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Dear Editor In Chief
Journal Food Processing Techniques and Technology,

Greetings from Indonesia,

I am pleased to submit an original research article entitled "**The characteristic of gelatin jelly candy from mackerel skin (*Scomberomorus commersonii*) based on proximate and sensory analysis**" for publication in the Journal Food Processing Techniques and Technology. Jelly candy is children's favourite snack because it has an attractive colour and delicious flavour. Using mackerel skin gelatine is expected to increase the protein content in jelly candy. The present study aimed to determine consumer acceptance, proximate value, and the quality of gelatin jelly candy with various natural flavours. We believe that this manuscript is appropriate for publication in Journal Food Processing Techniques and Technology because one of the peer-reviewed journals is about Fisheries Food Product Technology.

Each of the authors confirms that the manuscript has not been previously published and is not currently under consideration by any other journal. Additionally, all of the authors have agreed on this manuscript to the Journal Food Processing Techniques and Technology submission policies. I hope our manuscript is appropriate for your journal.

I appreciate your consideration

Sincerely,

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Original article

The characteristic of gelatin jelly candy from mackerel skin (*Scomberomorus commersonii*) based on proximate and sensory analysis

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Abstract: Jelly candy is children's favourite snack because it has an attractive colour and delicious flavour. Using mackerel skin gelatine is expected to increase the protein content in jelly candy. The present study aimed to determine consumer acceptance, proximate value, and the quality of gelatin jelly candy with various natural flavours. Gelatin was extracted from mackerel fish skin. Gelatin jelly candy was formulated and added with eight different natural flavours: honey, date juice, olive oil, soy milk, goat's milk, grape juice, avocado, and pumpkin. The quality of gelatin jelly candy was analyzed based on the water, ash, fat, protein content and the absence of bacterial colonies. The sensory analysis was done through the hedonic test with 10 panellists to determine the acceptance of natural ingredients. The results showed that panellists accepted all the variations in the flavour of gelatin jelly candy, including appearance, odour, flavour, and texture, with a hedonic scale of 7.00 (really like). The best formulation of gelatine gelly candy was the addition of natural flavouring containing soy milk with water (9.76 ± 0.70%), ash (0.21 ± 0.02%), protein (16.20 ± 0.37%), fat (2.32 ± 0.50%), carbohydrate (51.61 ± 0.80%), and reducing sugar (0.14 ± 0.01%). Gelatin jelly candy was free from Salmonella sp. and Escherichia coli, with a total plate count of 1 × 102 colonies/g. The mackerel skin gelatin jelly candy contains high protein and is well received by panellists. Based on these findings, gelatin jelly candy met the Indonesia National Standard for jelly candy.

Keywords: characteristics, jelly candy, nutritional value, mackerel skin

Introduction

Gelatin is a protein commonly extracted from cartilage, skin, and scales from several animals, such as cows, pigs, and fish [1]. One halal gelatin or without gelatin pig can be extracted from fishery resources such as mackerel (*Scomberomorus commersonii*) [2]. Gelatin preparations can be used in various food and non-food fields. Several research results explain that gelatin can be used as an emulsifier, stabilizer, biodegradable packaging, and microencapsulation agent. Gelatin is used due to its ability to form gels, viscosity, melting point, and form gels. One of the food products made from gelatin is candy [1].

Candy is divided into hard and soft candy (jelly). Generally, candy is made from cane sugar, corn sugar, flavourings, dyes, and gelling agents. Gelatin-based candy is called jelly candy and has a higher sugar content [3]. Jelly candy is preferred among children to introduce consuming vitamins and minerals [4]. Some jelly candies utilize natural flavourings such as nutmeg extract [5], strawberries [6] and mangoes [7]. However, some confectionery industries use synthetic acid flavourings such as citric acid [8], tartaric acid, and lactic acid as food additives whose safety has not been guaranteed. According to Fajarini and Wahyani [9], the food industry does not meet these nutrition standards because producers want to maximize profit by ignoring consumer safety. In addition, jelly candy is rarely found with a high enough protein content. Therefore, adding fish skin gelatin is expected to produce jelly candy with high protein content.

Adding natural flavourings containing vitamins and minerals is an alternative to making gelatin jelly candies. According to Kia et al. [10], food products with natural additives can improve health. The natural ingredients added in this study were honey, date juice, olive oil, soy milk, goat's milk, grapes, avocado, and pumpkin. Consumers are expected to prefer the new variants of gelatin candy flavours. Furthermore, eating jelly candy from mackerel skin gelatin will provide protein intake and reduce sugar consumption. Therefore, this study aimed to determine consumer acceptance, proximate value, and the quality of mackerel skin gelatin candy with eight flavours.

Objectives and Methods

Extraction of Mackerel Fish Skin Gelatin

The process of extracting gelatin from mackerel fish skin is referred to the Rahmawati and Pranoto [11]. Dried mackerel skin samples were soaked in water for ± 5 h. The sample was heated for ± 1 min to remove the remaining impurities. Soak the sample in 0.05 M of CH_3COOH acid solution for 10 h. The extraction process used H2O at 80°C for 2 h. The gelatin extract was then dried for three days at 55°C.

Preparation of Gelatin Jelly Candy

This study designed eight different natural flavor treatments (honey, date juice, olive oil, soy milk, goat's milk, grape juice, avocado, and pumpkin) with three repetitions. The procedure for making gelatin jelly candy from mackerel fish gelatin was referred to by Eletra et al. [12] with some modifications. All materials, such as 75 g of gelatin, 85 g of sucrose, 5 g of salt, 85 g of natural ingredients, were mixed. After that, 300 mL of cold water was added and stirred until evenly distributed. The sample was heated at 100°C for 2 min, then moulded with soft silicone bear-shaped until the sample cooled.

Water Content Assay

The water content was analyzed based on AOAC [13] method. The sample was weighed up to ± 2 g in a porcelain dish of known mass. The samples were dried in an oven at 105°C for 3 h. The sample was then cooled in a desiccator; then, the sample was weighed for further analysis.

Ash Content Assay

The sample was weighed up to ± 2 g on a porcelain dish of known mass. Then it was ignited on a burner flame and burned in an electric furnace at a maximum temperature of 550°C until complete combustion. Then, samples were cooled in a desiccator and weighed until a constant mass was obtained [13].

Fat Content Assay

The sample was weighed to ± 2 g and then placed in a cotton-lined paper bag. The sample's paper sleeve was covered with cotton, dried in an oven at a temperature of not more than 80°C for ± 1 h, and then put into the Soxhlet apparatus connected to an oil bottle containing boiling chips. Samples were dried, the weight was determined, and then extracted with hexane for ± 6 h. The hexane was filtered, and the fat extract was dried in an oven at 105°C. The fat extract was cooled and weighed. The cooling process was repeated until a constant weight was reached [13].

Protein Content Assay

The analysis of protein content is based on the AOAC method [13]. During the digestion, the sample was weighed as much as ± 1 g and put into a 100 mL Kjehdahl flask, and then 10 mL of concentrated sulfuric acid was pipetted into a Kjeldahl flask. A catalyst was added to speed up the digestion. The distillation stage was continued by diluting the digestion results with distilled water up to 100 mL. After homogenization and cooling, 5 mL was pipetted into a distillation flask. A total of 10 mL of 30% sodium hydroxide solution was added through the walls of the still flask until a layer formed under the acid solution. The container was filled with 10 mL of 0.1 N hydrochloric acid solution and drained with a methyl red indicator. The titration step resulted from the distillation, which was accommodated in an Erlenmeyer containing 0.1 N hydrochloric acids with 5 drops of methyl red indicator added and titrated directly using 0.1 N of sodium hydroxide solution. The titration step resulted in a pink-to-yellow colour. This treatment was carried out three times for each sample.

Total Plate Count

The total plate count method was referred to Salanggon et al. [14] method. A total of 25 g of sample was weighed aseptically, added with 225 mL of Butterfield's Phosphate Buffered, and then homogenized for 2 min. This homogenate was diluted to 10-1. 1 mL of the homogenate was pipetted using a sterile pipette and was put into a vial containing 9 mL of Butterfield's Phosphate Buffered solution to obtain a sample with a dilution of 10-2. Each diluent was stirred at least 25 times, the same for the 10-3, 10-4, 10-5, and other dilutions. The volume of each diluent was 1 mL and was repeated in a sterile petri dish using a sterile pipette. In each petri dish containing the sample, 12-15 mL of PCA medium was cooled to 5°C. After the agar hardened, it was incubated for 8 h at 35°C. The number of bacterial colonies in a petri dish was counted.

Screening of *Escherichia coli*

Twenty-five grams of the sample was homogenized with 225 mL of peptone buffer and then enriched at 37°C for 18 h. Next, 1 mL of the sample was inoculated directly into 9 mL of MacConkey broth (CM5a; Oxoid) and then incubated at 37°C for 18 h [15]. The enriched broth preparations were sprayed directly onto Eosin Methylene Blue Agar (EMBA) and then incubated at 37°C for 18-24 h. The isolates were confirmed biochemically using the E. coli antiserum rapid diagnostic Kit. Escherichia coli O antiserum consisted of polyclonal antibodies used for zero-classification of *Escherichia coli* O antigens.

Screening of *Salmonella* sp.

At the pre-enrichment stage, the collected samples were serially diluted (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and so on) using peptone water [16]. At the enrichment stage, it was planted on sterile Selenite Cystine Broth (SCB) selective media and was incubated at 37°C for 24 h. From the enrichment stage in each dilution, 1 mL was taken and planted on Xylose Lysine Deoxycholate (XLD) selective media. Bacteria growth was analyzed by counting the colonies and observing the morphology of the colonies. Purification of bacteria was using quadrant streaking technique on XLD media so that it was then incubated at 37°C for 48 h. Purification targets were colonies with different colony morphology and belonged to Gram-negative bacteria.

Furthermore, two types of colonies were selected to characterize the colonies and bacteria. Each colony was made in duplicate so that 40 colonies were obtained. The purification results were grown on slanted Nutrient Agar (NA) media, incubated at 37°C for 24 h, and stored at -20°C as stock culture. Storage of pure bacterial isolates with the addition of 60% glycerol in a ratio of 1:1 at a temperature of -80°C.

Sensory Analysis

In the study, gelatin jelly candy with the addition of natural ingredients (honey, date juice, olive oil, soy milk, goat's milk, grapes, avocado, and pumpkin) was placed on a white plastic plate with a glass of water, coded, and served to

panellists randomly at under the light. The sample was evaluated by 10 trained panellists from the laboratory of testing and quality control of fishery products, Banjarbaru, South Kalimantan. The descriptive terms chosen were appearance, odour, texture, and flavour. Panellists rated acceptance of gelatin jelly candy on a 9-point hedonic scale (1. very dislike; 2. dislike; 3. rather dislike; 4. Ordinary; 5. rather like; 6. Likes; 7. really like; 8. very much like; and 9. really like it very much)

Data Analysis

All data which passed the homogeneity and normality test were further analyzed using SPSS for Windows version 20.0. ANOVA (Analysis of Variance) further analyzed the significance of the parameters ($p<0.05$) followed by the Duncan Test.

Results and Discussion

Mackerel Skin Gelatin Characterization

The water content of fish skin gelatin was 6.45%, which is lower than the raw material (Table 1). The water content of gelatin was higher than that of Viji et al. [17] from the fish skins (4.81 ± 0.41%) and lower than the research by Ismail and Abdullah [18] from the blackfish skins (6.93%). However, the water content of the gelatin meets the Indonesian National Standard (SNI) No. 01-3735-1995 [19]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has a maximum of 18% [20], and the Gelatin Manufacturers Institute of America (GMIA) has a 10.5 ± 1.5% [21].

Table 1

Mackerel skin gelatin proximate

Proximate (%)	Mackerel raw skin	Mackerel dry skin	Mackerel gelatin
Water	60.74	20.18	6.45
Ash	5.23	2.36	0.86
Protein	35.63	69.76	91.52
Fat	4.85	2.24	0.73
Carbohydrate	60.74	20.18	6.45

According to Esfahani et al. [22], water content plays a role in determining the stability of dry products. High water content causes particle agglomeration and accelerates microbial growth and oxidation. While ash content was essential for evaluating gelatin quality, especially mineral and gelatin purity. The ash content of fish skin gelatin (Table 1) meets the SNI standards (3.25%) [19]; JECFA (maximum 2.00%) [20], and GMIA (0.5 ± 0.4) – (1.5 ± 0.5) %. An aquatic environment, habitat, and species affect the ash content of fish skin gelatin. The extraction process influenced gelatin ash content [23].

