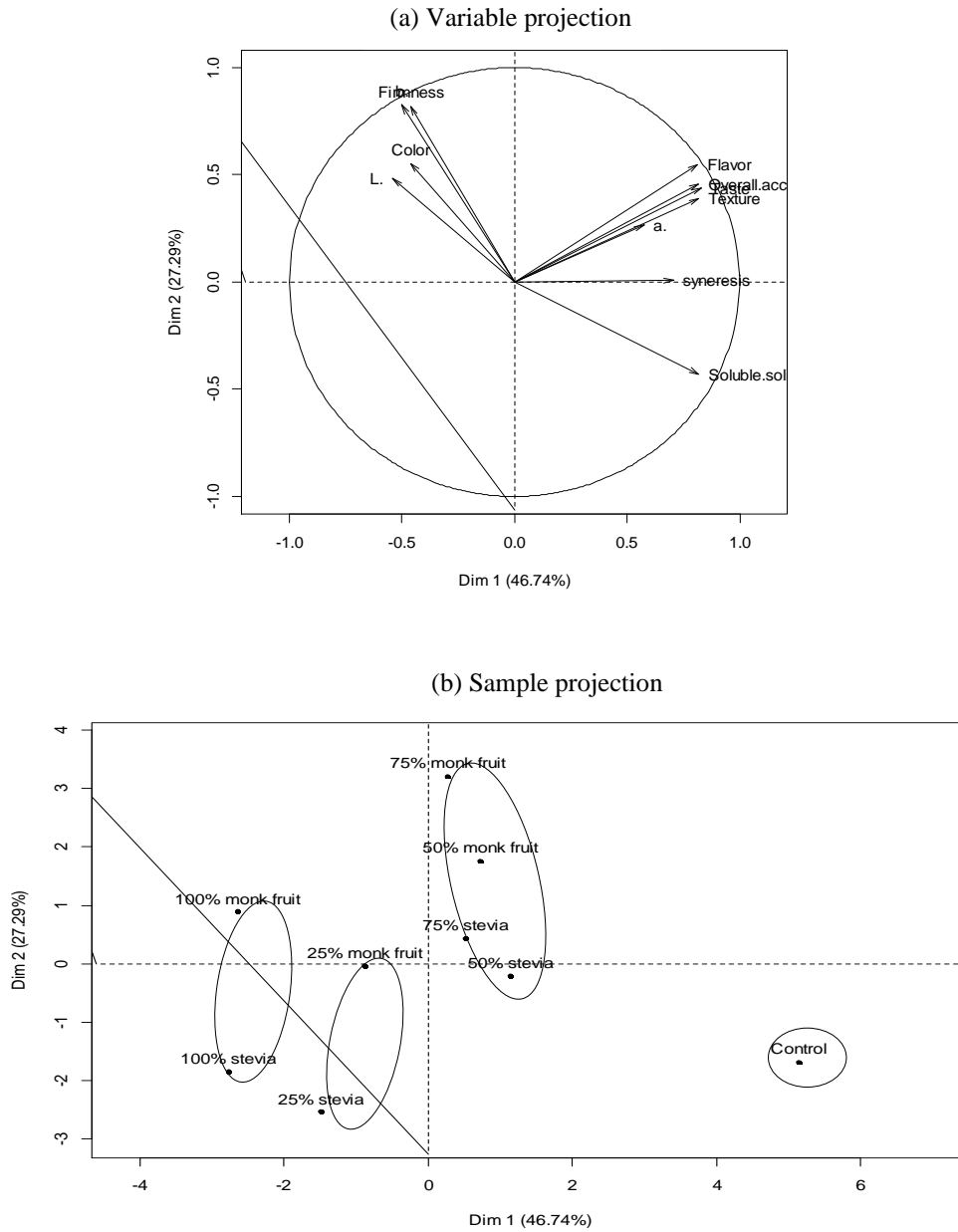


Figure 2 Preference mapping of agar jellies: (a) variable projection and (b) sample projection

b. Preference mapping

Figure 2a shows that Dim 1 can explain 46.74% of the total variability, followed by Dim 2 with 27.29%, and thus the total is 74.03% of the total variability. The high value assured that consumers could satisfactorily discriminate among the jellies. Most sensory attributes, such as taste, flavor, texture, and overall acceptability, were positively correlated in Dim 1. Still, Dim 2 positively correlated with firmness and L^* and b^* values. The result demonstrated a clear distinction between the four groups. The first is the control, followed by samples with 50% and 75% stevia and monk fruit replacement, samples with 25% stevia and monk fruit replacement, and samples with 100% stevia and monk fruit replacement are the last, respectively (Figure 2b). It indicates a significant distribution of the prominent discrimination subjecting to the level of sugar replacement. The control, positioned in the positive quadrant, was characterized by the preferable sensory attributes (Figure 2b). Moreover, the control was also characterized by the higher soluble solids, which linked to a full-sugar recipe. This observation emphasizes the role of sugar on the agar jelly texture, as the gel is brittle with a reduced firmness value, corresponding to a highly desirable texture. The more sugar replacement, the lower the sensory perception because the sugar influences the taste and flavor of the samples. Most panelists were not accustomed to the taste quality and negative attributes, such as unpleasant aftertaste of the sweeteners, reflecting lowered their sensory evaluation. Consequently, samples with 100% sugar replacement were far from the control, showing less acceptable (Figure 2b). At the same time, the sample with 50% stevia replacement was located close to the control against other samples, implying that it was more preferred.

CONCLUSION

Stevia and monk fruit can be used to partially replace sugar content in green tea agar jellies. The level of sugar replacement was significant on the quality of the jellies. Total soluble solids and syneresis were decreased in reduced-sugar samples, but instrumental firmness increased. The combination of sugar and stevia or monk fruit produced a mixture that has a unique taste and flavor. Jellies with stevia and monk fruit had more transparency and lower scores for taste and flavor than the control. Panelists preferred the samples with 50% and 75% sugar replacement with stevia or monk fruit to those with 25% replacement. In contrast, they denied the samples with 100% replacement. This result suggests basic information on development of reduced-sugar jellies made with stevia or monk fruit. The storage stability of these jellies should be further study.

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PRELIMINARY STUDY OF FISH OIL FROM MILKFISH SATAY BY PRODUCT USING DRY RENDERING EXTRACTION

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ABSTRACT

Milkfish satay processing has been left viscera waste that may causes environmental pollution. The viscera waste has contained omega-3 which can be extracted as fish oil. Dry rendering is a method of fish oil extracting using temperature without water addition. The temperature of extraction greatly affects to quality of fish oil. The purpose of this study was to determine the optimal temperature of extraction and characterized fish oil quality extracted from milkfish viscera. This study used dry rendering extraction methode with three different temperatures (40°C, 50°C, and 60°C) and tested the yield, free fatty acids, peroxide value, p-anisidine and total oxidation, for the best fish oil will be tested for its fatty acid profile. The best treatment for extracting fish oil from milkfish viscera used extraction temperature of 50°C with yield (6.88%), free fatty acid (4.89%) peroxide value (29.35 mEq/kg), anisidine value (4.61 mEq/kg), and total oxidation (63.53 mEq/kg). The fatty acid profile of fish oil was dominated by palmitic acid (31.17%) and also contains omega-3 such as linoleic acid, docosahexaenoate acid (DHA), and eicosapentaenoate acid (EPA).

Keywords: dry rendering extraction, milkfish visceral, temperature

INTRODUCTION

Serang City, Banten is one of the centers for milkfish product precessing with milkfish raw material that needs 133.14 tons/year (DKP 2017). One of the populer products is milkfish satay. The milkfish satay processing produced viscera waste. According to DKP (2017) the amount of viscera waste that produced in milkfish satay processing in Serang were of 49.6 kg/day.

Milkfish satay processing waste has not used optimally, if not managed properly will have a negative impact on the environment. The most effective and efficient alternative to solve this problem is optimizing the utilization of viscera waste to be fish oil. Suseno (2014) viscera of fish contain omega-

3 such as Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA).

Fish oil is a fat component in fish body tissue and produced by extraction, one of the fish oil extraction methods is the dry rendering method which uses temperature treatment without the addition of water (Estiasih 2009). According to Putra (2018), temperature greatly affects the fish oil extraction process and the use of high temperatures (>70°C) can cause quality degradation that affects the quality of fish oil that does not comply with the International Fish Oil Standard (IFOS (2011)). The purpose of this study was to determine the optimal temperature and characterize the quality of fish oil extraction from milkfish viscera.

MATERIALS AND METHODS

Materials

Viscera of milkfish collected from the processing industry of milkfish satay, located at Serang, Banten. The chemical material used in this study are ethanol 96%, n-heksan (Merck), phenolphthalein indicator, aquades, NaOH (Merck) 0.1 N, chloroform (Merck), acetic acid glacial (Merck), anisidine reagent (Aldrich chemistry), Bromine Cresol Green-Methyl Red indicator, KOH (Merck) 0.1 N, KI, starch 1%. The tools used in this study are oven, sieve, blender, knife, measuring cup, tray, aluminium foil and others.

Sample Preparation

The viscera of milkfish were taken from the milkfish satay processing industry. The viscera transported to the laboratory by being put into a cool box with the addition of ice (1:1 w/w). The sample washed with water and crushed using blender, then weighed.

Extraction Methods

This extraction used dry rendering extraction method according to Rozi *et al.* (2016). Viscera milk fish was heated in an oven with a temperature variation of 40°C, 50°C and 60°C for 8 hours and then pressed for collected the fish oil. The extract of fish oil was stored in the refrigerator for 5 minutes at 15 °C, then the fish oil was centrifuged at 4.000 rpm for 15 minutes. After that, the fish oil was weighed and put into a dark glass bottle that had been coated with aluminum foil, then stored at low temperature. The optimum extraction temperature was determined from the results of the yield test, peroxide value (PV), free fatty acid (FFA), anisidine value (p-AV) and total oxidation. The best temperature treatment would be characterized by the fatty acid profile.

Determination of Yield

The fish oil yield was calculated based on the following equation:

$$\% \text{ Yield} = \frac{\text{Fish oil weight (g)}}{\text{Viscera weight (g)}} \times 100\%$$

Determination of Peroxide Value (BSN, 2018)

Peroxide value was calculated based on the following equation:

$$\text{Peroxide value (mEq/kg)} = \frac{S \times M \times 1000}{\text{Sample weight (g)}}$$

S = Amount of sodium thiosulfate (mL)

M = Sodium thiosulfate concentration (0,01 N)

Determination of Free Fatty Acid (AOAC, 2005)

Free fatty acid was calculated based on the following equation:

$$\% \text{ Free fatty acid} = \frac{A \times N \times M}{10G}$$

A = Amount of KOH titration (mL)

N = Normality of KOH (0,1 N)

G = Sample weight (g)

M = Dominant fatty acid molecular weight

Determination of p-anisidine value (Watson, 1994)

Determination of anisidine value was calculated based on the following equation:

$$\text{Anisidine value (mEq/kg)} = \frac{25 \times (1,2 A_2 - A_1)}{\text{weight of sample}}$$

A1 = Absorbance of test solution 1

A2 = Absorbance of test solution 2

Determination of Total Oxidation Value (AOCS, 1998)

The total oxidation value was obtained by adding twice the peroxide number plus the anisidine value. The total oxidation value is calculated based on the following equation:

Total oxidation = 2PV + p-AV

PV = Peroxide value (mEq/kg)

p-AV = Anisidine value (mEq/kg)

Determination of Fatty Acid profile (AOAC, 2005)

Analysis of the fatty acid profile using a gas chromatograph. This tool uses the principle of converting fatty acids into methyl esters which are more volatile. The transformation of this research sample was carried out through methylation to obtain Fatty Acid Methyl Ester (FAME) which was then analyzed by gas chromatography.

Data Analysis

The experiment was run in triplicate with a completely randomized design. Fish oil data from dry rendering extraction (yield, PV, FFA, p-anisidine, total oxidation value) were subjected to analysis of variance (ANOVA). The differences among the data were determined using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Yield

The results showed that the difference in extraction temperature had a significant effect ($p < 0.05$) on the percentage of fish oil yield (Table 1). The higher of extraction temperature would increase the yield of fish oil produced. This is presumably because the temperature in the heating process affects the protein denaturation of the raw material. According to Nugroho et al. (2014) high temperature heating can damage protein and cell membranes, making it easier for deposited fat to come out more easily. According to Triyono (2010) protein denaturation will occur in the heating process at a temperature of 50°C-80°C and every 10°C increase in temperature the protein denaturation rate can reach 600 times, so the higher the temperature in the extraction process the greater the yield of oil produced.

Free Fatty Acid Value (FFA)

The results showed that the extraction temperature had a significant effect ($p < 0.05$)

on the value of free fatty acids in the fish oil produced (Table 2). The value of free fatty acids of fish oil in this study does not meet the standard of free fatty acids in consumption fish oil set by IFOS (2011), which is $< 1.13\%$. Free fatty acids are produced due to hydrolysis of triglycerides so that fatty acids are released from bonds with glycerol and also caused by splitting and oxidation of fatty acid double bonds (Crexi et al. 2010; Deepika et al. 2014). Increased hydrolysis can increase the potential for damage oil so that the oil smells rancid (Kamini et al. 2016).

Table 1. Yield of viscera fish oil

Temperature (°C)	Yield (%)
40	2,88 ± 0,35 ^a
50	6,88 ± 0,32 ^b
60	7,54 ± 0,81 ^b

*Different superscript letters in the same column indicate a significant difference ($p < 0,05$)

Table 2. Free fatty acid value of viscera fish oil

Temperature (°C)	Free Fatty Acid Value (FFA) (%)
40	10,47 ± 0,36 ^c
50	4,89 ± 0,36 ^a
60	5,86 ± 0,18 ^b

*Different superscript letters in the same column indicate a significant difference ($p < 0,05$)

Table 3. Peroxide value of viscera fish oil

Temperature (°C)	Peroxide Value (mEq/kg)
40	25,58 ± 0,26 ^a
50	29,35 ± 0,76 ^b
60	46,50 ± 0,88 ^c

*Different superscript letters in the same column indicate a significant difference ($p < 0,05$)

Peroxide Value

The results showed that the extraction temperature had a significant effect ($p < 0.05$) on the the peroxide value of the fish oil produced (Table 3). The higher of extraction temperature will affect increasing peroxide

value. The fish oil peroxide value from milkfish satay waste does not meet the consumption fish oil standard set by IFOS (2011), which is 3.75 mEq/kg.

According to Aidos et al. (2002) and Suseno et al. (2015) temperature is one of the supporting factors that can accelerate the oxidation process in fish oil. The value of peroxide is very dependent on the extraction temperature, the higher the temperature used, the faster the oxidation process occurs. The higher peroxide value indicates the level of damage to fish oil. Peroxide levels are closely related to the quantity of hydroperoxides. Hydroperoxides can occur due to the presence of double bonds (unsaturated fatty acids) in the oil that binds oxygen from the surrounding air. Other factors that affect the peroxide value are the type and freshness of the raw materials used, the length of storage and the extraction method used.

Table 4. Anisidine value of viscera fish oil

Temperature (°C)	Anisidine Value (mEq/kg)
40	10,67 ± 0,87 ^b
50	4,61 ± 0,04 ^a
60	13,68 ± 0,94 ^c

*Different superscript letters in the same column indicate a significant difference (p<0,05)

Table 5. Total oxidation value of fish oil

Temperature (°C)	Total oxidation value (mEq/kg)
40	61,90 ± 0,48 ^a
50	63,53 ± 0,96 ^b
60	107,36 ± 0,83 ^c

*Different superscript letters in the same column indicate a significant difference (p<0,05)

Anisidine Value

The results showed that temperature had a significant effect (p<0.05) on the anisidine value (Table 4). The lowest anisidine value was obtained at the extraction temperature of 50 oC. The anisidine value in all fish oil produced in this study met the IFOS (2011) standard, which is 15 mEq/kg.

The anisidine value is an indicator of secondary oxidation, so the higher the peroxide value produced from the primary oxidation process, the faster it decomposes into secondary oxidation products. (Panagan et al. 2012, Deepika et al. 2014).

Total Oxidation Value

The results showed that the extraction temperature had a significant effect (p<0,05) on the total oxidation value of fish oil (Table 5). The higher of extraction temperature causes increasing of total oxidation value. The total oxidation value in this study did not meet the consumption fish oil standard set by IFOS (2011), which is 20 mEq/kg. According to Kamini et al. (2016), the total oxidation value was used to estimate lipid oxidative damage. The total oxidation value is influenced by the primary oxidation value (peroxide number) and secondary oxidation value (anisidine). The higher of peroxide value and anisidine value causes increasing of total oxidation value.

Fatty Acid Profile

The fish oil selected for the fatty acid profile test was fish oil extracted using a temperature of 50 oC. This selection was based on the fact that extraction at 50 oC produced the best yield, free fatty acids and anisidine values. The fatty acid profile of viscera fish oil showed in Table 6. The fatty acid composition of milkfish viscera fish oil consists of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The fatty acid composition of viscera fish oil was dominated by SFA>MUFA>PUFA.

SFA are dominated by palmitic acid with the highest percentage of 31.17%. Estiasih (2009) stated that palmitic acid is one of the two dominant fatty acids and is most easily obtained in fish bodies with high enough levels, exceeding 30% or more in all types of fish.

Table 6. Fatty acid profile of viscera fish oil

Fatty acid	Percentage (%)
Capric Acid, C10:0	0,04
Lauric Acid, C12:0	1,60
Myristic Acid, C14:0	2,55
Pentadecanoic Acid, C15:0	0,27
Palmitic Acid, C16:0	31,17
Heptadecanoic acid, C17:0	0,20
Stearic Acid, C18:0	6,59
Arachidic Acid, C20:0	0,20
Heneichocyclic Acid, C21:0	0,02
Behenic Acid, C22:0	0,08
Tricosanoic Acid, C23:0	0,02
Total SFA	42,74
Myristolic Acid, C14:1	0,05
Palmitoleic Acid, C16:1	5,42
Cis-10-Heptadecanoic Acid, C17:1	0,23
Elaidic Acid, C18:1n9t	1,14
Oleic Acid, C18:1n9c	23,56
Cis-11-Eicocenoic Acid, C20:1	2,08
Erucic Acid, C22:1n9	0,13
Nervonic Acid, C24:1	0,04
Total MUFA	32,65
Linoleic Acid, C18:2n6c	6,38
Y-Linolenic Acid, C18:3n6	0,15
Linolenic Acid, C18:3n3	0,83
Cis-11,14- Eicosedienoate Acid, C20:2	0,57
Cis-8,11,14- Eikocetrienoate Acid, C20:3n6	1,12
Arachidonic Acid, C20:4n6	0,52
Cis-13,16- Docosadienoate Acid, C22:2	0,03
Cis-5,8,11,14,17- Eicosapentaenoate Acid, C20:5n3	0,74
Cis-4,7,10,13,16,19- Docosahexaenoate Acid, C22:6n3	0,32
Total PUFA	10,66
Total identified fatty acids	86,04
Total unidentified fatty acids	13,96
Total omega-3	1,89
Total omega -6	8,17
Total omega -9	24,83

MUFA are dominated by oleic acid (23.56%) which is included in omega 9 fatty acids. Oleic acid (MUFA) has a better role than omega 3 and omega 6 in lowering blood cholesterol levels. Oleic acid plays a role in lowering LDL blood cholesterol and

increasing HDL blood cholesterol (Sartika 2008).

Omega 3 and omega 6 are included in PUFA fatty acids. Omega 6 is dominated by linoleic acid with a percentage of 6.38%. Omega 6 plays an important role in fat transport and metabolism, immune function,

maintaining the function and integrity of cell membranes (Sartika 2008). Omega 3 is dominated by linolenic acid by 0.83%. Omega 3 plays an important role for intellectual development (Suseno and Saraswati 2015).

Omega fatty acids in fish oil from milkfish viscera consist of omega 9 (24.83%), omega 6 (8.17%) and omega 3 (1.89%). Based on these results, the highest percentage is omega 9 fatty acid. In accordance with Hafiludin's research (2015) the composition of omega fatty acids in milkfish is dominated by omega 9 fatty acids. Factors that affect the fatty acid content of milkfish are the type of food and fish habitat.

CONCLUSION

The best treatment for extracting fish oil from milkfish viscera used extraction temperature of 50 °C. This was because the extraction using a temperature 50 oC produced the best yield, free fatty acids and anisidine values. Fish oil from milkfish viscera was dominated by palmitic acid and still contains relatively small amounts of omega-3 such as linoleic acid, docosahexaenoate acid (DHA) and eicosapentaenoate acid (EPA).

The fish oil in this study needs to be further purified, so that its quality can be improved and meet the fish oil standards set by IFOS.

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ORGANOLEPTIC CHARACTERISTIC OF BROWNIES FROM MOCAF AND GREEN BEAN FLOUR

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ABSTRACT

The development of local food is one of the programs being promoted by the ministry of agriculture and as an effort to diversify food in the midst of the Covid-19 Pandemic. One of the local food commodities developed was cassava. Based on data from the Central Statistics Agency related to cassava productivity in Indonesia (2014 – 2018) it was in the range of 229.51 – 246.50 tons/year. the average percentage of flour consumption per capita from 2014 –2018 is 19.92%. In addition, the cassava industry had also begun to develop, namely the processing of cassava into Modified Cassava Flour (Mocaf). Organoleptically, the value of Mocaf (in terms of aroma and taste) was almost equivalent to wheat. (Nesia, 2009). One way to control the value of imported flour is by developing products based on local food ingredients. Based on this, local food preparations based on modified cassava flour were developed, namely mocaf and fortified green bean flour as a source of protein. Processed products from cassava and green bean are able to substitute the use of flour 10-100%. This study aims to determine the effect of MOCAF formulation and green bean flour on the organoleptic characteristics of brownies. The flour formulations used for the four treatments (MOCAF wheat flour and green bean flour) were based on the ratio of the composition of each flour. Furthermore, observations were made on the organoleptic characteristics of roasted brownies consisting of color, scent, texture, taste and after taste. Organoleptic test involved 30 untrained panelists using the hedonic method. Organoleptic test results illustrate that the overall product is acceptable, both in terms of taste, aroma, color, texture and after taste. In treatment A, which is brownies with a composition ratio of flour, mocaf and green bean flour (1:3:0), the panelists are generally preferable. In Treatment C, brownies with a composition ratio of flour, mocaf and green bean flour (1:2.33:0.75) only tasted better by the panelists. This indicates that the panelists prefer the addition of green bean flour less than mocaf.

Keywords: Mocaf, green bean flour, organoleptic, brownies

INTRODUCTION

In Indonesia, the average consumption of wheat flour per capita from 2014 –2018 was 19.92%. Every year there is an increase in consumption of about 200 kg (Komalasari, 2018). This causes the import value of wheat and wheat to be high and drains the country's foreign exchange (Ariani 2010). Most of the import demand is derived from the form of

noodles, bread, and biscuits. One way to control the value of wheat imports is to develop products based on local food ingredients. Groups of local foodstuffs developed are tubers and seeds such as cassava, sweet potato, corn, green beans (Ratnawati, 2001). Cassava production in Indonesia is 246.50 tons/year (BPS, 2018). green bean production is 234,720 tons with

consumption needs of 304,000 tons per year (Pusdatin, 2018).

Based on this, local food preparations based on modified cassava flour were developed, namely Modified Cassava Flour (MOCAF) and fortified green bean flour as a protein source. Processed products from cassava and green beans are able to substitute the use of 10-100% wheat both in fresh and intermediate products (flour). The addition of various types of flour in addition to improving the rheological properties of flour can also increase the nutritional value of the resulting product (Mohankumar, 2009). In addition to realizing the concept of food diversification, it also creates public awareness of healthy food and the development of agro-industry made from local raw materials (Ginting, 2011).

Mocaf is an intermediate product (flour) of cassava which is processed with the principle of fermentation using LAB (Lactic Acid Bacteria). This process produces pectinolytic and cellulolytic enzymes as well as lactic acid, so that the characteristics of the final product are similar to wheat flour. In addition, MOCAF does not contain gluten, so it is safe to consume for people with autism (Subagio, 2010). The texture of the mocaf is smooth, the color is whiter, and it doesn't smell musty like cassava flour in general. The source of cassava used can also affect the characteristics of mocaf, such as the Genotype Butter 2 which has a more yellow color characteristic (the presence of beta carotene content is around 52 mg/kg) than mango (Fathonia, 2016).

Green bean (*Vigna radiata* L.) contains good carbohydrates, protein and fiber (Kenawi, 2009). The protein content in green beans is 22.9% and has a digestibility of 81%. Green bean protein is rich in the amino acid lysine but contains a small amount of sulfur amino acids (methionine and cystine) (Astanto, 2006). The use of green beans in

processed foods can increase the nutritional value and taste.

One of the processed food products made from flour is brownies. Making brownies can also take advantage of local food ingredients. Brownies are an intermediate moisture food (IMF) product group with a moisture content of 10-20% (Cauvain, 2006). The consumption pattern of cake/bakery that is very popular with the people of Bandung is brownies. Brownies can be processed by baking or steaming (Sumarwan, 2011). In general, the difference in processing lies in the water content. Baked brownies have a lower water content than steamed brownies, so they have a longer shelf life. Baked brownies are more savory in terms of taste, while steamed brownies are safer and healthier because free radicals are not formed due to the roasting process (Saragih, 2011).

A touch of technological innovation is needed in an effort to reduce imports of flour, diversify food and utilize local food. One of them is making brownies made from local food, namely the substitution of mocaf and green bean flour. Furthermore, this product is subjected to a sensory test, which is a test method using the human senses to measure texture, appearance, aroma, and taste. Organoleptic test aims to determine consumer acceptance or preference for a product (Setyaningsih, 2010). The purpose of this study was to determine the organoleptic properties of brownies from mocaf and green bean flour.

MATERIALS AND METHODS

This research has been carried out at the Food Engineering and Processing Laboratory, Food Technology Study Program, Sultan Ageng Tirtayasa University.

Materials and Tools

The materials used for made brownies were flour, sugar, egg, margarine, cocoa

powder, chocolate bar, milk powder, salt, baking soda and SP. While type of flour used are whea, MOCAF and green bean flour. Other materials used The tools used in this research include tools for made brownies were oven, mixer, scale, baking sheet, measuring spoon, bowl, measuring cup, and others. Procedures and formulations for made brownies refer to Setiawati's research (2015). The treatment formulation in this research consisted of four formulations. Formulations are presented in Table 1.

Table 1. Research Treatment Formulation

Treatment	Flour formulation per 300 grams		
	wheat flour	mocaf	green bean flour
A	75	225	0
B	75	200	25
C	75	175	50
D	75	250	75

Methods

The stages of the research have been carried out in two stages, namely the stages of made brownies and organoleptic test. Organoleptic test was carried out by the hedonic test method. This method was the most widely used test to measure the level of preference for the product. The method was designed to directly select one product among other products. Organoleptic parameter includes changes that occur in color, aroma, texture, ta ste and after taste (Setyaningsih, 2010). This test was carried out by 30 panelists from students of the Sultan Agung Tirtayasa University. The scale used is a number from 1 to 5, where 1 = very dislike, 2 = dislike, 3 = netral, 4 = like, 5 = very like. The data has been processed using Microsoft Excel 2010. Sensory test results data were analyzed descriptively using the average value and the percentage of panelists' acceptance of the brownies formula.

RESULTS AND DISCUSSION

Organoleptic Test

Organoleptic tests use the hedonic method to explain the characteristics of the preferred product and describe the expected product characteristics (Nurmalasari, 2019). The research data illustrates that the product as a whole can be accepted, both in terms of taste, aroma, color, texture and after taste. In treatment A, namely brownies with a composition ratio of flour, mocaf and green bean flour (1:3:0) the panelists generally preferred. It's just that the average value in terms of taste is not liked but can be tolerated in terms of after taste. Treatment formulation A used MOCAF without green bean flour so that it produced a neutral taste like wheat flour or brownies in general. In Treatment C, brownies with a ratio of flour, mocaf and green bean flour (1:2.33:0.75) were only preferred by the panelists in taste. This shows that the panelists prefer the addition of green bean flour less than mocaf. The formulation of green bean flour that does not dominate, makes the brownie taste soft with the characteristic of green bean. In addition, if the composition of mung bean flour is added more then the level of preference for brownie texture decreases. The graph of the hedonic quality test of mocaf brownies and green bean flour is presented in Figure 1.

Color is the main component in determining the level of consumer acceptance of the overall product (Winarno, 2004). Color affects the assessment of the characteristics and quality of a food (Setyaningsih, 2010). Brownies color is usually influenced by the ingredients and the roasting process. The level of color produced in this study is more influenced by differences in the amount of use of green bean flour. The dark brown color which was less glossy in treatment C was not liked by the panelists. The average range of preference values for color is between 2.63 – 3.43 which is included in the neutral category

and tends to like. The average preference value for color brownies can be seen in Table 2.

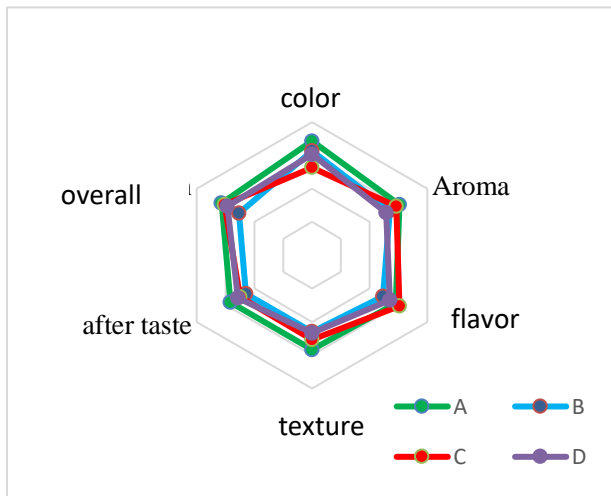


Figure 1. Graph of hedonic quality test of brownies from mocaf and green bean flour

Table 2. Average score of panelists' preference for brownies form mocaf and green bean flour

Treatment	Average score of panelists' preference for brownies					
	Color	aroma	Flavor	texture	After Taste	overall
A	3.43	3.03	2.9	2.83	2.93	3.13
B	3.13	2.7	2.47	2.3	2.3	2.53
C	2.63	2.93	3.03	2.53	2.5	3.03
D	3.03	2.57	2.7	2.33	2.57	2.93

Scale: 1 = very dislike, 2 = dislike, 3 = netral, 4 = like, 5 = very like

Aroma brownies has an average range of preference values between 2.57 – 3.03 including in the slightly neutral category. The strong and unpleasant aroma of green beans in treatment D was not liked by the panelists. Assessment of the characteristics of the scent is identical to the aroma of volatile compounds or certain products known (Setyaningsih, 2010). Panelists who are accustomed to consuming green bean will prefer the distinctive aroma of green bean flour. The average preference value for scent brownies can be seen in Table 2.

Taste is the most important factor in the final decision of the panelists to accept or reject a food (Kusumaningrum, 2016). Determination of food taste is influenced by the product formulation (Fellows 2000). The average range of preference values for the flavor brownies is 2.47 – 3.03 including in the slightly neutral category. The average value of preference for brownies can be seen in Table 2. From the average value for taste, it can be seen that the brownie formulation with the addition of green bean flour affected the panelists' preferences.