The protein content of gelatin was influenced by the time and concentration of chemicals used. This concentration caused more amino acid bonds to be broken, so more protein was broken down in the extraction process. The results showed the protein content of gelatin was 91.52%, which increased from the initial raw material for dry skin (69.76%) and 35.63% for wet skin. The protein content in gelatin meets the SNI (87.25%), which was higher than Zarubin et al. [23] by 73.2 ± 0.9%. The difference in the protein content is caused by differences in the concentration of acid and base used in gelatin extraction. The combination of acid and base concentration and immersion time produces high protein content between acid-base, concentration, and length of soaking time [24].

The content of fat affected the quality of raw materials during storage. The fat content of skin gelatin was 0.73%, lower than the initial raw materials for dry skin (4.85%) and wet skin (2.24%). This result was almost close to the research by Gunawan et al. [24], in which fat content was 0.71 ± 0.07%. High-fat content affects the shelf life of gelatin to be relatively short [23]. The high-fat content in gelatin jelly candy affects the quality of gelatin in the application process. The value of carbohydrates in gelatin was 6.45%, which was very small compared to the raw material for dry skin (20.18%) and wet skin (60.74%). According to Khirzin et al. [25], carbohydrates are not an essential parameter in gelatin but only supporting data. The essential parameters of gelatin were protein, water, and ash.

Sensory of Gelatin Jelly Candy

The sensation is a psycho-physiological process in which sensory recognition of object characteristics is carried out through stimuli received by the senses. The panellists' assessment showed the "really like" to "very much like" category of the appearance of the gelatin jelly candy. Figure 1 shows that adding honey has the highest value compared to date palm juice, olive oil, and grape juice, both liquid forms. This was because the natural colour of honey, clear brown when added, would give a light brown colour. Adding olive oil and date juice makes the jelly candy blackish brown [26], while adding grape juice makes the jelly candy yellowish [27]. The appearance of the jelly candy with the addition of soy milk and goat's milk was insignificant different; both were yellow-brown but very different from the addition of honey. This was because the milk powder had a colour similar to that of the jelly candy formulation. According to Charoenphun [28], milk powder in jelly candy produces a light yellow to

pale white colour. With the addition of avocado and pumpkin, gelatin jelly candy will turn black. This is because avocado naturally produces ethylene gas closely related to ripening which converts methionine to S-adenosylmethionine, which causes blackness [29] when added to food.

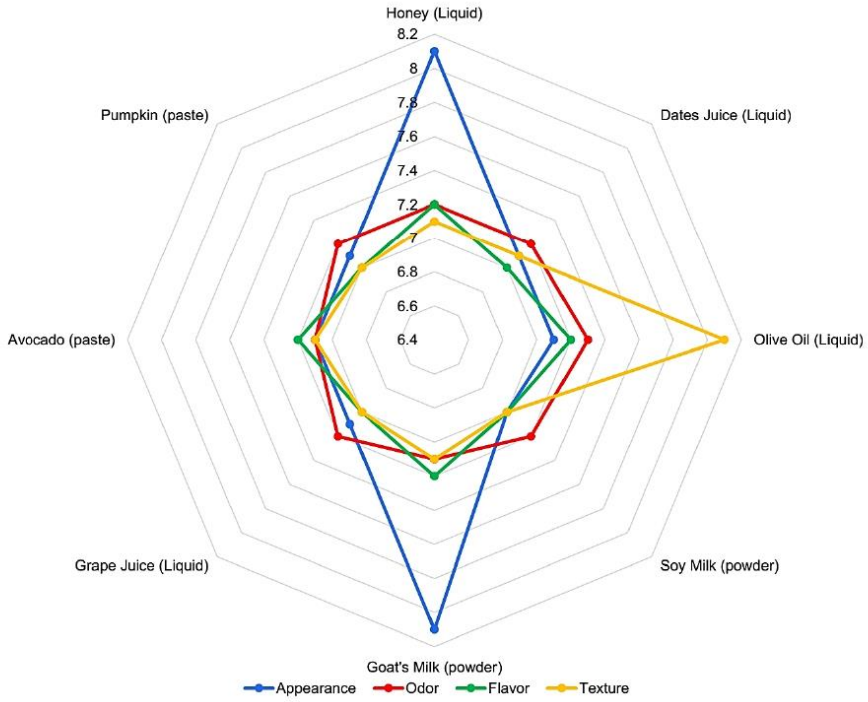

Figure 1. Sensory analysis of gelatin jelly candy

The panellist gave a "really like" category rating of all flavour variants from the odour of gelatin jelly candy. The addition of goat's milk powder had the highest odour value (7.6) with olive oil (7.3) and other natural additives such as honey, grape juice, date palm juice, soy milk, avocado, and pumpkin (7.1 – 7.2). This was because the aroma of goat's milk was stronger than the other ingredients. Even though it is not processed, goat's milk has a strong aroma and taste [30]. This aroma is caused by caproic acid in goat's milk. The goaty aroma can be removed by adding rare sugar (D-psychose, D-tagatose, D-sorbose) to neutralize caproic acid (goaty smell) through a glycation reaction. Znamirowska et al. [31] stated that fresh goat milk contains protein (2.69 ± 0.22%), fat (2.98 ± 0.53%), and general acidity (6.20 ± 1.20%).

The panellists assessed the "really like" category on the value of flavour. The addition of natural ingredients has varying values (7.0-7.4). The addition of avocado paste has the highest value (7.4). This was because avocados had a naturally sweet taste, soft and savoury. The savoury flavour is obtained from the fat vegetable content in avocados between 0.71 – 2.15% and the total fatty acid content (37 – 85%) [32].

From the gelatin jelly candy texture, the panellists assessed the "really like" to “very much like” category, with olive oil adding a significant difference from other natural ingredients. This was because olive oil had a characteristic yellowish-gold colour and even greenish, a relatively thick texture, and was more oily. According to Bermúdez-Oria et al. [33], adding gelatin serves as a stabilizer, adhesive and gelling agent in jelly candy, while olive oil gives an oily and shiny chewy texture to jelly candy.

Gelatin Jelly Candy Proximate Analysis Results

Proximate analysis of gelatin jelly candy results with the addition of various natural ingredients can be seen in Table 2. Based on the statistical analysis of the water content of gelatin jelly candy with various types of natural ingredients, there was no significant effect ($p>0.05$). These results indicated that each added natural ingredient had different water content. Adding soy milk and powdered goat's milk produced the best water content between 9.76 ± 0.70 – 9.92 ± 0.68%. This is because powdered soy milk contains 3.31 ± 0.27% water [34] and powdered goat's milk is 5.48 ± 0.23% [35]. The addition of natural ingredients in liquid form (honey, dates, olive oil, and

grapes) had a very high water content compared to pasta ingredients (avocado and pumpkin). This is strongly influenced by the water content of added natural raw materials such as honey containing 5.20 ± 0.33% of water [36], grapes 21.17 ± 0.76% [37], avocado 34.28 ± 0.95% [38], and pumpkin 14.18 ± 0.22% [39].

Table 2

Proximate analysis of gelatin jelly candy

Treatment of natural ingredients	Proximate (%)					
	Water	Ash	Protein	Fat	Carbohydrate	Sugar Reduction
Honey (liquid)	10.25 ± 0.42^{a}	$0.13 + 0.01^{a}$	$15.67 + 0.52^{a}$	2.20 ± 0.23^{a}	63.93 ± 1.28^{a}	0.18 ± 0.07^{a}
Dates juice (liquid)	14.09 ± 0.84^{b}	0.32 ± 0.01^{b}	15.82 ± 0.53^{a}	2.35 ± 0.26^{a}	64.03 ± 1.14^{b}	0.26 ± 0.03^{a}
Olive oil (liquid)	10.71 ± 0.60^{a}	0.18 ± 0.01^{c}	15.77 ± 0.67^{a}	2.09 ± 0.22^{a}	58.26 ± 1.60^{c}	0.13 ± 0.05^{a}
Soy Milk (powder)	9.76 ± 0.70^{a}	0.21 ± 0.02^{d}	16.20 ± 0.37^{a}	2.32 ± 0.50^{a}	51.61 ± 0.80^{c}	0.14 ± 0.01^{a}
Goat's milk (powder)	9.92 ± 0.68^{a}	0.20 ± 0.03^{cde}	13.97 ± 0.36^{b}	1.99 ± 0.28^{a}	57.57 ± 0.79^{ad}	0.17 ± 0.09^{a}
Grape juice (liquid)	10.82 ± 0.78^{a}	0.15 ± 0.01^{a}	13.62 ± 0.37^{b}	2.31 ± 0.33^{a}	62.55 ± 0.59^{ade}	0.18 ± 0.05^{a}
Avocado (paste)	10.23 ± 0.46^{a}	0.18 ± 0.01^{cef}	14.19 ± 0.45^{b}	1.67 ± 0.30^{a}	63.94 ± 1.46^{adef}	0.12 ± 0.05^{a}
Pumpkin (paste)	10.48 ± 0.56^{a}	0.14 ± 0.02^{a}	14.39 ± 0.64^{b}	2.29 ± 0.35^{a}	61.81 ± 1.20^{aeg}	0.22 ± 0.05^{a}
SNI about jelly candy	20.00	3.00	–	–	–	25.00

Note: Means in the rows with different superscripts are significantly ($p \leq 0.05$) different.

The concentration of water content greatly affects the quality and durability of gelatin jelly candy [40]. Based on the study results, the overall water content of gelatin jelly candy still followed the standards set by SNI No. 3547-2-2008 about jelly candy [41], a maximum of 20.00%. The variance analysis shows that adding natural ingredients to manufacture jelly candy significantly affected the water content ($p<0.05$). The highest ash content was added with 0.21 ± 0.02% soy milk powder. The value of ash content in this study met the standards required by SNI for jelly candy, which was a maximum of 3.00%. The high ash content of jelly candy in the treatment of soy milk and powdered goat's milk was due to the initial raw materials containing high minerals. The ash content of powdered soybean milk is 0.40 ± 0.05% [34], and powdered goat's milk is 0.07 ± 0.00% [42].

During the jelly candy processing process, the total minerals in the raw materials did not change significantly. The ash content and gelling agents in making jelly candy will be higher. For the ash content of jelly candy with natural additives in liquid form (honey, olive oil, dates juice, and grapes) and paste form (avocado and pumpkin), the ash content tended to be lower. This is because the fruit extraction process decreases the mineral content of the fruit juice. The components are easily decomposed or evaporated during fruit ashing [43].

Table 2 showed that the value of protein content ranged from 13.62 ± 0.37 – 16.20 ± 0.37%. The variance analysis showed that adding natural ingredients to produce gelatin jelly candy significantly affected protein content ($p<0.05$). The highest protein content was added with 16.20 ± 0.37% powdered soy milk. This is because fresh soy milk contains 23.08 ± 0.16% protein [44], while powdered soy milk has a protein content of 5.09 ± 0.29% [34]. Interestingly, the protein content of gelatin produces a very high protein content of 91.52%.

Protein intake is needed to build muscle mass, especially for toddlers. Jelly candy is one of the media to deliver bioactive compounds required by the toddler's body. Protein content in the treatment of the addition of natural ingredients in liquid form (honey, olive oil, dates, and grapes) and paste form (avocado and pumpkin) also had a relatively high protein content [45]. According to Kia et al. [10], jelly candy with gelatin tends to have a higher protein content.

Adding natural ingredients to manufacture jelly candy did not significantly affect fat content ($p<0.05$). After adding natural ingredients or soy milk powder, the fat content was 2.32 ± 0.50%. The high and low-fat content of jelly candy was due to differences in the raw materials used. According to Nemo and Bacha [36], the fat content in honey is 0.27 ± 0.20%, soy milk powder is 11.36 ± 0.44% [34], powdered goat's milk is 1.02 ± 0.09% [42], grapes 0.64 ± 1.17% [37], avocado 6.66 ± 0.10 [38], pumpkin 4.50 ± 0.21% [39].

The total value of carbohydrates in the study ranged from 51.61 ± 0.80 – 64.03 ± 1.14%. The variance analysis showed that adding natural ingredients to manufacture gelatin jelly candy significantly affected total carbohydrates ($p<0.05$). The highest total carbohydrate value with the addition of dates juice was 64.03 ± 1.14%. This was because the calculation of carbohydrates was carried out using the by-difference method. The high value of carbohydrates in each treatment could meet the body's energy needs.