The texture of the brownies preferred by the panelists in this study was soft, sturdy and not easy to melt in the mouth. Texture in bakery products is a critical parameter in appearance, taste and overall acceptance (Setser, 1995). The average preference value for texture brownies can be seen in Table 2. The average range of preference values for brownies texture is 2.30 – 2.83 including in the less like category. The amount of flour used greatly affects the texture of baked brownies. The more the amount of flour, the stronger the texture of the brownies produced (Setyani, 2017). In addition, if the amount of green bean flour is increased, the level of preference for brownies texture will decrease. Green bean juice dregs affect the resulting texture because of the rough after taste in the esophagus. The maximum limit for panelists' acceptance of the addition of mung bean juice is 50% of the total use of mocaf and wheat flour (Kusumaningrum, 2016).

After taste is a stimulant substance that gives the impression that it is easy or not easy to lose after a product is finished being consumed (Widiantoko, 2014). The average range of preference values for after taste brownies is 2.30 – 2.93 including in the category of dislike close to neutral. The addition of green bean flour in the treatment showed an effect on the after taste of the

addition of green bean flour in each treatment.

CONCLUSION

In terms of overall, the panelists preferred treatment A brownies, while in terms of taste, panelists preferred treatment C. This shows that the panelists prefer the addition of green bean flour less than mocaf. This value indicates that the brownie product in this study can be accepted by consumers with a neutral and good preference score.

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CHARACTERIZATION OF DRIED NOODLES FROM SEAWEED (*Kappaphycus alvarezii*) AS POTENTIAL SUBSTITUTE FOR WHEAT FLOUR

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ABSTRACT

The main ingredient of dry noodles is wheat flour which made from milling wheat. However, we usually obtain wheat from overseas by import. Therefore, to achieve food resilience in Indonesia, it is necessary to find substitutes for local ingredients with high nutrition that can be used to make dry noodles. This research aims to investigate the characteristics of dry noodles made from *Kappaphycus alvarezii* seaweed flour. The method used in this study was to substitute *K. alvarezii* seaweed flour as much as 0, 5, 10, 15, 20, and 25% in the production of dry noodles. Analysis was carried out on its chemical composition, physical properties, and organoleptic characteristics. The results showed the best result of substitution of *K. alvarezii* in the 5% treatment. The chemical composition in dry noodles of this treatment showed water content of 8.39%; ash content 3.07%; fat content 0.59%; protein content 12.43%; and 0.29% crude fiber content. Meanwhile, the results of the physical test showed 7.95% cooking loss; 210.5 seconds cooking time; and 89.34% water absorption. The organoleptic values obtained are color 4.60 (like); fragrance 4.80 (like); texture 4.23 (like); and taste 4.20 (like).

Keywords: *Kappaphycus alvarezii*, seaweed flour, dry noodle

INTRODUCTION

Dried noodles are one of the favorite food products in Asia because it has good nutritional content, inexpensive, and practical or easy to consume. In Indonesia, dry noodles have been consumed by all levels of society and become one of staple ingredient other than rice (Juniawati, 2003). Dry noodles are made from wheat flour which comes from milling wheat seeds. Indonesia's climate which is not suitable for the growth of wheat crops makes Indonesia have to import wheat to meet people's demand. In 2019, Indonesia's wheat imports reached 10 million tons. This causes Indonesia to become the largest wheat importing country in the world (FAO, 2020).

The high number of wheat imports is also due to the fact that many food products in Indonesia use wheat as their raw material (Saajidah & Sukadana, 2020). The high number of import needs to be a concern because if there is an escalation price of wheat, then dried noodles producers will experience difficulties. Wheat flour substitute material is needed to reduce Indonesia's dependence on imported raw materials and also to create food resiliency.

Seaweed is one of the important commodities in Indonesia. It has a complete nutritional content, including vitamins, essential amino acids, fatty acids, and dietary fibers (Roohinejad et al., 2016). As a good source of nutrition, seaweed have been used

as many food products, such as *nori*, pickles, pasta, or salad dressings. Seaweed can also be processed into seaweed flour, such as *Gracillaria* spp. (Aditia et al., 2021).

Kappaphycus alvarezii is a seaweed that has commercial value and is an Indonesian export commodity. *K. alvarezii* is usually used as a natural preservative because it contains antioxidants and antibacterials (Tjahyaningsih et al., 2013). In addition, *K. alvarezii* also contains carrageenan so that it can be used as a thickener, stabilizer, and thickener (Williams & Philips, 2008). Based on these characteristics, *K. alvarezii* has the potential to be developed into flour for the manufacture of dry noodles. Carrageenan from *K. alvarezii* (Ulfah, 2009) and also combination between *K. alvarezii* and *Elops hawaiiensis*'s fish meat (Setiawati, 2019) has been previously used to enhance the elasticity of dried noodles. However, the proper formulation of *K. alvarezii* to produce good noodles have no't been found. Therefore, it is necessary to conduct research on the formulation of seaweed flour from *K. alvarezii* and the characteristics of the dry noodles produced from the substitution.

MATERIALS AND METHODS

Tools and Materials

The tools used in this study were dry noodle cast, analytical scale (Boeco BBI-32), drum dryer, disk mill (model FFC 15), oven (Cosmos CO-958), 100 mesh filter, Soxhlet

extractor, whiteness meter, and *Kjeltec* machine.

The main ingredient in this study was 30-days-old dry seaweed *Kappaphycus alvarezii* obtained from Lontar, Serang Regency, Banten. Other materials used in this study were wheat flour, salt, water, eggs, carboxyl methyl cellulose (CMC), baking soda (NaHCO₃), 5% calcium oxide (CaO), and distilled water.

Methods

Seaweed Flour Production

The method of making seaweed flour was modified from Agusman et al. (2013). Dried seaweed *K. alvarezii* was soaked in fresh water for 24 hours, then drained. *K. alvarezii* was then immersed in 5% CaO solution for 5 hours, then rinsed and drained. After that, it was milled using a blender and dried using a drum dryer. Finally, *K. alvarezii* seaweed flour was sieved using a 100-mesh sieve. After that, chemical characteristics of dried seaweed flour was analyzed. It consists of water, ash, fat, crude fiber, and protein content.

Dried Noodle Production

The initial stage of making dry noodles is making a dough consisting of *K. alvarezii* flour, wheat flour and other ingredients. In this study, 5 different seaweed flour formulatios were made, namely 0% (control), 5%, 10%, 15%, and 20%. The formulation of dry noodle dough with the substitution of *K.*

Table 1. Dried noodles formulation with substitute of *Kappaphycus alvarezii* flour

Composition	Treatment					
	Control (0%)	5%	10%	15%	20%	25%
<i>Kappaphycus alvarezii</i> flour (g)	0	5	10	15	20	25
Wheat flour (g)	100	95	90	85	80	75
Water (ml)	58	58	58	58	58	58
Salt	1	1	1	1	1	1
CMC (g)	1	1	1	1	1	1
Egg (g)	5	5	5	5	5	5
Baking soda (g)	1	1	1	1	1	1

alvarezii flour is presented in table 1. Next, the dough is milled and molded into noodles. The noodles that have been printed are then steamed at 100°C for 10 minutes and then drained. After that, noodles were dried using an oven at 100°C for 1 hour.

Data Analysis

Dry noodles analysis consisted of chemical composition, physical properties, and organoleptic tests. Chemical composition analysis carried out on dried noodles were its content of water, ash, protein, fat (BSN 2006), and crude fiber (BSN 1992). Physical properties analysis that carried out includes cooking time, cooking loss (Oh et al., 1985), whiteness degree, and water absorption power (Rasper & de Man, 1980). Last, organoleptic test adopted from Setyaningsih et al. (2010) was conducted with hedonic test for its color, texture, aroma, and flavor.

This research was conducted using a completely randomized design (CRD) method with two repetitions / duplo. Data were analyzed using one-way ANOVA and Duncan's test. Meanwhile, the data from the organoleptic test were analyzed using the Kruskal Wallis test and Duncan's test.

RESULTS AND DISCUSSION

Chemical Characteristics of Seaweed Flour

Chemical characteristics of dried seaweed flour are shown in Table 2. It was composed of whiteness, crude fiber, water, ash, fat, and protein content. The results showed that *K. alvarezii* flour had a higher water content than BSN standard. This was due to the short drying time. It also had higher ash content than BSN standard. According to Mayer et al (2011), ash content in seaweed is much higher than in terrestrial plants, such as wheat.

Chemical Composition

Chemical composition analysis results for dried noodles are shown in Table 3. It

consists of water, ash, protein, fat, and crude fiber content.

Table 2. Chemical characteristics of dried seaweed flour

Characteristics	BSN standard	<i>K. alvarezii</i> flour
Whiteness (%)	White	Yellowish-white
Crude fiber (%)	-	5,43 ± 0,47
Water (%)	≤ 14,50	17,56 ± 0,15
Ash (%)	≤ 0,70	16,90 ± 0,11
Fat (%)	-	0,13 ± 0,01
Protein (%)	≥ 7,0	4,07 ± 0,12

a. Water Content

The results showed that all treatments had a higher water content than control. The lowest water content was found in the 5% treatment, which was 8.39%. The higher the concentration of *K. alvarezii* flour used, the higher the water content. This is presumably because seaweed has a high water insoluble fiber content, hence water will be bound and trapped in the matrix (Nafiah, 2011). Even so, BSN (2015) applies a good standard of moisture content in dry noodles is ≤ 10%. So, the dry noodles with *K. alvarezii* flour at concentrations of 5 and 10% still meet the standards. The water content of a food product will affect its durability, quality, and texture.

b. Ash Content

Ash content shows the mineral contained in a food product, such as calcium, potassium, phosphorus, iron, and others. In this study, all treatments had higher ash content than controls. According to Santoso et al. (2013), seaweed *K. alvarezii* has high mineral and salt content which will be left after the dry ashing process. The higher the concentration of *K. alvarezii* flour used, the greater the ash content. The lowest ash content was found in the 5% treatment, which was 3.07%. However, this value does not

meet the BSN standard (2015) which stipulates that a good ash content in dry noodles is 3%.

control. The highest fiber content was found in the 25% treatment, which was 1.41%. This is because seaweed is a high source of dietary

Table 3. Chemical composition of dried noodles

Composition	Treatment						BSN standard
	Control	5%	10%	15%	20%	25%	
Water (%)	5.64 ^a	8.39 ^b	9.26 ^c	11.4 ^d	14.79 ^e	17.69 ^f	≤ 10%
Ash (%)	2.11 ^a	3.07 ^b	3.97 ^c	4.65 ^d	5.88 ^e	6.15 ^e	3%
Protein (%)	12.57 ^c	12.43 ^c	11.81 ^b	11.59 ^b	11.39 ^b	10.84 ^a	≥ 8%
Fat (%)	0.62	0.59	0.52	0.50	0.42	0.39	-
Crude fiber (%)	0.16 ^a	0.29 ^a	0.38 ^a	0.81 ^b	1.32 ^c	1.41 ^c	-

Note: Value with different notation in the same column has a significant differences at 5% (Duncan’s test)

c. Fat and Protein Content

Dried noodles with *K. alvarezii* flour substitution had lower fat content than the control. This is because in general, seaweed has a lower fat content compared to terrestrial plants, which is <4% (Ortiz et al., 2012). In this study, the highest fat content was found in the 5% treatment, which was 0.59%. Similar to fat content, dry noodles with *K. alvarezii* flour substitute also had lower protein content than control. The highest protein content was found in the 5% treatment, which was 12.43%. According to BSN (2015), a good protein content in dry noodles is 10%. Therefore, although the protein content in the treatment was lower than the control, the protein content in all treatments had met the standards set by BSN.

fiber, which is 78.94% (Astawan et al., 2004). This is also shown by the higher concentration of *K. alvarezii* flour used, the higher the crude fiber content. Crude fiber can help the digestive process in humans. The higher the fiber content, the shorter the time required for the digestive process (Dhingra et al., 2012).

Physical Properties

Physical properties analysis results are shown in Table 4. It consists of cooking time, cooking loss, and water absorption power.

a. Cooking time

The calculation of cooking time refers to the time it takes for dry noodles to rehydrate so that the texture becomes supple and elastic as before it was dried. Short cooking time

Table 4. Physical properties of dried noodles

Physical Properties	Treatment					
	Control	5%	10%	15%	20%	25%
Cooking time (s)	199.50 ^a	210.50 ^b	216.00 ^c	219.50 ^d	223.00 ^e	227.50 ^f
Cooking loss (%)	7.14 ^a	7.95 ^b	9.04 ^c	9.88 ^d	10.82 ^e	11.13 ^e
Water absorption (%)	95.29 ^f	89.34 ^e	88.23 ^d	86.3 ^c	83.20 ^b	80.48 ^a

Note: Value with different notation in the same column has a significant differences at 5% (Duncan’s test)

d. Crude Fiber Content

The results showed that the fiber content in all treatments had a higher value than

will be preferred by consumers because it is more practical. In this study, dry noodles substituted with *K. alvarezii* seaweed flour

with a concentration of 5% had the shortest cooking time, which was 210.5 seconds. Even so, the value of this cooking time is still lower than the control, which is 199.5 seconds. The length of cooking time in the treatment is due to the fact that seaweed has a high fiber content so that it can inhibit the gelatinization process and slow down the cooking time (Irviani & Nisa, 2015). This is also shown by the higher the concentration of seaweed flour, the longer the cooking time.

drying process which is indicated by the level of swelling of noodles (Safriani et al., 2013). If the value of water absorption is high, then the ability of noodles to absorb water is also high indicated by a swelling noodles. The results showed that all treatments had a lower water absorption value than the control. The highest water absorption value was found in the 5% treatment, which was 89.34%. According to Sugiyono et al. (2011), one of the factors that affect water absorption in noodles is gluten. The low gluten value in dry

Table 5. Organoleptic test results of dried noodles

Sensory Test	Treatment					
	Control	5%	10%	15%	20%	25%
Color	4.07	4.60	4.77	4.57	4.57	4.43
Aroma	4.90 ^c	4.80 ^c	4.50 ^{bc}	4.07 ^{ab}	4.23 ^b	3.73 ^a
Texture	4.53 ^b	4.23 ^b	4.43 ^b	4.03 ^b	4.17 ^b	2.80 ^a
Flavor	4.37 ^{bc}	4.20 ^{ab}	4.17 ^{ab}	4.37 ^{bc}	4.87 ^c	3.77 ^a

Note: Value with different notation in the same column has a significant differences at 5% (Duncan's test)

b. Cooking loss

Cooking loss refers to the lost amounts of solids (starch) during the cooking process. A high cooking loss value indicates that the intermolecular bonds in the noodles are getting weaker so that the noodles become more easily broken (Widiatmoko & Estiasih, 2015). In this study, the smallest value of cooking loss was found in the treatment with a concentration of 5%, which was 7.95%. The higher concentration of *K. alvarezii* flour used, the higher the cooking loss value. The value of cooking loss in all treatments was higher than the control, which was 7.14%. This is because wheat flour has the ability to form gluten which is formed from gliadin and glutenin complexes (Widianto et al., 2002) while seaweed flour does not have the ability to do so.

c. Water absorption power

Water absorption is the ability of dry noodles to reabsorb water after undergoing a

noodles made from seaweed flour causes the noodle mass to become less dense so that the water absorption value also become low.

Organoleptic Test

The most preferred dried noodles for panelists is the treatment of 5% *K. alvarezii* flour. The results of organoleptic test is shown in Table 5.

a. Color

The initial characteristic seen by consumers in choosing a food product is color. The results of organoleptic testing on the panelists showed that the 10% treatment had the highest level of preference, which was 4.77 (like). The higher the concentration of *K. alvarezii* flour used, the lower the panelists' preference level. However, all samples of dried noodles with *K. alvarezii* flour substitution had a higher level of preference than the control, which was 4.07 (like). The color of dried noodles from *K.*

alvarezii seaweed flour is yellowish-white with a whiteness level of 80.50% (Figure 1). This is in accordance with the characteristics of BSN (2015) which states that good dry noodles have a yellowish-white color.

b. Aroma

Aroma is a factor that is also important for consumers to choose a product. The results showed that the most preferred aroma of dried noodles with *K. alvarezii* flour substitution was in the 5% treatment with a value of 4.80 and was not significantly different from the control. The higher the concentration of seaweed flour used, the lower the value of the level of preference for aroma. The 25% treatment had the lowest level of preference, namely 3.73 (rather like). This is because seaweed has a distinctive fishy odor from sea water. The distinctive odor can be disguised through better processing, such as soaking, heating, or adding other substances (Ulfa, 2009).



Figure 1. The color of *K. alvarezii* flour and dry noodle

c. Texture

Texture is a characteristic that can affect the image of a food. The dry noodle texture assessment showed that the most preferred substitution of *K. alvarezii* flour was at a concentration of 10% with a value of 4.43 (like) and not significantly different from the control, which was 4.53 (like). Dried noodles with 25% seaweed concentration had the lowest preference value, which was 2.80 (rather dislike). This is presumably because seaweed flour has a high

fiber content, causing the dry noodle structure to become brittle and break easily (Supriadi, 2004).

d. Flavor

The most important characteristic of a food product is taste. The results showed that the most preferred concentration of *K. alvarezii* flour substitution by the panelists was at a concentration of 20% with a value of 4.87 (like). This value is higher than the control, which is 4.37 (like) but not significantly different. This is because seaweed has a bland taste so it does not affect the taste of dried noodles (Hudaya, 2008). However, the addition of a concentration that is too high can cause a fishy odor which can also affect the taste. This is indicated by the low level of preference for the 25% treatment, which is 3.77 (rather like).

CONCLUSION

In accordance with the objectives of the study, which is to investigate the chemical characteristics, physical properties, and panelists' preference of dried noodles made from *K. alvarezii* flour formulations, the best formulation is 5% substitution of *K. alvarezii* flour. As for chemical composition, *K. alvarezii* dried noodles in this treatment showed 8,39% water; 3,07% ash; 0,59% fat; 12,43% protein; and 0,29% crude fiber content. Physical properties test shown 7.95% cooking loss; 210.5 seconds cooking time; and 89.34% water absorption. Moreover, the organoleptic values obtained are color 4.60 (like); fragrance 4.80 (like); texture 4.23 (like); and taste 4.20 (like).

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ACUTE TOXICITY, PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL INVESTIGATION OF AQUEOUS EXTRACT OF SODOM APPLE TREE (*Calotropis procera* Ait.F) LEAF OBTAINED WITHIN ANCHOR UNIVERSITY, LAGOS

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ABSTRACT

Calotropis procera is a species of flowering plant, in the family Apocynaceae, the investigation of *Calotropis procera* for its medicinal usefulness has not been fully documented in the literatures. This study was therefore aimed at investigating the antimicrobial activity, phytochemical screening and acute toxicity of *Calotropis procera*. The antimicrobial activity, phytochemical screening and acute toxicity of the leaf was carried out using standard methods for the analyses. The antimicrobial activity of *Calotropis procera* leaf extract for bacterial showed that *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus candidus* were resistant to the plant extract, while *Penicillium corylophilum* showed susceptibility to the plant extract. The phytochemical screening showed the presence of flavonoids, saponins and reducing sugar in the aqueous leaf extract of the leaf, while alkaloids and phlobatannins were absent in the extract of *Calotropis procera*. The acute toxicity investigation showed that *Calotropis procera* concentration at 1424 mg/kg body weight resulted in scratching and shivering but was not toxic to cause death of the mice, while lower concentrations of 142, 352, and 712 mg/kg body weight did not cause any observable negative reactions. The plant of *Calotropis procera* therefore showed that the aqueous extract of the plant contained viable phytochemicals compounds, potent antimicrobial activity to some organisms while the studied concentrations for acute toxicity were observed to be safe.

Keywords: acute toxicity, antimicrobial activity, *Calotropis procera*, phytochemical screening

INTRODUCTION

Calotropis procera is a species of flowering plant, in the family Apocynaceae that is native to North Africa, tropical Africa, Western Asia, South Asia, and Indochina (Khairnar *et al.*, 2012). It is a spreading shrub or medium-sized tree reaching 2.5 to 6 m in height. It has a profound taproot, 3 - 4 m deep, and a secondary root structure with woody sidelong roots that may quickly restore unexpected shoots when the plant is harmed (Khairnar *et al.*, 2012). The Stems are crooked and covered with a fissured corky bark, the grey-green leaves are 15 - 30 cm long and 2.5 - 10 cm broad they are decussate, obviate, and acuminate (Khairnar *et al.*, 2012). The flowers are pentamerous, little, cream or greenish white at the base and purple violet at the edges

of the lobes. The fruit is a fleshy and inflated, sub-globose, ellipsoid or ovoid, recurved follicle, 7.5 - 10 cm or more in diameter (Neto *et al.*, 2013). The Seed is light-brown, broadly ovate, flattened, 3.2 cm with silky hairs. A white milky juice is released from any cut on the plant.

Calotropis procera is a multipurpose tree, the stems yield a fiber valuable for making ropes, sacks, nets and paper (Neto *et al.*, 2013). The silky sap (latex) is eminent for its ethno-restorative properties and as a food, especially as a coagulation factor for cheese making in West Africa. *Calotropis* yields 90 t of biomass two times per year and is a potential source of renewable energy. *Calotropis* is also used as fodder. Immature pericarp, old leaves and flowers can be fed to goats, camels, and

sheep in the midst of scarcity. The latex contains toxic components that may be harmful to livestock (Mainasara *et al.*, 2011; Goyal *et al.*, 2013).

It has been observed that different degenerative diseases are rampant in the world these days, and many of the synthetic drugs are too expensive and not in the reach of the average human beings as a results of poverty level. Therefore, this study seeks to investigate the phytochemical constituents of the aqueous extract of *Calotropis procera* leaf and the acute toxicity effect on mice with the aim of establishing the plant viability for drugs development and human health benefits.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of *Calotropis procera* was obtained within Anchor University environment, Ayobo-Ipaja, Lagos State in October 2020. It was authenticated and identified by the Chief Technologist in Department of Plant Science and Technology, Faculty of Science, Ekiti State University, Ado-Ekiti, Nigeria with voucher number 2020083.

Aqueous Extraction of *Calotropis procera* Leaves

After collection of plant, the leaves were hand plucked aseptically and was air dried in the laboratory for 3-4 days, then it was grounded using electric blender, for ratio 1 g of sample into 10 mL of distilled water, 20 g of powdered sample was weighed into 100 mL of distilled water which was mixed using mechanical shaker overnight, it was then filtered using muslin cloth and also filtered with No. 1 Whatman filter paper, then the extract was kept in a corked bottle for analysis.

Phytochemical Analyses

Test for Phlobatannins

4 drops of 1% solution of hydrochloric acid was added to 10 drops of *Calotropis procera* aqueous leaf extract, it was heated on hot plate. Formation of red colored precipitate indicates a positive result, and presence of phlobatannins (Goyal *et al.*, 2013).

Test for reducing sugar

1ml of ethanol was mixed in 5ml of *Calotropis procera* leaf extract. After that, 1ml of Fehling solution A and 1ml of Fehling solution B were mixed in a test tube and boiled at 100⁰C. It was then poured in the aqueous ethanol mixture. Presence of colour change shows a positive result (Adebayo *et al.*, 2015).

Test for flavonoids

2.5 ml of ammonia and 1ml of concentrated sulphuric acid was added to 5ml of *Calotropis procera* leaf extract. Indication of yellow colour indicates the presence of flavonoid in the plant sample (Adebayo *et al.*, 2015).

Test for alkaloids (Wagner's test)

5 drops of Wagner's reagent was added to 30 drops of *Calotropis procera* leaf extract. The formation of a reddish-brown precipitate indicates positive result (Mainasara *et al.*, 2011).

Test for Saponins (Foam test)

10 drops of distilled water was added to 20 drops of *Calotropis procera* leaf extract, then it was vigorously shaken for persistent foam. The formation of foam indicates positive result (Adebayo *et al.*, 2015).

Antimicrobial Activity

20 g of the leaf was weighed and blended with 100 mL of distilled water and filtered firstly with sterile muslin cloth, and also filtered with No. 1 Whatman filter paper to obtain the aqueous extract (Nenaah & Ahmed, 2011).

Microbial isolation

Standard clinical isolates of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus candidus*, *Aspergillus niger* and *Penicillium corylophilum* was obtained from the Nigerian Institute of Medical Research, Yaba, Lagos and the Department of Biological Sciences, Anchor University Lagos. Nutrient agar (NA) was used for the culture of bacteria at 37⁰C and potato dextrose agar (PDA) was used to culture the fungi at 28⁰C.

Introduction of the extract and antimicrobials into the isolates

Sterile discs were introduced into 2 ml of the extract, to be adsorbed/impregnated with the extract. Discrete colonies of the isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland standard suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared Mueller-Hinton agar plates prepared according to manufacturer's instructions. The extract impregnated discs were placed on the agar plates and incubated at 37°C and 28°C for 18-24 hours for bacteria and fungi respectively.

The antibiotic discs used were: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxicillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) for Gram positive isolates. while SXT; Septrin (30µg), CH; Chloramphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxicillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg) for Gram negative isolates. After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition (clear zone) was measured in millimeter (mm). The interpretation of the measurement was recorded as sensitive (>16mm), intermediate (11- 15mm) and resistant(<11mm) (Mainasara et al., 2011; Adebayo et al., 2015).

Acute Toxicity Investigation

Dilution was done on the extract of dried sample as described in the Table 1.

Sixteen (16) Swiss Albino mice were used in this experiment, they were housed in the Anchor University, Lagos Animal House, with average weight of 19.30 g, which were randomly assigned into four (4) groups. By

Table 3. Antibacterial effect on aqueous extract of *Calotropis procera* compared with standard antibiotics disc for gram positive bacterial.

No	Organism	<i>Calotropis procera</i> extract	Gram positive antibiotic disc								
			PEF	CN	APX	AM	R	CP	S	SXT	E
1	<i>Streptococcus pyogenes</i>	R	R	S	R	R	R	R	R	R	R
2	<i>Staphylococcus aureus</i>	R	S	S	S	R	S	S	S	S	S

oral administration, the control group received 0.2 % distilled water in the same volume, while the treated group received *Calotropis procera* leaf extract at 150, 350, 700 and 1400 mg/kg body weight using oral gavage feeding needle. The general physical observations started after the mice were given dosage, records were taken for at least 1 hour interval for 24 hours, physical observations like shivering, appetite, mortality and so on. Mortality of mice will show the toxicity of *Calotropis procera* leaf extract (Bagri et al., 2013).

Table 1. Sample Dilution Concentration

Concentration	Distilled H ₂ O	Sample Extract
1	0.90	0.10
2	0.75	0.25
3	0.50	0.50
4	0	1.00

RESULTS AND DISCUSSION

Phytochemical Activity of *Calotropis procera*

Different chemical tests were carried out on the aqueous extract of *Calotropis procera* to screen for the phytochemical constituents.

Table 2. Phytochemical screening result

Parameters	Result
Alkaloids	-
Flavonoids	+
Saponins	+
Reducing Sugar	+
Phlobatannin	-

Antimicrobial activity (Antifungal and Antibacterial activity) was carried out on the aqueous leaf extract of *Calotropis procera* to determine the level of susceptibility or resistance of the microorganisms to the leaf extract (Nenaah & Ahmed, 2011).

Key: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxicillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), R; Rocephin (10µg), (APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) S; Susceptible, R; Resistant.

Antibacterial effect on *Calotropis procera* was carried out to determine if the aqueous extract of the leaf can have effect on the bacteria and act as an antimicrobial to cure or inhibit any bacterial diseases. This result shows that according to the standard gram positive antibiotic disc, for *Streptococcus pyogenes* it is resistant to the pefloxacin, ampiclox, zinnacef, amoxicillin, rocephin,

ciprofloxacin, streptomycin, septrin and erythromycin, but susceptible to gentamycin. *Staphylococcus aureus* was susceptible to pefloxacin, ampiclox, gentamycin, rocephin, ciprofloxacin, streptomycin, septrin and erythromycin, but resistant to amoxicillin, and zinnacef. *Staphylococcus aureus* and *Streptococcus pyogenes* were both resistant to the aqueous leaf extract of *Calotropis procera*.

Table 4. Antibacterial effect on aqueous extract of *Calotropis procera* compared with standard antibiotics disc for gram negative bacterial.

No	Organism	<i>Calotropis procera</i> extract	Gram positive antibiotic disc									
			SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
1	<i>Escherichia coli</i>	R	R	R	R	S	R	R	R	R	R	R

Key: SXT; Septrin (30µg), CH; Chloramphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxicillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg); R= Resistance, S= Susceptible

This result shows that *Escherichia coli* was resistant to septrin, chloramphenicol, sparfloxacin, amoxicillin, augmentin, gentamycin, pefloxacin, tarivid and streptomycin, but susceptible to ciprofloxacin. *Escherichia coli* was also resistant to the aqueous leaf extract of *Calotropis procera*.

Table 5. Antifungal effect on aqueous extract of *Calotropis procera*

No	Organism	<i>Calotropis procera</i> extract
1	<i>Aspergillus candidus</i>	R
2	<i>Penicillium corylophilum</i>	S
3	<i>Aspergillus niger</i>	R

R= Resistance, S= Susceptible

Antifungal effect on *Calotropis procera* was carried out to determine if the aqueous extract of the leaf can have effect on fungi and act an antifungal to cure or suppress any fungal diseases. This result shows that *Aspergillus candidus* and *Aspergillus niger* was resistant to the leaf extract of *Calotropis procera*, while *Penicillium corylophilum* was susceptible to the aqueous leaf extract of *Calotropis procera*.

Acute Toxicity Investigation of *Calotropis procera*

Acute toxicity investigation was carried out to determine the toxic level/toxicity of the aqueous extract of *Calotropis procera* leaf on Swiss albino mice. After 24 hours investigation on Swiss albino mice, for the first 3 hours of the observation they were all fine without any physical changes, with continuous observation, the mice that were given the highest dose (1400 mg/kg BW) were scratching and shivering compared to the control that was given distilled water, they were fine and no physical changes, all other mice were shivering, weak and were not eating well (loss of appetite), and the last 2 hours during the 24 hours observation, they were all fine without any physical change, alongside with the control, they were all fine without physical changes throughout the 24 hours investigation. Finally there was no death recorded.

This result shows that higher concentration of the dose will yield change in reaction physically, which can lead to cold or scratching of the body, and also shows that the leaf extract is not toxic because there was no

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death recorded throughout the 24 hours investigation.

Phytochemical properties of *Calotropis procera*

Secondary metabolites are responsible for medicinal activity of plants. *Calotropis procera* is a potential medicinal plant which can be explored further for its various uses in pharmaceuticals, nutraceuticals, fiber, health, environment and many other areas. The results suggest that *Calotropis procera* possess phytochemical properties for curing various ailments and may lead to the isolation of novel compounds. Flavonoids are a diverse group of phytonutrients found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. Flavonoids are the largest group of phytonutrients, with more than 6,000 types. Some of the best-known flavonoids are quercetin and kaempferol. The findings in this report are in contrast with that of Shobowale *et al.*, (2013) who detected the presence of flavonoids in the leaf extract of *Calotropis procera* gotten from Khulais, Kingdom of Saudi Arabia. The report of Bruno *et al.*, (2013) agrees with the findings of this research, as flavonoids was absent in the aqueous extract of *Calotropis procera* leaf gotten from Fortaleza beaches, Ceara, Brazil.