Liu et al. (2019) explained that carbohydrates give food a sweet taste, especially monosaccharides and disaccharides that provide energy for the body. The value of carbohydrates in the study was influenced by raw materials containing high carbohydrates, such as grapes 49.17 ± 2.31% [37], avocado 54.23 ± 0.02% [38], and pumpkin 61.71 ± 0.10% [39].

Sugar residue was a substance left or left in a specific chemical process; this residue could be likened to salt. Based on the study results, the average value was 0.12 ± 0.05 – 0.26 ± 0.05%. The results followed the standard SNI set about jelly candy, a maximum of 25.00%. Based on a statistical analysis of the sugar reduction value of gelatin jelly candy with the treatment of various natural ingredients, there was no significant effect ($p>0.05$). This was because the sugar residue came from the sucrose produced by jelly candy. Garusti et al. [46] stated that palm sugar contains 87.10% sucrose content with 6.06% reducing sugar. The content of reducing sugars is closely related to the inversion of sucrose into reducing sugars. The low, reducing sugar in the study was due to the natural ingredients used. Reducing sugar in natural ingredients tends to be lower and can be easily synthesized by the body [47].

Microbiological Analysis

The total plate count (TPC) value was $\leq 1 \times 10^2$ colonies/g, which still followed the SNI for jelly candy which was 3×10^3 colonies/g [41]. This was because the addition of sucrose in making gelatin jelly candy had antibacterial properties. Balakrishnan et al. [48] explained that sucrose would be oxidized to form acetals in the heating process. The acetal group can release cation-charged ions that interact with the anionic charge of the microbial cell membrane through electrostatic bonds leading to increased cell permeability and cell leakage leading to cell death.

All treatments in gelatin jelly candy exhibited negative results for *E. coli* and *Salmonella* sp. The results also met SNI's standards about jelly candy, which stated that jelly candy must be negative for *E. coli* and *Salmonella* sp. There was no identification of *E. coli* and *Salmonella* sp. because the natural ingredients used in manufacturing jelly candy have a natural antibacterial activity. Handayani et al. [49] explained that some natural ingredients in liquid form have antibacterial properties against *Staphylococcus aureus* and *E. coli* with a maximum concentration of 0.04 g/mL.

Conclusions

The addition of natural flavour to the skin mackerel gelatin jelly candy could be well received by panellists, with the "really like" to "very much like" category. The nutritional and quality of gelatin jelly candy met the Indonesia National Standard about jelly candy. The best treatment was the addition of natural flavouring ingredients soy milk with the highest protein dan the lowest carbohydrate content. Furthermore, it is necessary to analyze the amino acids of each flavour variant of gelatin jelly candy.

Conflict of interest

The authors declare no conflict of interest.

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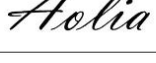
Сити Айсия д-р, доцент кафедры технологии рыбных продуктов факультета рыбного хозяйства и морского хозяйства Университета Ламбунг Мангкурат, Джалан А. Яни КМ 36.5 Simpang Empat Ban-jarbaru 70714, Южный	**Aolia Ramadhani** Assistance Professor, Department of Fisheries Product Technology, Faculty of Fisheries and Marine, Universitas Lambung Mangkurat, Jalan A. Yani KM 36.5 Simpang Empat Ban-jarbaru 70714, South Kalimantan, Indonesia, phone: +7 (3842) 39-68-57, e-mail:	Тел. +628-5240685886, e-mail: aolia.ramadhani@gmail.com 

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Original article

The characteristic of gelatin jelly candy from mackerel skin (*Scomberomorus commersonii*) based on proximate and sensory analysis

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Abstract: Jelly candy is children's favourite snack because it has an attractive colour and delicious flavour. Using mackerel skin gelatine is expected to increase the protein content in jelly candy. The present study aimed to determine consumer acceptance, proximate value, and the quality of gelatin jelly candy with various natural flavours. Gelatin was extracted from mackerel fish skin. Gelatin jelly candy was formulated and added with eight different natural flavours: honey, date juice, olive oil, soy milk, goat's milk, grape juice, avocado, and pumpkin. The quality of gelatin jelly candy was analyzed based on the water, ash, fat, protein content and the absence of bacterial colonies. The sensory analysis was done through the hedonic test with 10 panellists to determine the acceptance of natural ingredients. The results showed that panellists accepted all the variations in the flavour of gelatin jelly candy, including appearance, odour, flavour, and texture, with a hedonic scale of 7.00 (really like). The best formulation of gelatine gelly candy was the addition of natural flavouring containing soy milk with water (9.76 ± 0.70%), ash (0.21 ± 0.02%), protein (16.20 ± 0.37%), fat (2.32 ± 0.50%), carbohydrate (51.61 ± 0.80%), and reducing sugar (0.14 ± 0.01%). Gelatin jelly candy was free from *Salmonella* sp. and *Escherichia coli*, with a total plate count of 1 × 102 colonies/g. The mackerel skin gelatin jelly candy contains high protein and is well received by panellists. Based on these findings, gelatin jelly candy met the Indonesia National Standard for jelly candy.

Keywords: characteristics, jelly candy, nutritional value, mackerel skin

Introduction

Gelatin is a protein commonly extracted from cartilage, skin, and scales from several animals, such as cows, pigs, and fish [1]. One halal gelatin or without gelatin pig can be extracted from fishery resources such as mackerel (*Scomberomorus commersonii*) [2]. Gelatin preparations can be used in various food and non-food fields. Several research results explain that gelatin can be used as an emulsifier, stabilizer, biodegradable packaging, and microencapsulation agent. Gelatin is used due to its ability to form gels, viscosity, melting point, and form gels. One of the food products made from gelatin is candy [1].

Candy is divided into hard and soft candy (jelly). Generally, candy is made from cane sugar, corn sugar, flavourings, dyes, and gelling agents. Gelatin-based candy is called jelly candy and has a higher sugar content [3]. Jelly candy is preferred among children to introduce consuming vitamins and minerals [4]. Some jelly candies utilize natural flavourings such as nutmeg extract [5], strawberries [6] and mangoes [7]. However, some confectionery industries use synthetic acid flavourings such as citric acid [8], tartaric acid, and lactic acid as food additives whose safety has not been guaranteed. According to Yanchenko et al. [9], the food industry does not meet these nutrition standards because producers want to maximize profit by ignoring consumer safety. In addition, jelly candy is rarely found with a high enough protein content. Therefore, adding fish skin gelatin is expected to produce jelly candy with high protein content.

Adding natural flavourings containing vitamins and minerals is an alternative to making gelatin jelly candies. According to Kia et al. [10], food products with natural additives can improve health. The natural ingredients added in this study were honey, date juice, olive oil, soy milk, goat's milk, grapes, avocado, and pumpkin. Consumers are expected to prefer the new variants of gelatin candy flavours. Furthermore, eating jelly candy from mackerel skin gelatin will provide protein intake and reduce sugar consumption. Therefore, this study aimed to determine consumer acceptance, proximate value, and the quality of mackerel skin gelatin candy with eight flavours.

Objectives and Methods

Extraction of Mackerel Fish Skin Gelatin

The process of extracting gelatin from mackerel fish skin is referred to the Rahmawati and Pranoto [11]. Dried mackerel skin samples were soaked in water for ± 5 h. The sample was heated for ± 1 min to remove the remaining impurities. Soak the sample in 0.05 M of CH_3COOH acid solution for 10 h. The extraction process was done by heating with H_2O at 80°C for 2 h. The gelatin extract was then dried in oven for three days at 55°C.

Preparation of Gelatin Jelly Candy

This study designed eight different natural flavor treatments (honey, date juice, olive oil, soy milk, goat's milk, grape juice, avocado, and pumpkin) with three repetitions. The procedure for making gelatin jelly candy from mackerel fish gelatin was referred to by Eletra et al. [12] with some modifications. All materials, such as 75 g of gelatin, 85 g of sucrose, 5 g of salt, 85 g of natural ingredients, were mixed. After that, 300 mL of cold water was added and stirred until evenly distributed. The sample was heated at 100°C for 2 min, then moulded with soft silicone bear-shaped until the sample cooled.

Water Content Analysis

The water content was analyzed based on AOAC [13] method. The sample was weighed up to 2 ± 0.01 g in a porcelain dish of known mass. The samples were dried in an oven at 105°C for 3 h. The sample was then cooled in a desiccator; then, the sample was weighed for further analysis.

Ash Content Analysis

The sample was weighed up to 2 ± 0.01 g on a porcelain dish of known mass. Then it was ignited on a burner flame and burned in an electric furnace at a maximum temperature of 550°C until complete combustion. Then, samples were cooled in a desiccator and weighed until a constant mass was obtained [13].

Fat Content Analysis

The sample was weighed to 2 ± 0.01 g and then placed in a cotton-lined paper bag. The sample's paper sleeve was covered with cotton, dried in an oven at a temperature of not more than 80°C for ± 1 h, and then put into the Soxhlet apparatus connected to an oil bottle containing boiling chips. Samples were dried, the weight was determined, and then extracted with hexane for ± 6 h. The hexane was filtered, and the fat extract was dried in an oven at 105°C. The fat extract was cooled and weighed. The cooling process was repeated until a constant weight was reached [13].

Protein Content Analysis

The analysis of protein content is based on the AOAC method [13]. During the digestion, the sample was weighed as much as 1 ± 0.01 g and put into a 100 mL Kjehdahl flask, and then 10 mL of concentrated sulfuric acid was pipetted into a Kjeldahl flask. A catalyst was added to speed up the digestion. The distillation stage was continued by diluting the digestion results with distilled water up to 100 mL. After homogenization and cooling, 5 mL was pipetted into a distillation flask. A total of 10 mL of 30% sodium hydroxide solution was added through the walls of the still flask until a layer formed under the acid solution. The container was filled with 10 mL of 0.1 N hydrochloric acid solution and drained with a methyl red indicator. The titration step resulted from the distillation, which was accommodated in an Erlenmeyer containing 0.1 N hydrochloric acids with 5 drops of methyl red indicator added and titrated directly using 0.1 N of sodium hydroxide solution. The titration step resulted in a pink-to-yellow colour. This treatment was carried out three times for each sample.

Total Plate Count

The total plate count method was referred to Salanggon et al. [14] method. A total of 25 g of sample was weighed aseptically, added with 225 mL of Butterfield's Phosphate Buffered, and then homogenized for 2 min. This homogenate was diluted to 10-1. 1 mL of the homogenate was pipetted using a sterile pipette and was put into a vial containing 9 mL of Butterfield's Phosphate Buffered solution to obtain a sample with a dilution of 10-2. Each diluent was stirred at least 25 times, the same for the 10-3, 10-4, 10-5, and other dilutions. The volume of each diluent was 1 mL and was repeated in a sterile petri dish using a sterile pipette. In each petri dish containing the sample, 12-15 mL of PCA medium was cooled to 5°C. After the agar hardened, it was incubated for 8 h at 35°C. The number of bacterial colonies in a petri dish was counted.

Screening of *Escherichia coli*

Twenty-five grams of the sample was homogenized with 225 mL of peptone buffer and then enriched at 37°C for 18 h. Next, 1 mL of the sample was inoculated directly into 9 mL of MacConkey broth (CM5a; Oxoid) and then incubated at 37°C for 18 h [15]. The enriched broth preparations were sprayed directly onto Eosin Methylene Blue Agar (EMBA) and then incubated at 37°C for 18-24 h. The isolates were confirmed biochemically using the E. coli antiserum rapid diagnostic Kit. Escherichia coli O antiserum consisted of polyclonal antibodies used for zero-classification of *Escherichia coli* O antigens.

Screening of *Salmonella* sp.

At the pre-enrichment stage, the collected samples were serially diluted (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and so on) using peptone water [16]. At the enrichment stage, it was planted on sterile Selenite Cystine Broth (SCB) selective media and was incubated at 37°C for 24 h. From the enrichment stage in each dilution, 1 mL was taken and planted on Xylose Lysine Deoxycholate (XLD) selective media. Bacteria growth was analyzed by counting the colonies and observing the morphology of the colonies. Purification of bacteria was using quadrant streaking technique on XLD media so that it was then incubated at 37°C for 48 h. Purification targets were colonies with different colony morphology and belonged to Gram-negative bacteria.

Furthermore, two types of colonies were selected to characterize the colonies and bacteria. Each colony was made in duplicate so that 40 colonies were obtained. The purification results were grown on slanted Nutrient Agar (NA) media, incubated at 37°C for 24 h, and stored at -20°C as stock culture. Storage of pure bacterial isolates with the addition of 60% glycerol in a ratio of 1:1 at a temperature of -80°C.