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. According to Bruno *et al.*, (2013) there was absence of alkaloids from the aqueous extract of *Calotropis procera* leaf gotten from Fortaleza beaches, Ceara, Brazil. The findings in this report disagrees with that of Shobowale *et al.*, (2013) who detected the presence of alkaloids in the leaf extract of *Calotropis procera* gotten from Agbado, Ifo Local Government Area of Ogun State, Nigeria.

Phlobaphens can be formed under action of acids or heating of condensed tannins or of the fraction of tannins called Phlobatannins. According to Shobowale *et al.*, (2013), tannins was present in the aqueous extract of *Calotropis procera* leaf, but according to the findings of this research, phlobatannins was absent in the aqueous extract of *Calotropis procera* leaf.

Saponins may help reduce cholesterol levels, strengthen the immune system, treat diabetes and inhibit tumor growth. They also improve lipid metabolism and may help prevent and treat obesity. The findings of this research agrees with the report of Morsy *et al.*, (2016) and Shobowale *et al.*, (2013) who detected saponins was present in the aqueous extract of *Calotropis procera* leaf. *Calotropis procera* leaf helps to strengthen immune system, treat diabetes and inhibit tumor growth generally.

A reducing sugar is any sugar that is capable of acting as a reducing agent, because it has a free aldehyde group /a free ketone group. Reducing sugar was detected in the aqueous extract of *Calotropis procera* which shows there are presence of monosaccharides in the aqueous extract of the leaf.

Antimicrobial activity of *Calotropis procera*

Antimicrobial activity shows that the aqueous extract of *Calotropis procera* leaf, was not effective on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus candidus* and *Aspergillus niger*. Only *Penicillium corylophilum* was susceptible to the aqueous extract of *Calotropis procera* leaf.

Acute toxicity investigation of this research shows that the aqueous extract of *Calotropis procera* leaf is non-toxic to the mice, because there was no mortality recorded, only physical changes were observed to those that were given high dosage of 1400 mg/kg BW. They were shivering and scratching profusely, which shows that only high concentration of the extract given to the mice will yield physical changes.

CONCLUSION

Phytochemical screening, antimicrobial activity and acute toxicity investigation carried out on *Calotropis procera* aqueous leaf extract showed the presence of saponins and reducing sugars and the absence of alkaloids, flavonoids, and phlobatannins in the leaf extract of *Calotropis procera*. It also showed that the aqueous leaf extract of *Calotropis procera* was not toxic because there was no mortality of mice recorded throughout the 24 h observation of the mice, only physical changes

were observed like scratching and shivering of the group that were fed with higher concentration of the dose (i.e aqueous extract of *Calotropis procera* leaf). Finally, the antimicrobial activity of the aqueous extract of *Calotropis procera* leaf showed that there was marked resistance by *E. coli*, *S. pyogenes*, *S.aureus*, *Aspergillus niger* and *Aspergillus candidus* to the leaf extract, while *Penicillium corylophilum* was susceptible to the leaf extract.

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MICRO BUSINESS STUDY OF TRADITIONAL HEALTH DRINK PRODUCT “TELANG LIMAO BANGKAK” (GREEN BUSINESS CANVAS STUDY “ECOCANVAS”)

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ABSTRACT

This study aims to formulate a business development strategy that is appropriate and can be applied to the traditional Indonesian health drink business "Telang Limao Bangkak" which is a combination of Telang Flower (*Clitoria ternatea* L.) and Limao Calong from Bangka. The research method used is descriptive qualitative analysis. Data collection was carried out using interview and assessment questionnaire techniques. The results of this study indicate that the position of the health drink business "Telang Limao Bangkak" is in the very profitable position of the Aggressive quadrant. The strategy implemented is to support an aggressive growth policy (growth-oriented strategy) and has been implemented in the EcoCanvas Health Drink business model "Telang Limao Bangkak". The results of this research are expected to create a new air of green business that gives birth to health drink products that will improve people's welfare.

Keywords: Telang Flower, Limao Calong, EcoCanvas, Health Drink, People's Welfare

INTRODUCTION

Indonesia is a mega-biodiversity country with potential biodiversity to increase productivity, quality and competitiveness of commodity crops through plant breeding approaches. (Nurmayulis et al., 2021). Among the potential for megabiodiversity, flora in the territory of Indonesia, including part of Malesiana which is estimated to have around 25% of the world's flowering plant species, ranks as the seventh largest country with a number of species reaching 20,000 species, 40% of which are endemic or native to Indonesia. (Kusmana & Wisdom, 2015).

Telang flower or also known as Butterfly Pea, has the Latin name *Clitoria ternatea* L which is a plant of the Fabaceae family. The telang flower has bright dark blue petals, which is contributed by the presence of the anthocyanin delphinidin (Ab Rashid et al., 2021). Various previous studies have stated that the content of delphinidin anthocyanins is rich in health benefits and benefits. These benefits include improved eyesight, antioxidant properties, controlling type II diabetes, reduction of coronary heart disease, reduction of hypertension and prevention of

cancer. Bunga Telang also contains phytochemical constituents with pharmacological importance in medical activities (Oguis et al., 2019). The extract has benefits in various pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anesthetic, antidiabetic, and insecticidal activity. The diversification of pharmacological activity shows that it has outstanding properties in medical value (Gollen et al., 2018; Zhang et al., 2021).

But unfortunately the processing of telang flower in Indonesia is still not paid attention so that it is necessary to diversify processed products. The telang flower is less well known by the wider community because this plant is not used as a food product. One of the potential products to be developed is flower juice drink. Flower juice drink is one of the processed beverage products that are sourced from pressing or extracting flowers that have been filtered. This innovation with the use of telang flowers in the manufacture of flower juice drinks is not only intended to introduce to the public that there are still many flowers, namely ornamental flowers that need to be

improved in their use, but also the chemical compounds contained in them.(Spence, 2019)

Limao Calong or often known as the Bangka key orange is a plant that is optimally utilized by the people of Bangka Belitung. Limao Calong has a very distinctive taste, namely a fresh sour taste, like a combination of flavors between orange and lemon(Roanisca & Mahardika, 2020). This orange is often referred to as the "Cino Orange" because it is almost the same as the small orange that grows in China. This is indeed in line with the Chinese population as the second largest after the Malay population(Mariska et al., 2021). Limao Calong has several health benefits including being low in calories from sugar. Both fruits are traditionally used as traditional medicine in Asian countries to manage inflammation of the respiratory tract. In addition, Limao Calong contains flavonoids that have strong antioxidant and radical scavenging activities that appear to be associated with a reduced risk of certain chronic diseases, prevention of cardiovascular disorders and cancer (Chen et al., 2017).

Some Indonesian people, especially the Province of the Bangka Belitung Islands, take advantage of the opportunity for the economic value of a combination drink of telang flower and limao Calong by making an instant health drink product called "Telang Limao Bangkak" which is often served in two forms, namely drink bottles and bags containing telang and limao Calong which are served in two forms. dried. However, the problem is that due to lack of empowerment and access to marketing, this

product has some limitations, especially in product marketing and public knowledge. So that further analysis is needed as an effort to help provide solutions by producing a green business concept for health drink products with the best treatment that contributes to health and develops micro business potential for the people of Indonesia.

RESEARCH METHODS

The research method used is a descriptive method with a qualitative approach using tools based on EcoCanvas research by Daou et al. (2020) in designing a green business model. This study will begin with a SWOT analysis to analyze the "Telang Limao Bangkak" health drink business model obtained through written or oral data (interviews) with beverage business owners (5 people) as well as an assessment questionnaire from consumer respondents (57 consumers) in the Province of the Bangka Islands. Belitung and Banten during the research.

After that, it was continued with the design of a canvas business model using the 12-element EcoCanvas approach (needs and challenges, customer segment, key resources, circular value chain, environmental foresight, social foresight, cost structure, stakeholder relationship, communication and sales, unique circular value proposition, revenue streams, circular business model and innovation) on the Green Business Model Canvas to increase the business value of the health drink combination of telang flower and limao Calong.

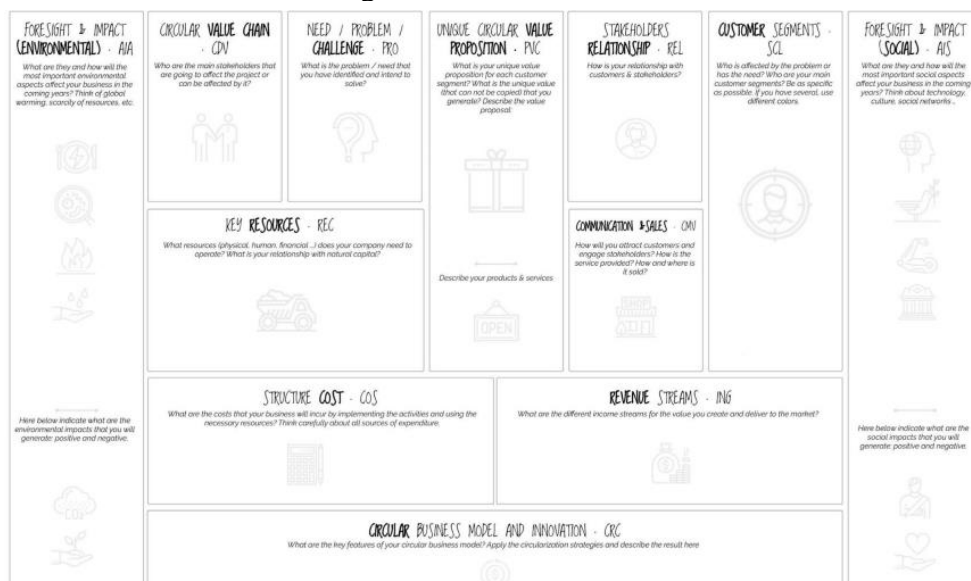


Figure 1. Green Business Model Canvas (Daou et al., 2020)

RESULTS AND DISCUSSION

SWOT Analysis Results

Written or verbal data (interviews) as well as assessment questionnaires from consumer respondents and beverage businessmen "Telang Limao Bangkak" in the Provinces of the Bangka Belitung and Banten Islands that have been collected and analyzed on internal and external factors are then used to determine business strategic factors for SWOT analysis. This study uses the basic analytical tools of EFAS and IFAS in order to analyze strategy formulation, product sales, and new business ideas (Kurniawan & Abidin, 2019; Sari & Oktafianto, 2017).

a. IFAS Analysis

Identification of internal factors in the health drink business "Telang Limao Bangkak" after being analyzed, then given a weight and rating. The following table structure is an Internal Factor Analysis Strategic Matrix (IFAS) used to find out how big the role of the internal factors in the business is. The IFAS matrix shows the company's internal conditions in the form of strengths and weaknesses which are calculated based on the rating and weight of the results of the analysis that has been carried out on the "Telang Limao Bangkak" business that can be used in the implementation of the SWOT strategy.

Table 1. Internal Factor Analysis Strategic Matrix

Internal Strategy Factors	Item Weight	Rating	Item weight X Rating
Strength factor			
Telang flower plants are easy to grow because they are a type of wild plant that does not require special care so that production costs are low	0.0612	4	0,2448
The process of extracting the content of the Telang Flower is easy because it only needs to be dried	0.0620	3	0.1860
Telang flower health drink products can last a long time	0.0615	3	1.8450
Limao Calong is easy to find at the Local Market	0.0623	3	0.1869
The process of filtering fruit juice is easy	0.0630	3	0.1890
The benefits contained in the "Telang Limao Bangkak" Health Drink are very many	0.0627	4	0.2508
Can help increase the body's immunity during the Covid-19 Pandemic	0.0610	3	0.1830
The harvest time of the pea flower does not know the season, making it easier for the availability of beverage production	0.0601	3	0.1803
The process of combining the Telang Flower and Limao Calong is easy	0.0620	3	0.1860
The combination effect of Telang Flower and Limao Calong further enriches the efficacy of the product	0.0645	3	0.1935
Maintained Product Cleanliness	0.0730	3	0.2190
TOTAL			3.8643
Weakness Factor			
Product efficacy education has not been widespread because the public is limited to only knowing about food coloring and eye pain medicine	0.0650	3	0.1950
The beverage product "Telang Limao Bangkak" is only known to be limited to the Bangka Belitung area	0.0625	2	0.1250
Telang flower has not been widely cultivated by the community	0.0612	2	0.1224
The product should not be consumed in excess because it has the effect of decreasing consciousness accompanied by anxiety	0.051	2	0.102
The product has not been fully standardized by BPOM	0.0670	1	0.0670
Total			0.6114

After knowing the total value of item weight x rating in Table 1, the next step is to find the value of the internal analysis coordinates. This is done by reducing the item weight value x Strength rating with item

weight value x weakness rating so that the internal analysis coordinate values are found as follows $3.8643 - 0.6114 = 3.2529$ which is used as a reference point for the internal coordinates of the health drink business "Telang Limao

Bangka". These results are used to see the strategic coordinates in the SWOT matrix.

b. EFAS Analysis

External Factor Analysis Strategic Matrix (EFAS) is used to find out how big the role of external factors in the business. The

EFAS matrix shows the company's external conditions in the form of opportunities and threats which are calculated based on the rating and weight of the results of the analysis that has been carried out.

Table 2. Matrix of External Factor Analysis Strategic

External Strategy Factors	Item Weight	Rating	Item weight X Rating
Opportunity Factor			
The market opportunity for the health drink "Telang Limao Bangka" is very large	0.0880	4	0.352
This drink contains a very wide pharmacological potential, so it is needed by the community, especially during the Covid-19 pandemic	0.0863	4	0.3452
Potential to be a new choice for Nusantara health drinks	0.0896	3	0.2688
Beverage products are often sought after as souvenirs	0.0817	3	0.2451
Beverage products can enter the online market	0.0842	4	0.3368
Products can be included in Jamu patents	0.0852	4	0.3408
Products can be exported abroad	0.0816	3	0,2448
Total			2.1335
Threat Factor			
The emergence of new manufacturers who have similar product innovations	0.0850	3	0.255
Competitive competition with similar products from abroad considering that the telang flower is spread across South Asia	0.0730	2	0.146
The price of additional raw materials (Sucrose) and supporting materials is not stable	0.0864	3	0.22592
Fear of hygiene and social limitations in the era of the covid-19 pandemic	0.0739	4	0.2956
Potential purchasing power decreases as other new products compete	0.0850	3	0.255
Total			1.2108

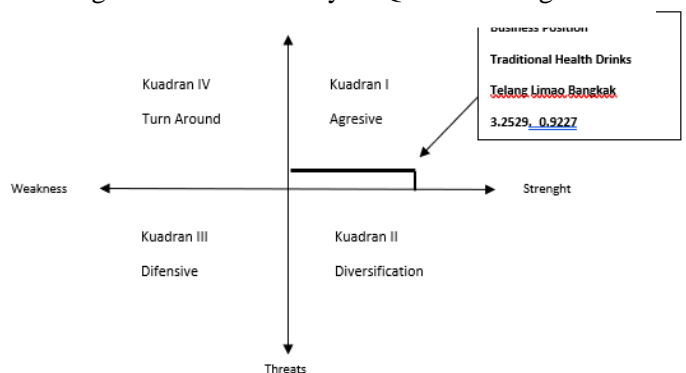
After knowing the total value of item weight x rating in table 2, the next step is to find the coordinates of the external analysis. This is done by reducing the value of the item weight x Opportunity rating with the item weight value x Threat rating so that the external analysis coordinates are found as follows $2.1335 - 1.2108 = 0.9227$ which is used as a reference point for the external health drink business "Telang Limao Bangkok". These results are used to see the strategic coordinates in the SWOT matrix.

c. SWOT Matrix

From the calculation of the data, it is known that the coordinates of the SWOT matrix of the health drink business "Telang Limao Bangkok" for Internal Analysis coordinates at 3.2529 while the coordinates for External Analysis are at 0.9227. The next stage is based on the coordinates obtained by the health drink business "Telang Limao Bangkok" in the SWOT matrix, it can be seen the company's position to implement a strategy that is in accordance with the company's

current conditions by entering the coordinates into the SWOT matrix as follows.