Sensory Analysis

In the study, gelatin jelly candy with the addition of natural ingredients (honey, date juice, olive oil, soy milk, goat's milk, grapes, avocado, and pumpkin) was placed on a white plastic plate with a glass of water, coded, and served to

panellists randomly at under the light. The sample was evaluated by 10 trained panellists from the laboratory of testing and quality control of fishery products, Banjarbaru, South Kalimantan. The descriptive terms chosen were appearance, odour, texture, and flavour. Panellists rated acceptance of gelatin jelly candy on a 9-point hedonic scale (1. very dislike; 2. dislike; 3. rather dislike; 4. Ordinary; 5. rather like; 6. Likes; 7. really like; 8. very much like; and 9. really like it very much)

Data Analysis

All data which passed the homogeneity and normality test were further analyzed using SPSS for Windows version 20.0. ANOVA (Analysis of Variance) further analyzed the significance of the parameters ($p<0.05$) followed by the Duncan Test.

Results and Discussion

Mackerel Skin Gelatin Characterization

The water content of fish skin gelatin was 6.45%, which is lower than the raw material (Table 1). The water content of gelatin was higher than that of Viji et al. [17] from the fish skins (4.81 ± 0.41%) and lower than the research by Ismail and Abdullah [18] from the blackfish skins (6.93%). However, the water content of the gelatin meets the Indonesian National Standard (SNI) No. 01-3735-1995 [19]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has a maximum of 18% [20], and the Gelatin Manufacturers Institute of America (GMIA) has a 10.5 ± 1.5% [21].

Table 1

Mackerel skin gelatin proximate

Proximate (%)	Mackerel raw skin	Mackerel dry skin	Mackerel gelatin
Water	60.74	20.18	6.45
Ash	5.23	2.36	0.86
Protein	35.63	69.76	91.52
Fat	4.85	2.24	0.73
Carbohydrate	60.74	20.18	6.45

According to Esfahani et al. [22], water content plays a role in determining the stability of dry products. High water content causes particle agglomeration and accelerates microbial growth and oxidation. While ash content was essential for evaluating gelatin quality, especially mineral and gelatin purity. The ash content of fish skin gelatin (Table 1) meets the SNI standards (3.25%) [19]; JECFA (maximum 2.00%) [20], and GMIA (0.5 ± 0.4) – (1.5 ± 0.5) %. An aquatic environment, habitat, and species affect the ash content of fish skin gelatin. The extraction process influenced gelatin ash content [23].

The protein content of gelatin was influenced by the time and concentration of chemicals used. This concentration caused more amino acid bonds to be broken, so more protein was broken down in the extraction process. The results showed the protein content of gelatin was 91.52%, which increased from the initial raw material for dry skin (69.76%) and 35.63% for wet skin. The protein content in gelatin meets the SNI (87.25%), which was higher than Zarubin et al. [23] by 73.2 ± 0.9%. The difference in the protein content is caused by differences in the concentration of acid and base used in gelatin extraction. The combination of acid and base concentration and immersion time produces high protein content between acid-base, concentration, and length of soaking time [24].

The content of fat affected the quality of raw materials during storage. The fat content of skin gelatin was 0.73%, lower than the initial raw materials for dry skin (4.85%) and wet skin (2.24%). This result was almost close to the research by Gunawan et al. [24], in which fat content was 0.71 ± 0.07%. High-fat content affects the shelf life of gelatin to be relatively short [23]. The high-fat content in gelatin jelly candy affects the quality of gelatin in the application process. The value of carbohydrates in gelatin was 6.45%, which was very small compared to the raw material for dry skin (20.18%) and wet skin (60.74%). Carbohydrates are not an essential parameter in gelatin but only supporting data. The essential parameters of gelatin were protein, water, and ash.

Sensory of Gelatin Jelly Candy

The sensation is a psycho-physiological process in which sensory recognition of object characteristics is carried out through stimuli received by the senses [25]. The panellists' assessment showed the "really like" to "very much like" category of the appearance of the gelatin jelly candy. Figure 1 shows that adding honey has the highest value compared to date palm juice, olive oil, and grape juice, both liquid forms. This was because the natural colour of honey, clear brown when added, would give a light brown colour. Adding olive oil and date juice makes the jelly candy blackish brown [26], while adding grape juice makes the jelly candy yellowish [27]. The appearance of the jelly candy with the addition of soy milk and goat's milk was insignificant different; both were yellow-brown but very different from the addition of honey. This was because the milk powder had a colour similar to that of the jelly candy formulation. According to Charoenphun [28], milk powder in jelly candy

produces a light yellow to pale white colour. With the addition of avocado and pumpkin, gelatin jelly candy will turn black. This is because avocado naturally produces ethylene gas closely related to ripening which converts methionine to S-adenosylmethionine, which causes blackness [29] when added to food.

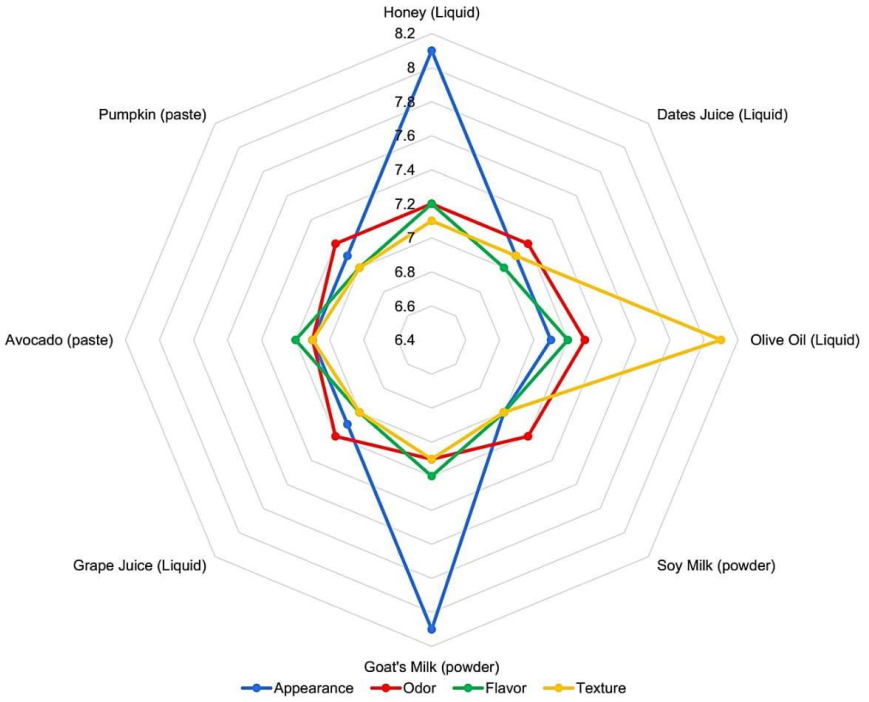

Figure 1. Sensory analysis of gelatin jelly candy

The panellist gave a "really like" category rating of all flavour variants from the odour of gelatin jelly candy. The addition of goat's milk powder had the highest odour value (7.6) with olive oil (7.3) and other natural additives such as honey, grape juice, date palm juice, soy milk, avocado, and pumpkin (7.1 – 7.2). This was because the aroma of goat's milk was stronger than the other ingredients. Even though it is not processed, goat's milk has a strong aroma and taste [30]. This aroma is caused by caproic acid in goat's milk. The goaty aroma can be removed by adding rare sugar (D-psychose, D-tagatose, D-sorbose) to neutralize caproic acid (goaty smell) through a glycation reaction. Znamirowska et al. [31] stated that fresh goat milk contains protein (2.69 ± 0.22%), fat (2.98 ± 0.53%), and general acidity (6.20 ± 1.20%).

The panellists assessed the "really like" category on the value of flavour. The addition of natural ingredients has varying values (7.0-7.4). The addition of avocado paste has the highest value (7.4). This was because avocados had a naturally sweet taste, soft and savoury. The savoury flavour is obtained from the fat vegetable content in avocados between 0.71 – 2.15% and the total fatty acid content (37 – 85%) [32].

From the gelatin jelly candy texture, the panellists assessed the "really like" to “very much like” category, with olive oil adding a significant difference from other natural ingredients. This was because olive oil had a characteristic yellowish-gold colour and even greenish, a relatively thick texture, and was more oily. According to Bermúdez-Oria et al. [33], adding gelatin serves as a stabilizer, adhesive and gelling agent in jelly candy, while olive oil gives an oily and shiny chewy texture to jelly candy.

Gelatin Jelly Candy Proximate Analysis Results

Proximate analysis of gelatin jelly candy results with the addition of various natural ingredients can be seen in Table 2. Based on the statistical analysis of the water content of gelatin jelly candy with various types of natural ingredients, there was no significant effect ($p>0.05$). These results indicated that each added natural ingredient had different water content. Adding soy milk and powdered goat's milk produced the best water content between 9.76 ± 0.70 – 9.92 ± 0.68%. This is because powdered soy milk contains 3.31 ± 0.27% water [34] and powdered goat's milk is 5.48 ± 0.23% [35]. The addition of natural ingredients in liquid form (honey, dates, olive oil, and

grapes) had a very high water content compared to pasta ingredients (avocado and pumpkin). This is strongly influenced by the water content of added natural raw materials such as honey containing 5.20 ± 0.33% of water [36], grapes 21.17 ± 0.76% [37], avocado 34.28 ± 0.95% [38], and pumpkin 14.18 ± 0.22% [39].

Table 2

Proximate analysis of gelatin jelly candy

Treatment of natural ingredients	Proximate (%)					
	Water	Ash	Protein	Fat	Carbohydrate	Sugar Reduction
Honey (liquid)	10.25 ± 0.42^{a}	$0.13 + 0.01^{a}$	$15.67 + 0.52^{a}$	2.20 ± 0.23^{a}	63.93 ± 1.28^{a}	0.18 ± 0.07^{a}
Dates juice (liquid)	14.09 ± 0.84^{b}	0.32 ± 0.01^{b}	15.82 ± 0.53^{a}	2.35 ± 0.26^{a}	64.03 ± 1.14^{b}	0.26 ± 0.03^{a}
Olive oil (liquid)	10.71 ± 0.60^{a}	0.18 ± 0.01^{c}	15.77 ± 0.67^{a}	2.09 ± 0.22^{a}	58.26 ± 1.60^{c}	0.13 ± 0.05^{a}
Soy Milk (powder)	9.76 ± 0.70^{a}	0.21 ± 0.02^{d}	16.20 ± 0.37^{a}	2.32 ± 0.50^{a}	51.61 ± 0.80^{c}	0.14 ± 0.01^{a}
Goat's milk (powder)	9.92 ± 0.68^{a}	0.20 ± 0.03^{cde}	13.97 ± 0.36^{b}	1.99 ± 0.28^{a}	57.57 ± 0.79^{ad}	0.17 ± 0.09^{a}
Grape juice (liquid)	10.82 ± 0.78^{a}	0.15 ± 0.01^{a}	13.62 ± 0.37^{b}	2.31 ± 0.33^{a}	62.55 ± 0.59^{ade}	0.18 ± 0.05^{a}
Avocado (paste)	10.23 ± 0.46^{a}	0.18 ± 0.01^{cef}	14.19 ± 0.45^{b}	1.67 ± 0.30^{a}	63.94 ± 1.46^{adef}	0.12 ± 0.05^{a}
Pumpkin (paste)	10.48 ± 0.56^{a}	0.14 ± 0.02^{a}	14.39 ± 0.64^{b}	2.29 ± 0.35^{a}	61.81 ± 1.20^{aeg}	0.22 ± 0.05^{a}
SNI about jelly candy	20.00	3.00	–	–	–	25.00

Note: Means in the rows with different superscripts are significantly ($p \leq 0.05$) different.

The concentration of water content greatly affects the quality and durability of gelatin jelly candy [40]. Based on the study results, the overall water content of gelatin jelly candy still followed the standards set by SNI No. 3547-2-2008 about jelly candy [41], a maximum of 20.00%. The variance analysis shows that adding natural ingredients to manufacture jelly candy significantly affected the water content ($p<0.05$). The highest ash content was added with 0.21 ± 0.02% soy milk powder. The value of ash content in this study met the standards required by SNI for jelly candy, which was a maximum of 3.00%. The high ash content of jelly candy in the treatment of soy milk and powdered goat's milk was due to the initial raw materials containing high minerals. The ash content of powdered soybean milk is 0.40 ± 0.05% [34], and powdered goat's milk is 0.07 ± 0.00% [42].

During the jelly candy processing process, the total minerals in the raw materials did not change significantly. The ash content and gelling agents in making jelly candy will be higher. For the ash content of jelly candy with natural additives in liquid form (honey, olive oil, dates juice, and grapes) and paste form (avocado and pumpkin), the ash content tended to be lower. This is because the fruit extraction process decreases the mineral content of the fruit juice. The components are easily decomposed or evaporated during fruit ashing [43].

Table 2 showed that the value of protein content ranged from 13.62 ± 0.37 – 16.20 ± 0.37%. The variance analysis showed that adding natural ingredients to produce gelatin jelly candy significantly affected protein content ($p<0.05$). The highest protein content was added with 16.20 ± 0.37% powdered soy milk. This is because fresh soy milk contains 23.08 ± 0.16% protein [44], while powdered soy milk has a protein content of 5.09 ± 0.29% [34]. Interestingly, the protein content of gelatin produces a very high protein content of 91.52%.

Protein intake is needed to build muscle mass, especially for toddlers. Jelly candy is one of the media to deliver bioactive compounds required by the toddler's body. Protein content in the treatment of the addition of natural ingredients in liquid form (honey, olive oil, dates, and grapes) and paste form (avocado and pumpkin) also had a relatively high protein content [45]. According to Kia et al. [10], jelly candy with gelatin tends to have a higher protein content.

Adding natural ingredients to manufacture jelly candy did not significantly affect fat content ($p<0.05$). After adding natural ingredients or soy milk powder, the fat content was 2.32 ± 0.50%. The high and low-fat content of jelly candy was due to differences in the raw materials used. According to Nemo and Bacha [36], the fat content in honey is 0.27 ± 0.20%, soy milk powder is 11.36 ± 0.44% [34], powdered goat's milk is 1.02 ± 0.09% [42], grapes 0.64 ± 1.17% [37], avocado 6.66 ± 0.10 [38], pumpkin 4.50 ± 0.21% [39].

The total value of carbohydrates in the study ranged from 51.61 ± 0.80 – 64.03 ± 1.14%. The variance analysis showed that adding natural ingredients to manufacture gelatin jelly candy significantly affected total carbohydrates ($p<0.05$). The highest total carbohydrate value with the addition of dates juice was 64.03 ± 1.14%. This was because the calculation of carbohydrates was carried out using the by-difference method. The high value of carbohydrates in each treatment could meet the body's energy needs.

Liu et al. (2019) explained that carbohydrates give food a sweet taste, especially monosaccharides and disaccharides that provide energy for the body. The value of carbohydrates in the study was influenced by raw materials containing high carbohydrates, such as grapes 49.17 ± 2.31% [37], avocado 54.23 ± 0.02% [38], and pumpkin 61.71 ± 0.10% [39].

Sugar residue was a substance left or left in a specific chemical process; this residue could be likened to salt. Based on the study results, the average value was 0.12 ± 0.05 – 0.26 ± 0.05%. The results followed the standard SNI set about jelly candy, a maximum of 25.00%. Based on a statistical analysis of the sugar reduction value of gelatin jelly candy with the treatment of various natural ingredients, there was no significant effect ($p>0.05$). This was because the sugar residue came from the sucrose produced by jelly candy. Garusti et al. [46] stated that palm sugar contains 87.10% sucrose content with 6.06% reducing sugar. The content of reducing sugars is closely related to the inversion of sucrose into reducing sugars. The low, reducing sugar in the study was due to the natural ingredients used. Reducing sugar in natural ingredients tends to be lower and can be easily synthesized by the body [47].

Microbiological Analysis

The total plate count (TPC) value was $\leq 1 \times 10^2$ colonies/g, which still followed the SNI for jelly candy which was 3×10^3 colonies/g [41]. This was because the addition of sucrose in making gelatin jelly candy had antibacterial properties. Balakrishnan et al. [48] explained that sucrose would be oxidized to form acetals in the heating process. The acetal group can release cation-charged ions that interact with the anionic charge of the microbial cell membrane through electrostatic bonds leading to increased cell permeability and cell leakage leading to cell death.

All treatments in gelatin jelly candy exhibited negative results for *E. coli* and *Salmonella* sp. The results also met SNI's standards about jelly candy, which stated that jelly candy must be negative for *E. coli* and *Salmonella* sp. There was no identification of *E. coli* and *Salmonella* sp. because the natural ingredients used in manufacturing jelly candy have a natural antibacterial activity. Handayani et al. [49] explained that some natural ingredients in liquid form have antibacterial properties against *Staphylococcus aureus* and *E. coli* with a maximum concentration of 0.04 g/mL.

Conclusions

The addition of natural flavour to the skin mackerel gelatin jelly candy could be well received by panellists, with the "really like" to "very much like" category. The nutritional and quality of gelatin jelly candy met the Indonesia National Standard about jelly candy. The best treatment was the addition of natural flavouring ingredients soy milk with the highest protein dan the lowest carbohydrate content. Furthermore, it is necessary to analyze the amino acids of each flavour variant of gelatin jelly candy.

Conflict of interest

The authors declare no conflict of interest.

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Сведения об авторах

Ф.И.О.(полностью), Ученая степень (если имеется), ученое звание (если имеется), должность, место работы / учебы (полное название учреждения в именительном падеже), адрес учреждения, рабочий тел., e-mail для связи		Информация для быстрой связи с автором (в журнале не публикуется): тел., e-mail
на русском языке	*на английском языке*	
Пример оформления		
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Примечание. Фамилия автора, с которым следует вести переписку, обозначается звездочкой (*).

Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit (17 Oktober 2023)

SUBMIT MANUSCRIPT (Agustiana et al.)_Journal Food Processing Techniques and Technology

agustiana agustiana <fpkulm.agustiana@gmail.com> Tue, Oct 17, 2023 at 3:02 PM
To: редакция Техника и технология пищевых производств <fptt98@gmail.com>

Dear Editor-In-Chief
Journal Food Processing Techniques and Technology,

I have revised my manuscript according to reviewer's suggestion.
The revision has been made in highlighted yellow color.
I also provide a rebuttal letter to answer each comment of the reviewer.
I hope the revision can improve our manuscript.
Thank you in advance.

Best regards
[Quoted text hidden]

3 attachments

Agustiana_Cover letter with letter head and date.PDF
810K

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REBUTTAL LETTER.docx
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Gmail **agustiana agustiana <fpkulm.agustiana@gmail.com>**

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редакция Техника и технология пищевых производств <fptt98@gmail.com> Tue, Oct 17, 2023 at 4:14 PM
To: agustiana agustiana <fpkulm.agustiana@gmail.com>

Здравствуйте, уважаемые авторы!
Доработанная рукопись получена и направлена на повторное рецензирование.
Но мы не получили Рисунок 1 в редактируемом формате excel. Мы должны иметь возможность редактировать рисунки под требования журнала.

С уважением,
ответственный за выпуск
Кирякова Алена Алексеевна
КемГУ, г. Кемерово, пр. Советский, 73, ауд. 2149
тел. (3842) 58-80-24

вт, 17 окт. 2023 г. в 15:02, agustiana agustiana <fpkulm.agustiana@gmail.com>:

Terjemahan:

Hello, dear authors!
The revised manuscript has been received and sent for re-review.
However, we did not receive Figure 1 in editable Excel format. We must be able to edit figures to suit the requirements of the magazine.

Sincerely,
release officer
Kiryakova Alena Alekseevna
Kemerovo State University, Kemerovo, Sovetsky Ave., 73, room. 2149
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KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,
RISET DAN TEKNOLOGI

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CORPORATE LETTERHEAD

To: A.Yu.Prosekov,
Editor-in-Chief of
Food Processing: Techniques and Technology
Kemerovo State University,
6, Krasnaya St., 650000, Kemerovo, Russia

Registration number 114/23-TiCCI 18/08/2023

Dear Dr Prosekov,

We would like to submit the manuscript entitled "The characteristic of gelatin jelly candy from mackerel skin (*Scomberomorus commersonii*) based on proximate and sensory analysis" by Agustiana, Siti Aisyah, Hafni Rahmawati, D.E. Anggraini, and Aolia Ramadhani to be considered for publication in the *Food Processing: Techniques and Technology*. The paper features the topical issues of the characteristic gelatin jelly candy from mackerel skin. Jelly candy is children's favourite snack because it has an attractive colour and delicious flavour. Using mackerel skin gelatine is ex-pected to increase the protein content in jelly candy. The present study aimed to determine consumer acceptance, proximate value, and the quality of gelatin jelly candy with various natural flavours.

We possess exclusive copyright rights and know of no conflict of interest that may be associated with its publication in the *Food Processing: Techniques and Technology*.

We declare that the manuscript does not violate the copyright law of the Russian Federation and contains all the necessary references to scientific sources and results obtained by other scientists.

We declare ourselves responsible for any possible wrongful use of intellectual property and copyright-protected items in accordance with the current legislation of the Russian Federation. The manuscript contains no content prohibited for publication under the current law.

We declare ourselves liable for the truthfulness of the scientific data. The paper has been examined for classified and expert controlled information; the manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We are familiar and agree with the publication ethics and editorial policy established by the Editorial Board of the *Food Processing: Techniques and Technology*.

We believe that the manuscript can be recommended for publication in the *Food Processing: Techniques and Technology* in the section *"Original Article"*

Authors:	Agustiana_		08/09/2023
	Siti Aisyah _		08/09/2023
	Hafni Rahmawati		08/09/2023
	D.E. Anggraini		08/09/2023
	Aolia Ramadhani		08/09/2023

Head of organization : Rabiatul Adawyah
Vice Dean

REBUTTAL LETTER

Reviewer comments	Author response	Changes
Reviewer's comments and recommendations: According to the annotation: the abstract meets the requirements. For work in general: - In the introduction it is necessary to indicate the purpose of the work.	The aim of the study was added in the introduction	The last sentence in the last paragraph of introduction. Highlighted in yellow color
- In the "Objects and methods" section, it is correct to present the extraction and heating time ranges (for example, 5±0.1 hours). From the presented extraction technique, it is not clear by what method and at what installation the gelatin extract was dried.	I have added in the object and methods	The extraction process was done by heating with H_2O at 80°C for 2 h. The gelatin extract was then dried in oven for three days at 55°C.
- In the "Objects and Methods" section, correctly present the ranges in the methods: Water content analysis, Fat content analysis, Protein content analysis (for example, 2±0.01 g).	I have added the ranges of Water content analysis, Fat content analysis, Protein content analysis	2 ± 0.01 g
- The text of the article does not describe the method of degreasing raw materials to obtain gelatin extracts, and whether this process was carried out.	It has been added	Dried mackerel skin samples were soaked in water for ± 5 h. The sample was heated for ± 1 min to remove the remaining impurities. Soak the sample in 0.05 M of CH3COOH acid solution for 10 h. The extraction process was done by heating with H2O at 80°C for 2 h. The gelatin extract was then dried in oven for three days at 55°C.
- The list of references should be supplemented with the latest publications of Russian scientists in the field under study. Reasoned conclusion:	I have added 2 references from Russian publication	9. Yanchenko EV, Volkova GS, Kuksova EV, Virchenko II, Yanchenko AV, Serba EM, Ivanova MI. Chemical Composition and Sensory Profile of Sauerkraut from Different Cabbage Hybrids.

b) recommend the article for publication in a journal after revision;		Food Processing: Techniques and Technology. 2023; 53(1): 131-139. https://doi.org/10.21603/2074-9414-2023-1-2420 25. Sergev AI, Kalinina IG, Shilkina NG, Barashkova II, Gradova MA, Motyakin MV, Ivanov VB. Effect of Elevated Storage Temperatures on the Physicochemical and Sensory Properties of Apple Puree. Food Processing: Techniques and Technology. 2023; 53(2):259–271. https://doi.org/10.21603/2074-9414-2023-2-2430

Bukti konfirmasi review dan hasil review kedua (19 Oktober 2023)

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SUBMIT MANUSCRIPT (Agustiana et al.)_Journal Food Processing Techniques and Technology

agustiana agustiana <fpkulm.agustiana@gmail.com> Thu, Oct 19, 2023 at 11:59 AM
To: редакция Техника и технология пищевых производств <fptt98@gmail.com>

Dear Editor,

Please find the attached file about the editable figure in Excel format.
Thank you

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SUBMIT MANUSCRIPT (Agustiana et al.)_Journal Food Processing Techniques and Technology

редакция Техника и технология пищевых производств <fptt98@gmail.com> Thu, Oct 19, 2023 at 12:41 PM
To: agustiana agustiana <fpkulm.agustiana@gmail.com>

Добрый день, уважаемые авторы!
У вас в статье один рисунок, а в присланном документе 2 ???