Figure 2. SWOT Analysis Quadrant Diagram



From the results of the SWOT analysis on the health drink business "Telang Limao Bangkok", it is necessary for how we determine the strategy so that we can compete in the market. The analysis is described as follows:

Table 3. SWOT Matrix

	<p>Strength (S):</p> <ol style="list-style-type: none"> 1. Telang flower plants are easy to grow because they are a type of wild plant that does not require special care so that production costs are low 2. The process of extracting the content of the Telang Flower is easy because it only needs to be dried 3. Telang flower health drink products can last a long time 4. Limao Calong is easy to find at the local market 5. The process of filtering fruit juice is easy 6. The benefits contained in the "Telang Limao Bangkok" Health Drink are very many 7. Can help increase the immunity of the community during the Covid-19 Pandemic 8. The harvest time of the telang flower knows no season, making it easier for the availability of beverage production 9. The process of combining Telang Flower and Limao Calong is easy to do 10. The combination effect of Telang Flower and Calong Limao further enriches the efficacy of the product 	<p>Weaknesses (W):</p> <ol style="list-style-type: none"> 1. Education on the efficacy of the product has not been widespread because the public is limited to only knowing about food coloring and eye pain medicine 2. The beverage product "Telang Limao Bangkok" is only known to be limited to the Bangka Belitung area 3. Telang flower has not been widely cultivated by the community 4. The product should not be consumed in excess because it has a decreasing effect of consciousness accompanied by anxiety 5. The product has not been fully standardized by BPOM
<p>Opportunity (O):</p> <ol style="list-style-type: none"> 1. The market opportunity for the health drink "Telang Limao Bangka" is very large 2. The very wide pharmacological potential of this drink is needed by the community, especially during the Covid-19 pandemic 3. Potential to be a new choice for Nusantara health drinks 4. Beverage products are often sought after as souvenirs 5. Beverage products can enter the online market 6. Products can be included in Jamu patents 7. Products can be exported abroad 	<p>SO Strategy:</p> <ol style="list-style-type: none"> 1. Creating Telang Limao Bangkok beverage products with new serving variants (such as powder or syrup) which are guaranteed cleanliness and further maintain the efficacy and quality of the products contained to make them last longer (S2,S3,S4,S5,S6,S7,S9,S10,O2,O3,O4,O7) 2. Make product packaging more beautiful with a touch of Bangka coal graphics and easier to carry as souvenirs (S3, O3,O4,O5,O7) 3. Promoting through various media to attract new customers in developing their business and utilizing Instagram Influencers in terms of Product Endorsment (S6, S7, S9, S10, O1, O3, O5) 4. Ensure the legality of the efficacy and safety of product combinations through patents and BPOM licensing (S6,S7,S10,O1,O6) 	<p>WO Strategy:</p> <ol style="list-style-type: none"> 1. Take advantage of large market opportunities, especially online marketplaces to expand product access and take a position as a contemporary health drink typical of the archipelago (W2, O1, O3,O5,O7) 2. Spread the message of the potential pharmacological properties of products that have been tested in clinical research to provide education on how important it is to increase body health intake through the Telang Limao Bangka health drink (W1,O1,O2,) 3. Cooperating with BPOM and the Ministry of Law and Human Rights to test the compound content and legalize the Telang Limao Bangkok Beverage product to find out recommendations for drinking products and

meet existing health drink
product standards
(W4,W5,O2,O6)

Threats (T):

1. The emergence of new manufacturers who have similar product innovations
2. Competitive competition with similar products from abroad considering that the telang flower is spread across South Asia
3. The price of additional raw materials (Sucrose) and supporting materials is not stable
4. Fear of hygiene and social limitations in the era of the covid-19 pandemic
5. The potential for purchasing power decreases with the competition of other new products

ST Strategy:

1. Carrying out research on product development innovations for the Telang Limao Bangkak beverage with different color variations (S8,S9,T1,T2,T4)
2. Ensuring that the facilities (Place, Tools and Materials) and the production process of the Telang Limao Bangkak drink are guaranteed to be hygienic (S2,S5,S9,T4)

WT Strategy:

1. Create an appropriate promotional strategy during the pandemic so that it can expand the market. (S2,S3,T1,T2,T5)
2. Coordinate with stakeholders to formulate prices, needs and distribution of the required raw materials (W3,T3,T5)

This SWOT matrix is used as a comparison between internal and external strategic factors to obtain a strategy for each factor, based on the results obtained to determine the focus of the strategy recommendations, it can be described as follows:

SO Strategy (strengths and opportunities)

This strategy is carried out to take advantage of the company's strengths in order to capture the opportunities the company has. Some of the strategies proposed are creating Telang Limao Bangkak beverage products with new serving variants such as syrup or beverage powder, making product packaging more beautiful with a touch of Bangka coal graphics and easier to carry as souvenirs, conducting promotions through various media to attract new customers. developing business including utilizing Instagram Influencers in terms of Product Endorsment and ensuring the legality of the efficacy and safety of product

combinations through patents and BPOM licensing

ST strategy (strengths and threats)

This strategy is applied where the strengths of the company are used to overcome threats that may be faced. Some of the strategies proposed are making research and innovation development of Telang Limao Bangkak beverage products with different color variations and ensuring that the facilities (Place, Tools and Materials) and the production process of the Telang Limao Bangkak drink are guaranteed to be hygienic.

WO Strategy (weaknesses and opportunities)

This strategy is applied when there are opportunities for the company to overcome business threats. Businessmen take advantage of large market opportunities, especially online marketplaces to expand product access and take a position as a contemporary health drink typical of the archipelago. In addition,

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businessmen need to spread marketing messages containing the potential pharmacological properties of products that have been tested in clinical research to provide education on how important it is to increase body health intake through the Telang Limao Bangkok health drink. Businessmen need to cooperate with BPOM and the Ministry of Law and Human Rights to test the content of compounds and legalize the Telang Limao Bangkok Beverage product to find out recommendations for drinking products and meet existing health drink product standards.

WT strategy (weaknesses and threats)

This strategy is applied when the company must be able to overcome the weaknesses of the company in order to avoid the business threats that will be faced. Businessmen need to make appropriate promotional strategies during the pandemic so that they can expand the market and coordinate with stakeholders to formulate prices, needs and distribution of the raw materials needed.

After identifying several attributes that describe the strengths and weaknesses of the product/business being run. From the results of weighting for each attribute by comparing the level of importance of each attribute the importance value of internal factors (S + W), as well as external factors (O + T) shows that the condition and potential of the health drink business "Telang Limao Bangkok" is in Quadrant 1 so that the strategy used based on the SWOT matrix strategy analysis is the SO strategy. The SO strategy is characterized by the company optimizing its strengths to take advantage of existing opportunities by taking into account all the threats made by competitors in the market.

Telang Limao Bangkok EcoCanvas Design

Based on the results of the SWOT analysis, the position of the health drink business "Telang Limao Bangkok" is in quadrant 1, namely the company has opportunities and strengths. The strategy that must be taken in this condition is to support an aggressive growth policy or growth oriented strategy by taking advantage of existing opportunities and the company's internal strengths. Based on the results of the SWOT analysis, a business model canvas-based business model development plan was prepared using the EcoCanvas design which is

an innovative sustainable green business planning tool specifically designed to support entrepreneurs who are interested in creating a circular value proposition for their business.

Ecocanvas has many advantages that set it apart from other models. Ecocanvas gives us the opportunity to address the weaknesses of both conceptual approaches, sustainability, and circular economy, by placing the focus on personalization and rethinking how the economy, society, and environment integrate into particular entities and contexts. Additionally, compared to the original business model canvas, it has a more complete and inclusive approach (Daou et al., 2020; Ramos Leon, 2020).

a. Need/Problem/Challenge

To set a course for circularity, the first step is to identify business needs, problems, and challenges. Business Needs and Challenges can guide companies to fill this block. Companies are required to list all environmental, social, customer/market, and personal/motivational business needs and challenges. They can decide to focus on the most pressing later.

In the health drink business "Telang Limao Bangka" there are several challenges that must be answered, namely to reduce the negative environmental impact of the waste of limao Calong Bangka by processing it as raw material for organic fertilizer for the cultivation of telang plants. In addition, it is necessary to overcome the potential vulnerabilities of production systems and supply chains accompanied by eco-design of eco-friendly label products, lightweight and reusable bottles with a graphic touch of Bangka coal cloth. In addition to legality, the efficacy and safety of the product combination needs to be enforced through patents and BPOM licensing.

b. Customer Segments

This block consists of dividing the market share into its constituent segments and defining social, economic, and behavioral needs and wants. It answers the following questions: Who is affected by the problem or has a need? Who are your main customers? Segment?.

The target market segment is all people at any age, especially people who like a healthy lifestyle by liking health drinks with

environmentally friendly beverage product packaging and techniques.

c. Key Resources

The key Resources component involves identifying the physical, human, financial, and natural capital required by the company to ensure operations. This tool helps define a user's product lifecycle IN and OUT by mapping the process from buying raw materials to manufacturing, selling, using and disposing of it, in addition to all the logistics and management in between. The life cycle is customized based on the type and amount of resources, energy, and water that the company needs to create a product, service, or process. The types and amounts of by-products (ie, effluent, air, water, and soil emissions) are also determined.

Table 4. EcoCanvas of Health Drink “Telang Limao Bangkak”

Environmental Foresight and Impact	Circular Value Chain	Need/Problem/Challenge	Unique Circular Value Proportion	Stakeholder Relationship	Customer Segments	Social Foresight and Impacts
<p>1. Climate change that can damage the quality of the telang flower</p> <p>2. The quality of Limao Calong is affected by weather conditions and storage of perishable supplies</p> <p>3. Scarcity of resources and potential increase in the price of production energy and complementary materials such as sucrose</p> <p>4. Environmental regulations that will be applied from time to time in the midst of strict health protocol conditions</p>	<p>1. Consumer Health Drinks Telang Limao Bangka</p> <p>2. Healthy Living Movement Community</p> <p>3. Investors</p> <p>4. Suppliers and Distributors</p> <p>5. Market and Competitors</p>	<p>1.Reducing the environmental impact of the waste of limao Calong Bangka as a raw material for organic fertilizer for the cultivation of telang plants</p> <p>2.Reducing the vulnerability of production and supply chain systems, and eco-designing eco-friendly labels for lightweight and reusable bottles with a graphic touch of Bangka cual fabric.</p> <p>3.Ensuring the legality of the efficacy and safety of product combinations through patents and BPOM licensing</p>	<p>1.branded bottles in a sustainable, youthful and dynamic way to target consumers who love health drinks with environmental care</p> <p>2. Changing the waste of limao Calong that has been squeezed into organic fertilizer for telang flower cultivation</p>	<p>1. Trustworthy, close and transparent relationship to retain customers</p> <p>2. Direct and transparent relationship with suppliers and distributors</p> <p>3. Guarantee good quality and sustainable oriented products</p> <p>4. Direct and transparent relationship with the government (Ministry of Health, Ministry of Tourism and Kemenkop UMKM) and Research Institute for Product Quality Testing (BPOM)</p>	<p>The target of the business is all people at any age, especially for people who like a healthy lifestyle by liking health drinks with environmentally friendly beverage product packaging and techniques.</p>	<p>1.Consumer awareness of environmental issues will increase through social and mass media, especially after the Covid-19 Pandemic</p> <p>2. Shifting consumer attitudes and trends towards environmental and organic products</p> <p>3.Business trends that will evolve towards a sustainable model</p> <p>4. An increase in the flower portfolio and business trends that make the air of competition heat up</p> <p>5. Consumers will be increasingly expected to check the product footprint, creating environmental labeling of the “Telang Limao Bangkak” Health Drink product</p>
<p>Impact</p> <p>1. Restrictions on the use of chemical fertilizers and pesticides</p> <p>2. Do not use bottles that have a damaging impact on the environment</p> <p>3. Product Processing Operational System that is more organized, healthy and</p>	<p>Key Resources</p> <p>1. Continuing and further expanding the cultivation of independent telang flower plants</p> <p>2. Supplying Limao Calong supplies directly from Limao Calong plant cultivators</p> <p>3. lightweight bottles, environmentally friendly labels and bottle caps</p> <p>4. Composting system for self-sufficient pea flower cultivation from natural ingredients</p> <p>5. Product Processing System and Equipment Cleanliness by taking into</p>			<p>Communication and Sales</p> <p>1. Social Media (Instagram, Facebook) and Campaigns (TV, Billboards and Google Ads)</p> <p>2. Marketplace Online Application (Shopee and Tokopedia)</p> <p>3. Influencers and Verbal Communication</p> <p>4. Supermarkets, Organic Shops and Restaurants</p> <p>5. Hospital Canteen and Clinic</p> <p>6. Gym and Sport Center</p>		<p>Impact</p> <p>1. Improving the welfare of the community in terms of health through reducing the risk of disease indications through processed products that are more sustainable and strive to maintain efficacy</p> <p>2. The taste of drinks is fresher, authentic and in accordance with the tastes of the Indonesian people</p> <p>3. Creation of new jobs</p>

<p>does not damage the environment</p> <p>4. Working closely with suppliers of supporting materials such as sugar (Sucrose) for production continuity</p>	<p>account sustainable environmental aspects (such as the use of detergents in post-processing equipment hygiene)</p>				
<p style="text-align: center;">Structure Cost</p> <p>1. General Expenses (Labor, Rent, Administration and Marketing)</p> <p>2. Depreciation (Extraction Machinery, Trucking, Vinification and Bottling Costs, Tanks, Tongs and Telang Blossoms)</p> <p>3. Variable Costs (Packaging and Eco-Label, Organic Compost of Telang Flower Plants, Equipment, Fuel and Product Certification)</p>		<p style="text-align: center;">Revenue Streams</p> <p>1. Sales of Telang Health Drinks in Supermarkets, Restaurants, Bars and Cafés,</p> <p>2. The results of ordering beverage products online through the Marketplace and Contact Person</p> <p>3. Profit sharing system from product storage at the RS-Klinik Canteen and Gym-Sport Center</p>			
<p style="text-align: center;">Circular Business Model and Innovation</p> <p>Beverage production resources so that the waste produced can be of sustainable use, such as waste water that can be used for irrigation of flower plants, Limao Calong waste can be used as an ingredient for making organic fertilizer for telang flower plants, design boxes and packaging bottles that are more environmentally friendly and can create innovative serving variants. drinks in the form of drink bottles, syrup bottles to powder drinks</p>					

In the health drink business "Telang Limao Bangkok" there are several resource component points that are concentrated in the design of this EcoCanvas, namely continuing and further expanding the cultivation of independent telang flower plants, supplying Limao Calong supplies directly from Limao Calong plant cultivators, making lightweight bottles, labels and environmentally friendly bottle caps, creating an independent telang flower plant compost system from natural ingredients and a Product Processing and Equipment Cleanliness system by paying attention to sustainable environmental aspects (such as the use of detergents in post-processing equipment hygiene).

d. Circular Value Chain

The Circular Value Chain involves all the specific agents that influence or are affected by any sector. The tool used to help companies fill this block is the Stakeholder Map (Do tool). Stakeholders can be defined as internal or external stakeholders. They include public authorities, media, and social networks, customers and users, competitors and market agents, local communities, supporters, suppliers and financiers, and knowledge centers.