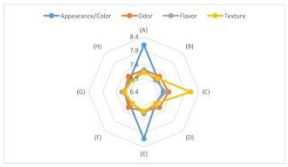
С уважением,
ответственный за выпуск
Кирякова Алена Алексеевна
КемГУ, г. Кемерово, пр. Советский, 73, ауд. 2149
тел. (3842) 58-80-24

Terjemahan:

Good afternoon, dear authors!
You have one picture in your article, but 2 in the document you sent???

Sincerely,
release officer
Kiryakova Alena Alekseevna
Kemerovo State University, Kemerovo, Sovetsky Ave., 73, room. 2149
tel. (3842) 58-80-24

Treatments	Code	Appearance/Color	Odor	Flavor	Texture	Average
Honey	(A)	8.1	7.2	7.2	7.1	7.40
Dates Juice	(B)	7.1	7.2	7.0	7.1	7.10
Olive Oil	(C)	7.1	7.3	7.2	8.1	7.43
Soy Milk	(D)	7.0	7.2	7.0	7.0	7.05
Goat's Milk	(E)	8.1	7.1	7.2	7.1	7.38
Grape Juice	(F)	7.1	7.2	7.0	7.0	7.08
Avocado	(G)	7.1	7.1	7.2	7.1	7.13
Pumpkin	(H)	7.1	7.2	7.0	7.0	7.08



Appearance/Color

Panelist	A	B	C	D	E	F	G	H
1	8	7	7	7	8	8	7	7
2	8	7	7	7	8	8	7	7
3	9	8	8	7	9	7	8	8
4	7	7	7	7	8	8	7	7
5	8	7	7	7	8	7	7	7
6	8	7	7	7	8	6	7	7
7	9	7	7	7	8	7	7	7
8	8	7	7	7	8	7	7	7
9	8	7	7	7	8	7	7	7
10	8	7	7	7	8	6	7	7
Average	8.1	7.1	7.1	7.0	8.1	7.1	7.1	7.1

Odor

Panelist	A	B	C	D	E	F	G	H
1	7	7	7	7	7	7	7	7
2	7	7	7	7	7	7	7	7
3	8	7	8	7	8	7	8	7
4	7	7	7	7	7	7	7	7
5	7	7	7	7	7	7	7	7
6	7	7	7	7	7	7	7	7
7	8	7	8	7	8	7	8	7
8	7	7	7	7	7	7	7	7
9	7	7	7	7	7	7	7	7
10	7	7	7	7	7	7	7	7
Average	7.2	7.0	7.2	7.0	7.2	7.0	7.2	7.0

Flavor

Panelist	A	B	C	D	E	F	G	H
1	7	7	7	7	7	7	7	7
2	7	7	7	7	7	7	7	7
3	8	8	8	8	7	8	7	8
4	7	7	7	7	7	7	7	7
5	7	7	7	7	7	7	7	7
6	8	8	9	8	8	8	8	8
7	7	7	7	7	7	7	7	7
8	7	7	7	7	7	7	7	7
9	7	7	7	7	7	7	7	7
10	7	7	7	7	7	7	7	7
Average	7.2	7.2	7.3	7.2	7.1	7.2	7.1	7.2

Texture

Panelist	A	B	C	D	E	F	G	H
1	7	7	8	7	7	7	7	7
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3	8	8	8	7	8	7	8	7
4	7	7	8	7	7	7	7	7
5	7	7	8	7	7	7	7	7
6	7	7	8	7	7	7	7	7
7	7	7	8	7	7	7	7	7
8	7	7	8	7	7	7	7	7
9	7	7	9	7	7	7	7	7
10	7	7	9	7	7	7	7	7
Average	7.1	7.1	8.1	7.0	7.1	7.0	7.1	7.0

Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit

(23 Oktober 2023)

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Dear Editor,

There is only one figure. I have revised this.
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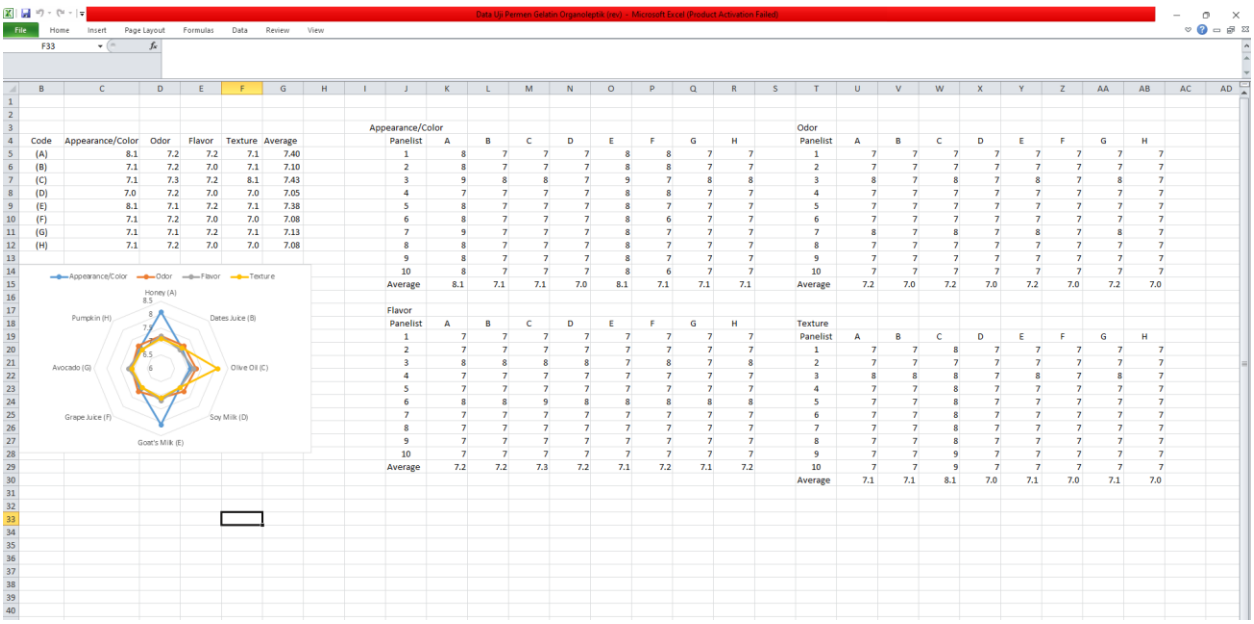

Data Uji Permen Gelatin Organoleptik (rev) - Microsoft Excel (Product Activation Failed)

Code	Appearance/Color	Odor	Flavor	Texture	Average
(A)	8.1	7.2	7.2	7.1	7.40
(B)	7.1	7.2	7.0	7.1	7.10
(C)	7.1	7.3	7.2	8.1	7.43
(D)	7.0	7.2	7.0	7.0	7.05
(E)	8.1	7.1	7.2	7.1	7.38
(F)	7.1	7.2	7.0	7.0	7.08
(G)	7.1	7.1	7.2	7.1	7.13
(H)	7.1	7.2	7.0	7.0	7.08

Appearance/Color

Panelist	A	B	C	D	E	F	G	H
1	8	7	7	7	8	8	7	7
2	8	7	7	7	8	8	7	7
3	9	8	8	7	9	7	8	8
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7	9	7	7	7	8	7	7	7
8	8	7	7	7	8	7	7	7
9	8	7	7	7	8	7	7	7
10	8	7	7	7	8	6	7	7
Average	8.1	7.1	7.1	7.0	8.1	7.1	7.1	7.1

Odor

Panelist	A	B	C	D	E	F	G	H
1	7	7	7	7	7	7	7	7
2	7	7	7	7	7	7	7	7
3	8	7	8	7	8	7	8	7
4	7	7	7	7	7	7	7	7
5	7	7	7	7	7	7	7	7
6	7	7	7	7	7	7	7	7
7	8	7	8	7	8	7	8	7
8	7	7	7	7	7	7	7	7
9	7	7	7	7	7	7	7	7
10	7	7	7	7	7	7	7	7
Average	7.2	7.0	7.2	7.0	7.2	7.0	7.2	7.0

Flavor

Panelist	A	B	C	D	E	F	G	H
1	7	7	7	7	7	7	7	7
2	7	7	7	7	7	7	7	7
3	8	8	8	8	7	8	7	8
4	7	7	7	7	7	7	7	7
5	7	7	7	7	7	7	7	7
6	8	8	9	8	8	8	8	8
7	7	7	7	7	7	7	7	7
8	7	7	7	7	7	7	7	7
9	7	7	7	7	7	7	7	7
10	7	7	7	7	7	7	7	7
Average	7.2	7.2	7.3	7.2	7.1	7.2	7.1	7.2

Texture

Panelist	A	B	C	D	E	F	G	H
1	7	7	8	7	7	7	7	7
2	7	7	7	7	7	7	7	7
3	8	8	8	7	8	7	8	7
4	7	7	8	7	7	7	7	7
5	7	7	8	7	7	7	7	7
6	7	7	8	7	7	7	7	7
7	7	7	8	7	7	7	7	7	7
8	7	7	8	7	7	7	7	7
9	7	7	9	7	7	7	7	7
10	7	7	9	7	7	7	7	7
Average	7.1	7.1	8.1	7.0	7.1	7.0	7.1	7.0

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To: agustiana agustiana <fpkulm.agustiana@gmail.com>

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На Вашу статью "**The characteristic of gelatin jelly candy from mackerel skin (*Scomberomorus commersonii*) based on proximate and sensory analysis**" поступила положительная рецензия. Статья находится в портфеле журнала и будет опубликована в первом полугодии 2024 г.

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Hello, dear authors!
Your article “The characteristic of gelatin jelly candy from mackerel skin (*Scomberomorus commersonii*) based on proximate and sensory analysis” received a positive review. The article is in the journal's portfolio and will be published in the first half of 2024.
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Уважаемые авторы!

Сообщаем Вам, что статья «The characteristic of gelatin jelly candy from mackerel skin (Scomberomorus commersonii) based on proximate and sensory analysis» авторов Agustiana, Siti Aisyah, Hafni Rahmawati, D.E. Anggraini, Aolia Ramadhani (регистрационный номер 114/23-ТиТПП от 18.08.2023 г.) будет опубликована в журнале «Техника и технология пищевых производств (Food Processing: Techniques and Technology)» в 2024 г.

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Gelatin Jelly Candy from Mackerel Skin (*Scomberomorus commersonii*)

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Abstract.
Jelly candies are colorful, delicious, and loved by children. Mackerel skin gelatin has a good nutritional potential to increase the protein content in jelly candy. The present study tested consumer acceptance, proximate value, and quality of gelatin jelly candy fortified with eight different natural flavorings: honey, date juice, olive oil, soy milk, goat's milk, grape juice, avocado, and pumpkin.

Gelatin was extracted from mackerel (*Scomberomorus commersonii*) skin. The quality assessment involved tests on the water, ash, fat, and protein contents, as well as bacterial contamination. The sensory evaluation involved a hedonic test with 10 panelists, who found all samples acceptable in appearance, smell, flavor, and texture.

The average score for each criterium was 7.00 out of 9.00. The sample with soy milk proved to have the most optimal formulation: water (9.76 ± 0.70%), ash (0.21 ± 0.02%), protein (16.20 ± 0.37%), fat (2.32 ± 0.50%), carbohydrate (51.61 ± 0.80%), reducing sugar (0.14 ± 0.01%). All samples were free from *Salmonella* sp. and *Escherichia coli*, with a total plate count of 1×10^2 colonies per 1 g.

The jelly candy with mackerel skin gelatin was high in protein, had a favorable sensory profile, and met the Indonesia National Standard for this type of food products.

Keywords. Gelatin, halal gelatin, jelly candy, nutritional value, quality, mackerel skin, *Scomberomorus commersonii*

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Жевательный мармелад с желатином из кожи скумбрии (*Scomberomorus commersonii*)

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Аннотация.
Жевательный мармелад – это яркое и вкусное лакомство, которое пользуется спросом у детей. Желатин из кожи скумбрии (*Scomberomorus commersonii*) обладает хорошими питательными свойствами и может способствовать увеличению содержания белка в жевательном мармеладе. Описали потребительскую привлекательность, пищевую ценность и качество жевательного мармелада с желатином из кожи скумбрии и 8 натуральными ароматизаторами: мед, финиковый сок, оливковое масло, соевое молоко, козье молоко, виноградный сок, авокадо и тыква.
Желатин экстрагировали из кожи скумбрии (*S. commersonii*). Качество мармелада оценивали по содержанию влаги, золы, жира, белка и наличию бактерий. Органолептическая оценка заключалась в проведении гедонического теста: десять экспертов оценили все образцы как приемлемые по внешнему виду, запаху, вкусу и текстуре.
Средний балл по каждому критерию составил 7,0 из 9,0. Оптимальным по составу оказался образец с соевым молоком: содержание влаги составило 9,76 ± 0,70 %, золы – 0,21 ± 0,02 %, белка – 16,20 ± 0,37 %, жира – 2,32 ± 0,50 %, углеводов – 51,61 ± 0,80 %, редуцирующего сахара – 0,14 ± 0,01 %. В образцах не обнаружены *Salmonella* sp. или *Escherichia* coli; общее количество бактерий составило 1×10^2 колоний на 1 г.
Жевательный мармелад с желатином из кожи скумбрии оказался богат белком, продемонстрировал хорошие органолептические свойства и соответствовал государственному стандарту, принятому в Индонезии для данного типа пищевых продуктов.

Ключевые слова. Желатин, халяльный желатин, желейные конфеты, пищевая ценность, качество, кожа скумбрии, *Scomberomorus commersonii*

Финансирование. Исследование было поддержано Программой обязательных исследований по схеме PNBP для преподавателей Университета Ламбунг Мангкурат в 2021 финансовом году (основной кластер), договор № 010.49/UN 8.2/PL/2021.

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Introduction

Gelatin is a protein commonly extracted from cartilage, skin, and scales of cows, pigs, and fish [1]. Halal gelatin cannot contain any pig-based products. As a rule, it is extracted from fish, e.g., mackerel (*Scomberomorus commersonii*) [2]. Gelatin preparations are part of various foods and non-food products. Gelatin serves as an emulsifier, a stabilizer, a microencapsulation agent, as a component of biodegradable packaging, etc. [3]. Its most useful property is the ability to form gels with convenient viscosity and melting point. As a result, gelatin is a popular component of various candy products [1].

Candy can be hard and soft. As a rule, candy contains cane sugar, corn sugar, flavorings, dyes, and gelling agents. Gelatin-based candy is called jelly candy and has a higher sugar content [4]. Jelly candy is often fortified with vitamins and minerals to improve children's diet [5]. Some sorts of jelly candy involve natural flavorings, e.g., nutmeg extract, strawberries and mangoes [6–8]. However, some confectionery industries prefer synthetic acid

flavorings with unreliable safety, e.g., citric acid, tartaric acid, and lactic acid [9]. According to Yanchenko et al., the food industry does not meet nutrition standards in this sphere because producers ignore consumer safety to maximize profit [10]. In addition, jelly candy is rarely rich in protein. Fish skin gelatin may solve this problem by fortifying jelly candy with protein.

Natural flavorings with vitamins and minerals offer good prospects for candy production. According to Kia *et al.*, food products with natural additives are health-beneficial [11]. In this research, we used such natural ingredients as honey, date juice, olive oil, soy milk, goat's milk, grapes, avocado, and pumpkin. These flavorings are expected to raise the consumer attractiveness of halal gelatin candy. Furthermore, jelly candy made from mackerel skin gelatin potentially provides protein intake and reduces sugar consumption. This study featured consumer acceptance, proximate value, and quality profile of jelly candy with mackerel skin gelatin fortified with eight different natural flavorings.

Study objects and methods

Extracting mackerel skin gelatin. We used the protocol described by Rahmawati & Pranoto to extract gelatin from mackerel skin [12]. After soaking dried mackerel skin in water for ± 5 h, we heated it for ± 1 min to remove the remaining impurities. Then, the sample was soaked in 0.05 M of ethanolic acid (CH_3COOH) solution for 10 h. The extraction process involved heating with H_2O at 80°C for 2 h. Finally, the gelatin extract remained three days in an oven at 55°C.

Preparing gelatin jelly candy. We designed eight different natural flavorings, i.e., honey, date juice, olive oil, soy milk, goat's milk, grape juice, avocado, and pumpkin. Each test was performed in triplicate. The formulation and technology for jelly candy with fish skin gelatin was borrowed from Eletra *et al.* with some modifications [13]. We mixed 75 g gelatin, 85 g sucrose, 5 g salt, and 85 g natural ingredients. After adding 300 mL cold water, we stirred the mix until it became homogeneous. The sample was then heated at 100°C for 2 min, molded with soft silicone bear-shape templates, and cooled.

Water content analysis. To analyze the water content, we appealed to the method published by the Association of Official Analytical Chemists [14]. The samples were weighed up to 2 ± 0.01 g on a porcelain dish of known weight and dried in an oven at 105°C for 3 h. After being cooled in a desiccator, the weighing was repeated.

Ash content analysis. The samples were weighed up to 2 ± 0.01 g on a porcelain dish of known weight, ignited on a burner flame, and burned in an electric furnace at ≤ 550°C until complete combustion. Then, they were cooled in a desiccator and weighed until constant mass [14].

Fat content analysis. We placed 2 ± 0.01 g of each sample into a cotton-lined paper bag. The paper sleeve was covered with cotton, dried in an oven at ≤ 80°C for ± 1 h, and put into the Soxhlet extraction apparatus connected to an oil bottle with boiling chips. After drying, we determined the weight and extracted the sample with hexane for ± 6 h. Then we filtered the hexane and dried the fat extract in an oven at 105°C, cooled it, and weighed. The cooling process continued until constant weight [14].

Protein content analysis. The analysis of protein content relied on the method recommended by the Association of Official Analytical Chemists [14]. During the digestion, we put 1 ± 0.01 g of each sample into a 100 mL Kjehdahl flask with 10 mL of concentrated sulfuric acid. A catalyst was added to speed up the digestion. After the distillation, the digestion results were diluted with distilled water up to 100 mL. After homogenization and cooling, we pipetted 5 mL into a distillation flask. A total of 10 mL of 30% sodium hydroxide solution penetrated through the walls of the still flask until a layer formed under the acid solution. The container was filled with 10 mL of 0.1 N hydrochloric acid solution and drained with a methyl red indicator. The titration was accommodated in an Erlenmeyer flask with 0.1 N hydrochloric acids and five drops of methyl red indicator. The mix was titrated directly using a 0.1 N sodium hydroxide solution. The titration resulted in a pink-to-yellow color. This treatment was repeated three times for each sample.

Total plate count. The total plate count method belonged to Salanggon *et al.* [15]. A total of 25 g of each sample was weighed aseptically. After adding 225 mL Butterfield's phosphate buffer, we homogenized the mix for 2 min and diluted it. The homogenate was put with a sterile pipette into a vial containing 9 mL of Butterfield's phosphate buffer solution to obtain a sample with a dilution of 10^{-2}. Each dilutant was stirred at least 25 times to obtain further dilutants (10^{-3}, 10^{-4}, 10^{-5}, etc.). The volume of each diluent was 1 mL, and the procedure was repeated in a sterile petri dish with a sterile pipette. In each petri dish, 12–15 mL of medium was cooled to 5°C for the plate count agar method. After the agar hardened, it was incubated at 35°C for 8 h to count the number of bacterial colonies in the petri dish.

Screening of *Escherichia coli*. We homogenized 25 g of each sample with 225 mL peptone buffer and then fortified it at 37°C for 18 h. Next, 1 mL of the sample was inoculated directly into 9 mL of MacConkey broth (CM5a; Oxoid) and then incubated at 37°C for 18 h [16]. After that, we sprayed the fortified broth preparations directly onto eosin methylene blue agar and incubated them at 37°C for 18–24 h. The isolates were confirmed biochemically using an *E. coli* antiserum express diagnostic kit. *E. coli* O antiserum consisted of polyclonal antibodies used for zero-classification of *E. coli* O antigens.

Screening *Salmonella* sp. At the pre-fortification stage, the collected samples were serially diluted (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, etc.) using peptone water [17]. At the fortification stage, we planted them on sterile selenite cystine broth selective media and incubated at

37°C for 24 h. After the fortification stage in each dilution, 1 mL was planted on xylose lysine deoxycholate. We analyzed bacteria growth by counting the colonies and observing their morphology. Purification involvedthe quadrant streaking method, with presupposed xylose lysine deoxycholate media and incubation at 37°C for 48 h. The purification process targeted colonies with different colony morphology that belonged to gram-negative bacteria.

After that, we selected two types of colonies. Each colony was duplicated so that eventually 40 colonies were obtained. The purification results were grown on slanted nutrient agar, incubated at 37°C for 24 h, and stored at –20°C as stock culture. The storage condition of pure bacterial isolates involved 60% glycerol in a ratio of 1:1 at –80°C.

Sensory analysis. Each sample was placed on a white plastic plate together with a glass of water, coded, and served to panelists randomly in a well-lit environment. The panel consisted of 10 trained panelists from the laboratory of testing and quality control of fishery products, Banjarbaru, South Kalimantan. The criteria included appearance, smell, texture, and flavor. The panelists rated the acceptance using a nine-point hedonic scale: 1 – dislike extremely, 2 – dislike very much, 3 – dislike moderately, 4 – dislike slightly, 5 – neither like nor dislike, 6 – like slightly, 7 – like moderately; 8 – like very much, 9 – like extremely.

Data analysis. All data that passed the homogeneity and normality tests were further analyzed using SPSS 20.0 for Windows and ANOVA Analysis of Variance ($p < 0.05$) followed by the Duncan's Test.

Results and discussion

Mackerel skin gelatin characterization. The water content in fish skin gelatin was 6.45%, which was lower than in the raw material (Table 1). In this research, the water content exceeded that reported by Viji *et al.* as 4.81 ± 0.41% [18]. However, it was lower than the data published by Ismail & Abdullah as 6.93% [19]. Yet, the water content met the Indonesian National Standard No. 01-3735-1995 Gelatin quality and test method. The Joint FAO/WHO Expert Committee on Food Additives defines the maximum of 18%, and the Gelatin Manufacturers Institute of America mentions 10.5 ± 1.5% [20, 21].

According to Esfahani *et al.*, water content determines the stability of dry products [22]. High water content causes particle agglomeration and accelerates microbial growth and oxidation. Ash content was essential for evaluating gelatin quality, especially in terms of mineral content and purity. The ash content of fish skin gelatin (Table 1) meets the standards specified by the Indonesian National Standard (3.25%), the Joint FAO/WHO Expert Committee on Food Additives (max. 2.00%), and the Gelatin Manufacturers Institute of America 0.5 ± 0.4 – 1.5 ± 0.5% (Indonesian National Standard No. 01-3735-1995) [20]. Specifics of aquatic environment, habitat, and species affect the ash content of fish skin gelatin. Its ash content also depends on the extraction process [23].

In this research, the protein content of gelatin depended on the time and concentration of chemicals used. This concentration broke more amino acid bonds, so that more protein broke down during extraction. The resulting protein content in gelatin was 91.52%, which exceeded the initial data for dry fish skin (69.76%) and wet fish skin (35.63%). The protein content in gelatin met the Indonesian National Standard (87.25%). However, our results exceeded those obtained by Zarubin *et al.* by 73.2 ± 0.9% [23]. The difference in the protein content resulted from the differences in the concentration of acid and base used during extraction. Acid and base concentration and immersion time combined were reported to produce high protein content [24].

Fat content is known to affect the quality of raw materials during storage. The fat content of skin gelatin equaled 0.73%, which was lower than the initial data for dry skin (4.85%) and wet skin (2.24%). This result was similar to that reported by Gunawan *et al.* as 0.71 ± 0.07% [24]. High-fat content shortens the shelf-life of gelatin and affects the quality of gelatin in the application process [23]. In our research, the value of carbohydrates in gelatin was 6.45%, which was much less than the initial data for dry fish skin (20.18%) and wet fish skin (60.74%). Carbohydrates are not considered as an essential parameter in gelatin production: the essential parameters include protein, water, and ash.