Telang Limao Bangkok Health Drink consumers, Healthy Living Movement community, investors, suppliers, distributors, markets and competitors are some of the parties that influence the "Telang Limao Bangkok" health drink business cycle.

e. Environmental Foresight and Impact

The Environmental Forecast component addresses all aspects of the environment that affect a business, both positively and negatively. The tool used to support this block is known as the PESTEL Tool which stands for Political, Economic, Social, Technological, Environmental Factors, and Law. In this component, users identify the factors that can affect the business and, above all, evaluate their positive or negative impact on their company. The purpose of this analysis is to be prepared to respond to future situations and increase business resilience.

There are several environmental factors that affect this business cycle, namely: climate change that can damage the quality of the telang flower, the quality of Limao Calong

which is affected by weather conditions and storage of supplies that can rot, scarcity of resources and potential increases in the price of production energy and complementary materials such as sucrose and environmental regulations that will be applied from time to time amid protocol conditions strict health. So that the predicted impact that will occur in the operational management body of this business is the limitation of the use of chemical fertilizers and pesticides, not using bottles that have an impact on the environment, the need for a more organized Product Processing Operational system,

f. Structure Cost

Cost Structure includes all the sources of expenditure that will be incurred by the business by implementing activities and using existing resources. In this health drink business, there are 3 main cost divisions, namely fixed, variable and depreciation with the following details:

1. General Expenses (Labor, Rent, Administration and Marketing)
2. Depreciation (Extraction Machinery, Trucking, Vinification and Bottling Costs, Tanks, Tongs and Telang Blossoms)
3. Variable Costs (Packaging and Eco-Label, Organic Compost of Telang Flower Plants, Equipment, Fuel and Product Certification)

g. Social Foresight and Impact

This block deals with all social aspects that affect business, whether positive or negative and includes new habits, enabling technology, values, etc. The PESTEL tool is also used to support the Social Foresight block.

There are several social issues that will affect the health drink business, namely growing consumer awareness of environmental issues through social and mass media, especially after the Covid-19 pandemic, there is a shift in consumer attitudes and trends towards environmental and organic products, business trends that will develop towards a sustainable model, an increase in the telang flower portfolio and business trends that make the competition heat up and consumers will be increasingly expected to examine the product footprint, the creation of environmental labeling of the "Telang Limao Bangkok" Health Drink product.

So that the predicted impact that will occur in the operational management body of this business is an increase in public welfare in terms of health through reducing the risk of disease indications through processed products that are more sustainable and strive to maintain the efficacy, taste of drinks that are fresher, authentic and in accordance with the tastes of the Indonesian people. and the creation of new jobs for the community

h. Stakeholder Relationship

The Stakeholder Relations block describes the business relationships with the stakeholders listed above, particularly with customers and beneficiaries.

There are several steps of business relations carried out in this business, namely:

1. trustworthy, close and transparent relationship to retain customers
2. Direct and transparent relationship with suppliers and distributors
3. Guarantee good quality and sustainable oriented products
4. Direct and transparent relationship with the government (Ministry of Health, Ministry of Tourism and Kemenkop UMKM) and Research Institute for Product Quality Testing (BPOM)

i. Communication and Sales

This component describes ways to engage and attract customers and stakeholders to deliver a value proposition. It also allows users to identify the various communication and sales channels used to provide or promote a product or service.

In this business, there are various business channels that are owned and will be optimized for a better company value proposition, namely from sales of Telang Health Drinks in Supermarkets, Restaurants, Bars and Cafés, the results of ordering beverage products online through the Marketplace and Contact Persons, a sharing system. the results of product storage at the RS-Klinik Canteen and Gym-Sport Center.

j. Unique Circular Value Proposition

Circular value creation of business value must be unique to be competitive in the market. This Ecocanvas component helps answer these questions: What is your unique value proposition for each customer segment? What unique (non-copyable) value do you generate?

There is a unique value that will be owned and optimized for the purpose of a sustainable green business, namely the existence of branded bottles in a sustainable, youthful and dynamic way to target consumers who like health drinks by caring for the environment and converting limao Calong waste that has been squeezed into organic fertilizer for plants. pea flower cultivation.

k. Revenue Streams

Revenue Streams refer to the different types of income and the resulting streams of value created and delivered to the market. There are several revenue streams generated from the sale of this "Telang Limao Bangkok" health drink, namely:

1. Sales of Telang Health Drinks in Supermarkets, Restaurants, Bars and Cafés,
2. The results of ordering beverage products online through the Marketplace and Contact Person
3. Profit sharing system from product storage at the RS-Klinik Canteen and Gym-Sport Center

l. Circular Business Model and Innovation

Through a series of circularization strategies, this block helps in defining the main features of the circular business model. Many sets of more environmentally friendly actions throughout the manufacturing process of the "Telang Limao Bangkok" health drink were implemented to transform the business into a more circular and sustainable one. However, in this particular case study, the researcher decided to tackle the waste aspect of the process. Many valuable resources in the process of making this health drink must be wasted. So we designed several systems of continuous innovation model in this business. Beverage production resources so that the waste produced can be of sustainable use, such as waste water that can be used for irrigation of flower plants, Limao Calong waste can be used as material for making organic fertilizer for telang flower plants.

CONCLUSION

The typical health drink of the archipelago "Telang Limao Bangkok" has enormous business potential if it is optimized with environmentally friendly, hygienic and sustainable business planning. The use of the

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Green Business Model Canvas (BMC) through EcoCanvas is expected to give birth to an operational plan for the health drink business “Telang Limao Bangkok” which pays attention to environmental and sustainable aspects. Moreover, based on the results of the SWOT analysis, it shows that the business position is in the very profitable aggressive quadrant which can bring the business to glory, increasing business profits by up to 50% every month. Moreover, in the midst of a pandemic crisis, people still want to maintain their body's immunity, one of which is through health drinks. The strategy implemented is to support an aggressive growth policy (growth oriented strategy) and has been implemented in the EcoCanvas Health Drink business model “Telang Limao Bangkok”.

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THE EFFECT OF ADDING GELATIN AND SALT SOLUTION TO OFF-GRADE SAPODILLA JELLY CANDY THROUGH THE PROCESS OF REDUCING ITS TANNIN CONTENT

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ABSTRACT

The young sapodilla fruit will take when harvesting is brownish green and has an astringent taste, called off-grade sapodilla fruit. The astringent taste of the fruit is due to the high tannin content. Soaking with a salt solution is a treatment done to reduce the astringent taste so that the fruit can continue as raw material for making jelly candy. Gelatin is added as a gelling agent to get a good jelly candy. This study aimed to determine the effect of adding gelatin and salt solution used in the manufacture of off-grade sapodilla fruit jelly candy, which has been studied for its tannin content reduction. The research method used is an experimental method followed by a Factorial Randomized Block Design test consisting of 6 treatments. The tested treatment consisted of two factors: the first factor was soaking sapodilla off-grade with variations in salt solution of 2%, 4%, and 6%, and the second factor was gelatin concentration of 18% and 20%. The results showed that the off-grade sapodilla sample gave the greater salt concentration was given, the more the salt could reduce the amount of tannin content. In jelly candy, the tannin content obtained was decreased compared to the tannin content of off-grade sapodilla fruit. The tannin is due to the unstable or too high cooking temperature. Based on the results of the texture test, found that the L2G2 treatment (4% salt solution, 20% gelatin) was closest to the commercial texture, while based on the organoleptic test, the L3G1 treatment (6% salt solution, 18% gelatin) had the highest value by the panellists

Keywords: Gelatin, jelly candy, off-grade sapodilla fruit, tannin, texture

INTRODUCTION

According to Arbaiah (2019), there are young sapodilla fruits that are also taken when harvesting. Generally, the sapodilla is discarded or used as feed by fruit farmers. This young sapodilla fruit has a size ranging from 4-5 cm, brownish-green, and when ripe, the young sapodilla fruit does not experience perfect ripening and has a sour taste. In other words, this type of young sapodilla fruit is said to be an off-grade sapodilla fruit.

According to Awika, Yang, Browning, & Faraj (2009), high tannin levels cause an astringent and bitter taste in foodstuffs. The fruit's bitter, astringent, and sour taste does not make it acceptable to the public, so it needs to be handled to reduce it so that the flavour is good to the panellists when the fruit is processed into a product. Soaking with the salt solution is the most common treatment to reduce the astringent taste in fruit. The price is relatively affordable in off-grade sapodilla

fruit that has gone through soaking treatment with a salt solution to continue to be the raw material for making jelly candy.

Jelly candy is one of the food products that is liked by everyone, from children to adults. Jelly candy is candy made by adding fruit juice, a mixture of gelling ingredients, or flavourings to create various flavours with clear and transparent physical forms (Atmaka, Nurhartadi, & Karim, 2013). Jelly candy is generally made from gelatin with the addition of gelatin as a thickener. Gelatin is a natural product obtained from the partial hydrolysis of collagen. The addition of gelatin can affect the physical and chemical properties of the product. One of the most important factors in gelling is the gelatin concentration in the mixture because the gel will only form to a certain extent. If the gelatin concentration is too low, the gel will become soft, or no gel is included, but if the concentration of gelatin used is too high, the gel formed will be stiff (Vail et al. 1978 in Herutami, 2002).

Based on the description above, the researcher studied the reduction of tannins in off-grade sapodilla fruit by using a salt solution so that could continue it in the manufacture of jelly candy by examining the concentration of gelatin used so that it could produce processed products in the form of off-grade sapodilla fruit jelly candy which the panellists favoured.

MATERIALS AND METHODS

Tools and Materials

The main ingredient used in this research is brown off-grade Sukatali variety with characteristics of 4-6 cm diameter, brownish-green skin, round shape, has a lot of sap, not sweet/astringent. Other ingredients used include gelatin, citric acid, high fructose syrup (HFS), and water. The chemicals used in this study were distilled water, table salt solution, methanol, folin denis reagent (sodium tungstate, phosphomolybdic acid,

phosphoric acid), saturated Na_2CO_3 , and tannic acid.

The tools used in this study were glass containers/glass jars, paper packaging, blenders, filters, knives, spatulas, bowls, pans, pans, stoves, basins, mixing spoons, scales, measuring cups, Texture Analyzer, spectrophotometer, vortex, cup aluminium, oven, desiccator, funnel, silica gel, analytical balance, stirring rod, magnetic stirrer, volumetric flask, krustang, Erlenmeyer, aluminium foil, filter paper, glassware for chemical analysis.

Methods

Jelly Candy Production

The sapodilla fruit was treated with 2%, 4%, and washed 6% salt solutions for 24 hours at room temperature (25°C) to remove the solution still attached to the fruit. Then the flesh of the fruit is separated from the seeds of the fruit. The flesh of the fruit is mashed using a blender with a ratio of water and fruit 10:1. The pulp is then filtered using a filter cloth to separate the pulp from the juice. Sapodilla juice as much as 500 ml is divided into two parts. Part I: 100 ml sapodilla juice added 50 grams of fructose syrup/HFS and 0.2 grams of citric acid into a Teflon pan and heated for 5 minutes to 90°C while continuously stirring using a wooden spoon. Part II: of sapodilla fruit juice 400 ml was used to dissolve gelatin whose concentration had been determined in the research preparation stage. Sapodilla juice II was heated at 60°C, then added gelatin little by little and heated for 3 minutes while stirring until all gelatin was dissolved. Sapodilla juice I mixed with sapodilla juice II, then heated into jelly candy dough. The jelly candy dough was cooked for 7 minutes at 90°C while continuously stirring. After that, the jelly candy mixture is poured into an aluminium pan. Then the jelly candy was allowed to cool for 1 hour at room temperature and continued cooling in the refrigerator at 5°C for 24 hours. Furthermore,

the jelly candy was placed at room temperature for 1 hour, cut into pieces, and then analyzed.

Experimental Design

The treatment carried out was making jelly candy with off-grade sapodilla, which had gone through the astringent reduction process with 2%, 4%, and 6% salt solutions. The study was conducted using an experimental method using a factorial Randomized Block Design consisting of 6 treatments and three replications. The treatment that was tried consisted of two factors.

The first factor is soaking sapodilla off-grade with a variation of salt solution (L):

L1 = Immersion of 2% salt solution

L2 = Immersion of 4% salt solution

L3 = Immersion of 6% salt solution

The second factor is gelatin concentration (G):

G1 = Addition of 18% gelatin

G2 = Addition of 20% gelatin

Data Analysis

The main response variables observed in this study are as follows Tannin content (AOAC, 2005 modification), Texture which includes hardness, springiness, cohesiveness, chewiness, and gumminess using the TA-XT2 Texture Analyzer (Stable Micro System, 2003) and Hedonic organoleptic test (Soekarto, 1985).

RESULTS AND DISCUSSION

Effect of Immersion in Salt Solution on Tannin Content of Sapodilla Off grade

Tannin compounds that are quite high in fruits can provide astringent taste, so treatment is needed to reduce the astringent taste in the sapodilla. Off-grade sapodilla fruit that has been treated with immersion in several concentrations of salt solution, then tested and measured for tannin content. Table 1 shows that the greater the salt concentration is given to the off-grade sapodilla sample, the

more the salt can reduce the number of tannin contents. Because the greater the concentration of salt given and the long immersion time, the more tannin content will diffuse out of the cell so that the tannin remaining in the material decreases (Reza.S, 2016).

The mechanism of NaCl in reducing the tannin content of fruit is as follows; The gallic acid found in tannins, when reacted with a salt solution (NaCl), will produce sodium gallate. Similar to gallic acid, ellagic acid will react with NaCl and produce sodium elagate. Gallic acid and ellagic acid are compounds found in tannins that cause fruit's bitter and astringent taste. Sodium gallate and sodium ellagic acid are salts that are more soluble in water than in the form of gallic acid and ellagic acid, which are weak acids. Weak acids will partially dissociate in water, and if they are in the form of their salts, more parts will dissociate. Sodium gallate and sodium elagate will dissolve with the liquid from the fruit and cause a less bitter and astringent taste (Tortoe, 2010).

The addition of salt can increase the osmotic pressure so that losing water from the material is getting bigger. In addition, the addition of salt also causes the release of sugar, dissolved protein, minerals, and other substances carried by liquids that come out of food (Yuliana, 2007). The more salt is added, the more water and other implications come out of the food, but this osmosis process will stop when the concentration in the food and the environment has reached a state of equilibrium.

Effect of Addition of Gelatin on Tannin Levels of Sapodilla Jelly Candy Off grade after Immersion in Salt Solution

Tannins are a set of polyhydroxy phenols that can be distinguished from other phenolic compounds because of the nature of tannins that can precipitate protein (Pudjiaji and Tintin, 1994 in Sa'adah (2010). The

protein contained in off-grade sapodilla jelly candy is gelatin. Based on Table 1, the average tannin level in off-grade sapodilla jelly candy is not too high because it goes through the cooking process. Based on the research of Mutmainnah, Chadijah, & Qaddafi (2018), a temperature of 85oC increased tannin levels and at a temperature of 100oC showed a decrease in tannin levels

caused by too much water. In addition, according to Schuerch (1968), based on the chemical properties of tannins, they will decompose into pyrogallol, pyrocatechol, and phloroglucinol when heated to a temperature of 210°F-215°F (98, 89°C-101.67°C). So it is possible that the tannins contained in the jelly candy decrease due to the high temperature during cooking.