Sensory profile of gelatin jelly candy. Sensation is a psycho-physiological process in which sensory recognition of object characteristics is carried out through stimuli received by the senses [25]. In our research, the sensory evaluation results for the appearance ranged from "liked moderately" to "like very much". Figure 1 shows that the liquid honey-flavored sample received a bigger score than date juice, olive oil, and grape juice. The natural color of honey, clear brown when added, turned light brown. Adding olive oil and date juice made the jelly candy blackish-brown while adding grape juice made it yellowish [26, 27]. The appearance score of the soy milk and goat's milk samples was very similar; both were yellow-brown but not like the honey sample. The milk powder had a color similar to that of the jelly candy formulation. According to Charoenphun, milk powder

Table 1. Mackerel skin gelatin proximate

Таблица 1. Предварительный анализ состава кожи скумбрии

Proximate, %	Mackerel raw skin	Mackerel dry skin	Mackerel gelatin
Water	60.74	20.18	6.45
Ash	5.23	2.36	0.86
Protein	35.63	69.76	91.52
Fat	4.85	2.24	0.73
Carbohydrate	60.74	20.18	6.45

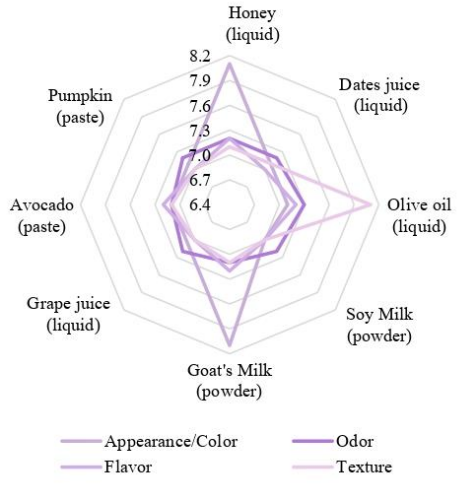

Figure 1. Sensory analysis of gelatin jelly candy

Рисунок 1. Органолептический анализ жевательного мармелада с желатином из кожи скумбрии

makes jelly candy light yellow or pale white [28]. Avocado and pumpkin turned the gelatin black. The problem is that avocado naturally produces ethylene gas, which is associated with ripening. It converts methionine to S-adenosylmethionine, which causes blackness when added to food [29].

The smell category received "like moderately" from all panelists. The samples with goat's milk powder had the highest score for smell (7.6), followed by olive oil (7.3) and honey, grape juice, date juice, soy milk, avocado, and pumpkin (7.1–7.2). The aroma of goat's milk turned out to be stronger than that of the other flavorings. Even unprocessed, goat's milk has a strong smell and taste caused by caproic acid [30]. The specific aroma can be removed by adding rare sugar (D-psychose, D-tagatose, D-sorbose): it would neutralize caproic acid with a glycation reaction. Znamirowska *et al.* stated that fresh goat's milk contains protein (2.69 ± 0.22%), fat (2.98 ± 0.53%), and general acidity (6.20 ± 1.20%) [31].

The flavor category also received "like moderately" from most panelists, the score ranging from 7.0 to 7.4. The avocado paste sample had the highest score of 7.4. Avocado has a naturally sweet taste, soft and savory. The savory flavor comes from the fat vegetable content of 0.71–2.15% and the total fatty acid content of 37–85% [32].

The texture evaluation ranged from "like moderately" to "like very much". The sample fortified with olive oil demonstrated a significant difference from other samples in this respect. Olive oil has a characteristic yellowish-gold color, sometimes greenish, and its relatively thick texture is rather oily. According to Bermúdez-Oria *et al.*, gelatin serves as a stabilizer, as well as an adhesive and gelling agent in jelly candy while olive oil gives it an oily and shiny chewy texture [33].

Gelatin jelly candy proximate analysis. Table 2 shows the proximate analysis of gelatin jelly candy with various natural ingredients. Water content had no significant effect ($p > 0.05$): each natural ingredient brought about different water content. The samples with soy milk and goat's milk powder produced the best water content between 9.76 ± 0.70 and 9.92 ± 0.68%. Initially, soy milk powder contains 3.31 ± 0.27% water, and goat's milk powder has 5.48 ± 0.23% water [34, 35]. Honey, dates, olive oil, and grapes added in liquid form resulted in very high water content compared to pasta ingredients (avocado and pumpkin). The correlation with the initial water content in the natural raw materials is very strong: honey contains 5.20 ± 0.33% of water while grapes contain 21.17 ± 0.76%, avocado contains 34.28 ± 0.95%, and pumpkin contains 14.18 ± 0.22% [36–39].

Table 2. Proximate analysis of gelatin jelly candy

Таблица 2. Предварительный анализ состава жевательного мармелада с желатином из кожи скумбрии

Natural ingredients, form	Proximate, %					
	Water	Ash	Protein	Fat	Carbohydrate	Sugar reduction
Honey (liquid)	10.25 ± 0.42^{a}	0.13 ± 0.01^{a}	15.67 ± 0.52^{a}	2.20 ± 0.23^{a}	63.93 ± 1.28^{a}	0.18 ± 0.07^{a}
Date juice (liquid)	14.09 ± 0.84^{b}	0.32 ± 0.01^{b}	15.82 ± 0.53^{a}	2.35 ± 0.26^{a}	64.03 ± 1.14^{b}	0.26 ± 0.03^{a}
Olive oil (liquid)	10.71 ± 0.60^{a}	0.18 ± 0.01^{c}	15.77 ± 0.67^{a}	2.09 ± 0.22^{a}	58.26 ± 1.60^{c}	0.13 ± 0.05^{a}
Soy milk (powder)	9.76 ± 0.70^{a}	0.21 ± 0.02^{d}	16.20 ± 0.37^{a}	2.32 ± 0.50^{a}	51.61 ± 0.80^{c}	0.14 ± 0.01^{a}
Goat's milk (powder)	9.92 ± 0.68^{a}	0.20 ± 0.03^{cde}	13.97 ± 0.36^{b}	1.99 ± 0.28^{a}	57.57 ± 0.79^{ad}	0.17 ± 0.09^{a}
Grape juice (liquid)	10.82 ± 0.78^{a}	0.15 ± 0.01^{a}	13.62 ± 0.37^{b}	2.31 ± 0.33^{a}	62.55 ± 0.59^{ade}	0.18 ± 0.05^{a}
Avocado (paste)	10.23 ± 0.46^{a}	0.18 ± 0.01^{cef}	14.19 ± 0.45^{b}	1.67 ± 0.30^{a}	63.94 ± 1.46^{adef}	0.12 ± 0.05^{a}
Pumpkin (paste)	10.48 ± 0.56^{a}	0.14 ± 0.02^{a}	14.39 ± 0.64^{b}	2.29 ± 0.35^{a}	61.81 ± 1.20^{aeg}	0.22 ± 0.05^{a}
Indonesian National Standard for jelly candy	20.00	3.00	–	–	–	25.00

Note: Means in the rows with different superscripts are significantly ($p \leq 0.05$) different.

Примечание: Средние значения с разными верхними индексами существенно различаются ($p \leq 0{,}05$).

Water content greatly affects the quality and durability of gelatin jelly candy [40]. In our study, the overall water content of gelatin jelly candy fell within the standards set by Indonesian National Standard No. 3547-2-2008 Jelly candy with its maximum of 20.00%. The variance analysis showed that adding natural ingredients affected the water content significantly ($p < 0.05$). The highest ash content of 0.21 ± 0.02% belonged to the sample fortified with soy milk powder. However, the value of ash content in this study met the standards required by the Indonesian National Standard (max. 3.00%). The high ash content in the samples with soy milk and goat's milk powder was due to the initial mineral content in the raw materials. The ash content of soy milk powder is 0.40 ± 0.05%, and that of goat's milk is 0.07 ± 0.00% [34, 41].

During processing, the total minerals in the raw materials did not change significantly. The ash content and that of gelling agents were higher in the final product. The ash content tended to be lower in the samples with liquid honey, olive oil, date juice, and grapes, as well as in avocado and pumpkin pastes. Obviously, the fruit extraction process reduced the mineral content in the fruit juice. The components are easily decomposed or evaporated during fruit ashing [42].

Table 2 showed that the value of protein content ranged from 13.62 ± 0.37 to 16.20 ± 0.37%. According to the variance analysis, the natural ingredients produced a significant effect on the protein content ($p < 0.05$). The highest protein content of 16.20 ± 0.37% belonged to the sample fortified with soy milk powder. Fresh soy milk contains 23.08 ± 0.16% protein while powdered soy milk has a protein content of 5.09 ± 0.29% [34, 43]. Interestingly, the protein content of gelatin produced a very high protein content of 91.52%.

Protein intake is needed to build muscle mass, especially in toddlers. Jelly candy can deliver bioactive compounds required by the toddler's body. The protein content in the samples fortified with natural ingredients in liquid form (honey, olive oil, dates, and grapes) and paste form (avocado and pumpkin) also had a relatively high protein content [44]. Kia *et al.* reported that jelly candy with gelatin had a higher protein content [11].

The natural ingredients produced no significant effect on fat content ($p < 0.05$). In the sample with soy milk powder, the fat content was 2.32 ± 0.50%. The high and low-fat content of jelly candy depended on the differences in the raw materials used. According to Nemo & Bacha, the fat content in honey is 0.27 ± 0.20% [36]. Other studies reported the following fat contents for different raw materials: soy milk powder – 11.36 ± 0.44%, goat's milk powder – 1.02 ± 0.09%, grapes – 0.64 ± 1.17%, avocado – 6.66 ± 0.10, pumpkin – 4.50 ± 0.21% [34, 37–39, 41].

The total value of carbohydrates in this study ranged from 51.61 ± 0.80 to 64.03 ± 1.14%. The variance analysis showed that adding natural ingredients to gelatin jelly candy had a significant impact on total carbohydrates ($p < 0.05$). The highest total carbohydrate value belonged to the sample fortified with date juice and equaled 64.03 ± 1.14%, probably because the calculation of carbohydrates was carried out using the by-difference method. The high value of carbohydrates in each treatment managed to meet the requirements for energy intake.

Liu *et al.* explained that carbohydrates give food a sweet taste, especially monosaccharides and disaccharides that provide energy for the body [44]. The value of carbohydrates in our study depended on the raw materials. The level of carbohydrates was quite high in grapes (49.17 ± 2.31%), avocado (54.23 ± 0.02%), and pumpkin (61.71 ± 0.10%) [37–39].

Sugar residue is a substance left after a specific chemical process; this residue could be likened to salt. In our research, the mean value ranged from 0.12 ± 0.05 to 0.26 ± 0.05%. The results followed the Indonesian National Standard for jelly candy with its maximum of 25.00%. The statistical analysis of the sugar reduction between the samples revealed no significant effect ($p > 0.05$). It was because the sugar residue came from the sucrose produced by jelly candy. Garusti *et al.* stated that palm sugar contains 87.10% sucrose with 6.06% reducing sugar [45]. The content of reducing sugars depends on the inversion of sucrose into reducing sugars. The low level of reducing sugar in the study was due to the natural ingredients used. Reducing sugar in natural ingredients tends to be lower and can be easily synthesized by the body [46].

Microbiological analysis. The total plate count value was $\leq 1\times10^2$ colonies per 1 g, which met the Indonesian National Standard for jelly candy, i.e., 3×10^3 colonies per 1 g (Indonesian National Standard No. 3547-2-2008). The low total plate count could be explained by the fact that sucrose has antibacterial properties. In Balakrishnan *et al.*, sucrose was oxidized to form acetals in the heating process [47]. The acetal group can release cation-charged ions that interact with the anionic charge of the microbial cell membrane through electrostatic bonds, thus increasing cell permeability, and cell leakage leads to cell death.

All samples of gelatin jelly candy exhibited negative results for *E. coli* and *Salmonella* sp. The results also met the Indonesian National Standard. The absence of *E. coli* and *Salmonella* sp. could be traced to the natural antibacterial activity of the ingredients added. According to Handayani *et al.*, some natural ingredients in liquid form have antibacterial properties against *Staphylococcus aureus* and *E. coli* at a maximum concentration of 0.04 g/mL [48].

Conclusion

The mackerel skin gelatin jelly candy fortified by different natural flavorings was well received by panelists, with evaluations ranging from "like moderately" to "like very much". The nutritional quality of the gelatin jelly candy met the Indonesian National Standard. The best results belonged to the samples fortified with soy milk: it had the highest protein and the lowest carbohydrate

contents. Further research will feature the amino acids in each flavor sample of gelatin jelly candy.

Contribution

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

Conflict of interest

The authors declare no conflict of interests regarding the publication of this article.

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Авторы заявляют об отсутствии потенциальных конфликтов интересов в отношении исследования, авторства и/или публикации данной статьи.

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