Table 1. Tannin content of Sapodilla Fruit and Off grade Sapodilla Jelly Candy

Off-Grade Sapodilla Fruit		Off grade Sapodilla Jelly Candy	
Treatment	Tannin Content (mg/g)	Treatment	Tannin Content (mg/g)
Immersion of 2% salt solution	21,186 ^c	L1G1	2,500 ^c
		L1G2	2,228 ^c
Immersion of 4% salt solution	19,114 ^b	L2G1	1,648 ^b
		L2G2	1,054 ^b
Immersion of 6% salt solution	16,118 ^a	L3G1	0,830 ^a
		L3G2	0,625 ^a

Note: The average treatment marked with the same letter states that it is not significantly different at the 5% test level. (Duncan Test)

However, the presence of gelatin affects the tannin levels in the jelly candy. The nature of tannins can precipitate protein, and all tannins cause a little or a lot of sediment when added with gelatin because it is a natural protein. Gelatin is a water-soluble

and digestible protein derived from collagen, which has been heated in boiling water by a dilute acid and base solution, consisting of 25% glycine and 25% proline and hydroxyproline (Noviyanty, Hepiyansori, & Agustian, 2020).

Table 2. Observation Result of Off grade Sapodilla Jelly Candy Texture

Treatment	Hardness (gF)	Springiness	Cohesiveness	Gumminess (gF)	Chewiness (mJ)
L1G1	418,935 ^a	0,946 ^a	0,817 ^a	337,797 ^a	313,542 ^a
L1G2	401,346 ^a	0,953 ^a	0,812 ^a	401,083 ^a	351,110 ^a
L2G1	359,056 ^a	0,945 ^a	0,813 ^a	331,874 ^a	282,984 ^a
L2G2	589,885 ^a	0,954 ^a	0,814 ^a	440,114 ^a	334,012 ^a
L3G1	436,194 ^a	0,925 ^a	0,829 ^a	368,402 ^a	274,858 ^a
L3G2	318,420 ^a	0,919 ^a	0,770 ^a	359,472 ^a	255,306 ^a

Note: The average treatment marked with the same letter states that it is not significantly different at the 5% test level

Jelly Sapodilla Candy Texture Off grade after Immersion in Salt Solution

a. Hardness Value

Hardness is the maximum peak at the first pressure or the first bite. Based on the data in the table, it shows that the results of the analysis of the hardness values of each treatment did not show a significant change, or it could say that the treatment did not affect the hardness value of the off-grade sapodilla jelly candy. However, compared to the average value obtained, jelly candy with the

addition of 20% gelatin has a higher hardness value than 18% gelatin. The hardness value of jelly candy is influenced by the concentration of gelatin added. Meanwhile, commercial jelly candy has an average hardness value of 311,221 gf. Based on the hardness value of commercial candy, the hardness of off-grade sapodilla jelly candy showed that the L3G2 treatment was close to the hardness of commercial jelly candy.

The more gelatin is added, the harder the candy will be. This is because the higher

the use of gelatin will produce a hard gel, while the lower the use of gelatin will produce a soft and sticky gel. According to Rahmi, SL., Tafzi, Fitry., Anggaraini (2012), gelatin concentration is one of the most important factors in gel formation. Gelatin concentration that is too low will cause the gel formed to be soft or not even gel. In candy with a higher gelatin concentration, the protein molecules will cross-link more closely to create a network, so the hardness of the confection will be higher.

The mechanism of gelatin in forming a gel is the crosslinking of polymer chains that form a cross-link (double helix) and produce large aggregates in the form of very strong nets, resulting in very small intermolecular spaces and free water in the gel being pushed out so that the gel become increasingly violent (Kaya, Küçükada, & Alemdar, 2019).

b. Springiness Value

The data in Table 2 shows that the analysis results of the springiness value of each treatment did not show a significant change, or it can say that the treatment did not affect the springiness value of off-grade sapodilla jelly candy. The springiness value for commercial candy is 1.092, while the springiness value for off-grade sapodilla jelly candy is 0.919-0.954. DeMan (1985) in Verawaty (2008) states that springiness/elasticity is expressed as the rate at which an object returns to its original shape after deformation (change of form). Springiness or elasticity can be defined as the recovery time between the end of the first bite and the beginning of the second bite. No units are used because this parameter calculates the time area difference.

c. Cohesiveness Value

Cohesiveness is defined as the ratio of the pressure area during the second compression to the first compression and has

no units. The data in Table 2 shows that the results of the analysis of the cohesiveness value of each treatment did not show a significant change, or it can say that the treatment did not affect the cohesiveness value of off-grade sapodilla jelly candy. However, when compared to the average value obtained. Commercial jelly candy has an average cohesiveness value of 0.86, which indicates its value is slightly above the off-grade sapodilla jelly candy. Jelly candy is easy to chew and swallow if it has a low cohesiveness value. Cohesiveness is the most important parameter in consumer acceptance of all ages (Kawano et al., 2017). Moreover, literature has shown that the cohesiveness of jelly candy is between 0.54-0.82 (Mutlu et al., 2018), which is similar to the results obtained in this study.

d. Gumminess Value

The hardness value influences the value of gumminess or stickiness. The higher the hardness value, the higher the gumminess value (Mutlu et al., 2018). The data in Table 2 shows that the results of the analysis of the gumminess value of each treatment did not show a significant change, or it can say that the treatment did not affect the gumminess value of off-grade sapodilla jelly candy. Based on the average value of gumminess in commercial candies of 266.32 gF, gumminess in off-grade sapodilla jelly candy has greater stickiness than commercial ones. The resulting data shows that the harder the texture of the jelly candy, the higher the stickiness. As in the research conducted by Oktavianti (2003), wherefrom the results of texture measurements, both hardness and stickiness, there is a tendency that the harder a candy is, the stickier it will be.

e. Chewiness Value

The chewiness is the energy needed to chew solid products until they can swallow them. The data in Table 2 shows that the

results of the analysis of the chewiness value of each treatment did not show a significant change, or it can say that the treatment did not affect the chewiness value of off-grade sapodilla jelly candy. The average weight of chewiness on commercial candy is 290.48. In contrast, the value of chewiness on off-grade sapodilla jelly candy, which has a value close to that of commercial candy, is in the L2G1

treatment. The highest chewiness value was in the L1G2 treatment with a 20% gelatin concentration. The factor that affects the chewiness parameter of jelly candy is gelatin. Gelatin is composed of polypeptides, resulting in higher elasticity, which results in increased elasticity when eaten (Mutlu et al., 2018).

Table 3. Results of Organoleptic Observations of Sapodilla Jelly Candy Off grade

Treatment	Color Hedonic	Flavor Hedonic	Aroma Hedonic	Texture Hedonic	Overall Hedonic
L1G1	1,780 ^a	1,913 ^a	1,861 ^a	3,933 ^a	2,792 ^a
L1G2	1,816 ^a	1,962 ^a	1,948 ^a	4,015 ^a	2,898 ^a
L2G1	1,896 ^b	2,062 ^b	1,947 ^b	4,021 ^b	3,009 ^b
L2G2	2,055 ^b	2,109 ^b	1,996 ^b	4,130 ^b	3,138 ^b
L3G1	2,137 ^c	2,183 ^b	1,996 ^b	4,132 ^c	3,146 ^b
L3G2	2,028 ^c	1,996 ^b	1,939 ^b	4,116 ^c	3,012 ^b

Note: The average treatment marked with the same letter states that it is not significantly different at the 5% test level. Value for each type of observation according to preference, with the following value provisions: 1= Dislike, 2 = Slightly Disliked, 3 = Ordinary, 4 = Like, 5 = Very Like

Organoleptic

a. Color

Colour is a factor that needs to be considered in product development because panellists judge a new food product first based on visual appearance. Colour is one of the visual forms that consumers feel (Winarno, 2002). Table 3 shows that the hedonic value of off-grade brown jelly candy colour ranges from 1.780-2.137 (do not like - somewhat dislike). In each treatment, different immersion has significantly different results. This is because the treatment has a significant colour difference. The resulting colour is almost other in each treatment because there is an influence from the concentration of gelatin or the concentration of salt solution during the sapodilla fruit soaking process.

Based on the hedonic value of the colour from the panellists who showed dislike and somewhat dislike because the colour of the candy produced generally had a yellowish-white colour and was transparent. As is the case, according to Atmaka et al.

(2013), jelly candy is a candy made from a mixture of fruit juices that has a clear and transparent physical form. The yellowish-white colour of sapodilla fruit juice and gelatin is thought to affect the colour produced from jelly candy products and the colour that is not attractive to consumers. The gelatin used in this study is beef gelatin in the form of granules. According to Aris et al. (2020), gelatin has a white or slightly pale yellow powder or granule colour. In sheet form, gelatin is transparent pale yellow.

b. Flavor

Taste has an important role in determining the acceptance of food. The sense of taste is divided into four flavours: sweet, salty, bitter, and sour. Panellists' acceptance of taste is influenced by chemical compounds, temperature, concentration, and interactions with other flavor components (Winarno, 2002). Table 3 shows that the hedonic value of off-grade sapodilla jelly candy flavor ranges from 1.913 to 2.183 (do not like - somewhat dislike). Based on the hedonic value of the taste from the panelists

who showed dislike and somewhat dislike because the taste of the candy produced was influenced by the sap from sapodilla that was still attached. So that the resulting taste has a slightly astringent taste. The resulting astringent taste indicates the content of tannins in the product. Another factor that also affects the taste of this off-grade sapodilla jelly candy is gelatin. Gelatin is used as a gelling agent in the food and pharmaceutical industries. The unique characteristic that gelatin can form is that it is "melt-in-mouth". This also affects the level of panellists' acceptance of the taste of the nutmeg jelly candy produced.

c. Aroma

Aroma is a very important factor in determining the level of consumer acceptance of a product. Before eating, consumers usually first smell the product's scent to assess whether or not the product is worth eating. According to Soekarto and Hubeis (2000), the scent in food is one factor that determines the delicacy of food-related to the sense of smell. Table 3 shows that the hedonic value of off-grade sapodilla jelly candy scent ranges from 1.861-1.996 (dislike). Based on the hedonic value of the panellists' taste, they did not like it because the scent of the candy produced was influenced by the off-grade sapodilla, which was still immature. So that the scent that appears is not the scent of brown. The unripe sapodilla fruit has not yet released an scent, while when it is ripe, it will emit a strong scent. In sapodilla, several compounds such as alcohol, phenols, alkanes, aldehydes, scentic compounds, secondary alcohols, scentic amino, and halogens will come out when the fruit is ripe (Orwa C, et al.,2009).

The scent of off-grade sapodilla jelly candy is influenced by the scent of gelatin because the concentration of gelatin given is quite large, namely 18% and 20%. According to Aris et al. (2020), gelatin is in sheets, pieces or pieces or coarse to fine powder,

weak yellow or light brown. The colour varies depending on the particle size. The solution smells weak like broth. If dry, stable in air, but easily decomposed by microbes if moist or in solution form.

d. Texture

The texture is one of the important characteristics of food products in influencing consumer acceptance. Table 3 shows the hedonic value of off-grade sapodilla jelly candy texture ranges from 3.933 to 4.132 (regular-like). This is because the treatment has a significant difference in texture. The resulting surface is almost different in each treatment because there is an influence from the concentration of gelatin or the concentration of salt solution during the sapodilla fruit soaking process.

The analysis results showed that the panellists favoured the high concentration of gelatin and the higher concentration of a salt solution. The higher the concentration of gelatin added to the off-grade sapodilla jelly candy, the hedonic quality value of the texture also increases. This is directly proportional to the surface of the jelly candy, which was tested using a texture analyzer, where the texture of the off-grade sapodilla jelly candy is almost close to that of commercial jelly candy. In addition, the higher the concentration of gelatin in a product will result in lower syneresis in a product (Mutlu et al., 2018), resulting in a gel with a higher gel consistency, and the jelly candy product obtained becomes chewy (not too hard) which the panellists preferred. According to Rahmi et al. (2012), if the gelatin concentration is too low, the gel will become soft, or even gel will not form. But if the concentration of gelatin is too high, the gel formed will be stiff.

e. Overall

Overall acceptance is the most important because it relates to the level of

endorsement of the product by the panellists. Table 3 shows that the overall hedonic value of off-grade sapodilla jelly candy ranges from 2.792 to 3.146 (rather unfavourable). The analysis of variance ANOVA showed that the four treatments were not significantly different at the 5% test level on the panellists' preference level. This indicates that the difference in treatment on off-grade sapodilla jelly candy does not affect the panellists' preference for overall acceptance. This shows that the treatment can be used because it has almost the same level of acceptance. Overall, found the highest value in off-grade sapodilla jelly candy with L3G1 treatment.

CONCLUSION

Following the study's objectives, the greater the concentration of salt given to the off-grade sapodilla sample, it showed that the salt was able to reduce the amount of tannin content. In jelly candy, the tannin content obtained is decreased compared to the tannin content of off-grade sapodilla fruit. This is due to the unstable or too high cooking temperature. Based on the results of the texture test, it found that the L2G2 treatment (4% salt solution, 20% gelatin) was closest to the commercial texture, while based on the organoleptic test, the L3G1 treatment (6% salt solution, 18% gelatin) had the highest value by the panellists.

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A research article is an original full-length research paper which should not exceed 5,000 words (including table and figures). Research article should be prepared according to the following order: title, authors name and affiliations, abstract, keywords, introduction, materials and method, result and discussion, conclusion, acknowledgement (optional), and references.

Short communication or Review

A short communication or review is up to 3,500 words (including table and figures) and consists of title, authors name and affiliations, abstract, keywords, introduction, materials and method, result and discussion, conclusion, acknowledgement (optional), and references. A short communication should contribute an important novelty for science, technology, or application.

The authors are fully responsible for accuracy of the content. Any correspondence regarding the manuscript will be addressed to the correspondent author who is clearly stated including his/her email address, telephone and fax number (including area code), and the complete mailing address. The correspondent author will handle correspondence with editor during reviewing process. The author are required to suggest three potential reviewer names including their email address..

Preparation of the manuscript

- The manuscript should be written in a good English. It must be type written on A4 paper by using Microsoft Word processor with Arial 11 font and 1.5 spaced.
- All graphics and table should be prepared in separate pages.
- If the manuscript has been presented in scientific meeting, please mention in the footnote the detail about the meeting (name of conference, date, place).
- When animal/human subject is involved in the in-vivo study, ethical clearance should be included in the manuscript by stating the number of ethical approval obtained from ethic committee.
- Soft copy of a manuscript should be sent to the editor by e-mail.

Guideline for the manuscript

content Title

- The title of the article should be brief and informative (max. 10 words).
- The title is written all in capital letters, except for the species name of organisms.
- The institution where authors are affiliated should be completely written (Laboratory/department, and institution name).

Abstract

- Abstract written in one paragraph in English and the Indonesian language (in italics), Abstract is not more than 250 words.
- The abstract should state briefly background, material and method, the main findings supported by quantitative data which is relevant to the title, and the major conclusions.

Keywords

The keywords consists of no more than 5 important words representing the content of the article and can be used as internet searching words and arranged in alphabetical order.

Introduction

The introduction states background of the research supported mainly by the relevant references and ended with the objectives of the research.

Materials and Methods

- The materials used should include manufacture and source.
- The reagents and equipment or instruments used should include manufacture name written in this section.

- The methods used in the study should be explained in detail to allow the work to be reproduced. Reference should be cited if the method had been published.
- Specification of the instruments and equipments (except for glass wares) should also be mentioned clearly.

Results and Discussion

- The title of tables and figures should be numbered consecutively according to their appearance in the text.
- The discussion of the results should be supported by relevant references.
- Decimals numbers adjusted to the type of analysis.
- The data presented figures and tables must Standard Deviation (SD) or Standard Error of Mean (SEM).
- A brief explanation on methods for sampling replication and statistical analysis is required in the methods section.

Conclusion

Conclusion is drawn based on the result, discussion, and the objectives of the research.

Acknowledgement (if necessary)

Acknowledgement contains the institution name of funding body/grants/sponsors or institution which provides facilities for the research project, or persons who assisted in technical work and manuscript preparation

References

- References are arranged in alphabetical.
- 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.
- The name of journal/bulletin is written using standard abbreviation according to ISI's list of journal title abbreviations.
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- Year, volume and pages should be completely written.
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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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