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Gelatin from Milkfish Scales for Food Application

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Abstract

The global demand for food gelatin is increasing each year. Recovery of valuable components from fish processing-by products could help solve problems on tons of wastes produced each year and address religious beliefs like Islam and Judaism. Gelatin can be made from different sources of collagen, mostly from hides and bones of pigs and cattle. In this study, gelatin was extracted from milkfish scales which were collected from Science City of Muñoz Public Market, Science City of Muñoz, Nueva Ecija. The physical properties (yield, strength, color, clarity and pH) of extracted gelatin were studied and compared with commercially-available bovine gelatin. Marshmallows were developed from these gelatin sources. Sensory evaluation was done to determine the appropriateness of the level of a specific attribute and the consumer's preference using the nine-point hedonic and just about right (JAR) scales. The extracted fish gelatin was comparable to bovine gelatin in terms of strength but they differ in terms of color, clarity and pH. The fish gelatin had a yield of 8.7%, high bloom value of 505g, white appearance and an acidic pH (5.25). The marshmallow developed from fish gelatin is comparable to bovine gelatin in all attributes (color, aroma, texture, taste, sweetness and aftertaste) except for sweetness. Overall, the gelatin extracted from milkfish scales can be used as an alternative to bovine gelatin for food application such as in marshmallow production.

Key Words: bovine, gelatin, gel strength, marshmallow, milkfish

Introduction

Gelatin is a highly digestible protein compound that complements certain types of diets (Gomez-Guillén et al., 2002; Johnston-Banks, 1990). This natural macromolecule is translucent, colorless, brittle when dry, flavorless, edible and serves a wide variety of functions: as a dietary food, salt reducer, flocculating agent, protein enrichment, and adhesives (Arpi et al., 2018). These many functions together with its excellent gelling property make the demand for gelatin for food, pharmaceutical, and photographic applications continuously increasing (Saeed et al., 2013; Gomez-Guillen et al., 2011). Gelatin is extracted by controlled thermal hydrolysis of collagen from various animals such as pigs, cattle and fish (Arpi et al., 2018). The properties of gelatin are influenced by several intrinsic factors such as source, age of the animal, and the type of collagen (Johnston-Banks, 1990).

The quality of gelatin depends on its physicochemical properties: gel strength, viscosity, thermal stability (setting and melting point), amino acid composition, and peptide size. Commercial gelatins have bloom values (gel strength) ranging from 50–200 g (Meng & Cloutier, 2014), viscosities between 2.0 cP to 7.0 cP (Johnston-Banks, 1990; Rafieian et al., 2015), setting points between 31.6–31.80°C (Ninan et al., 2014), melting points between 60.42–61.71°C (Suderman et al., 2018), high amount of glycine, proline, and arginine, and a beta and alpha chain with molecular weights of approximately 220kDa and 100kDa, respectively (Nhari et al., 2012).The physical properties depend on the gelatin's chemical properties: molecular weight distribution and the amino acid composition (Johnston-Banks, 1990). For example, gelatins with high amino acid glycine and proline content will also have high gel strength (Siregar & Suprayitno, 2019). In addition, the higher the viscosity of the gelatin, the higher the amino acid level (Rera & Suprayitno, 2019). Compared to bovine and porcine gelatin, the disadvantages of fish-derived is its lower bloom value (Arnesen & Gildberg, 2002; Cho et al., 2005), lower yields (Herpandi et al., 2011), and lower stability which tend to limit its application in the field (Leuenberger, 1991). It is seldom used due to its dark color and fishy odor. However, several authors have claimed that physical properties like yield, strength, and even odor are greatly influenced and can be altered using extracting conditions or pretreatment methods to improve the quality of gelatin (Arpi et al., 2018; Herpandi et al., 2011; Montero & Gomez-Guillen, 2000).

Seafood processing plants generate numerous amounts of by-products that are rich in protein (Muyonga et al., 2004). About 70–85% of the total weight of catch are regarded as processing discards. Bones and skins which are high in collagen corresponds to 30% of these wastes (Shahidi, 2007).In addition, several authors reported that more than 50% of the material coming from the total fish capture is not used as food and about 32 million tons or 25% of the total production of marine fishery catch are considered as wastes (Kristinsson and Rasco, 2000; Arvanitoyannis and Kassaveti, 2008; Kim and Mendis, 2006). Many western countries prefer bone-free fillets and products made from bone-free flesh that necessitates the removal of flesh from fish. This in turn produces substantial amount wastes such as scales, bones, skin, and entrails corresponding to 55% of the fish weight (Arvanitoyannis & Tserkezou, 2014).

Fish gelatin is an important alternative of bovine and porcine gelatin and can be considered as a Halal food, acceptable to all religions and cultures (Herpandi et al., 2011). Moreover, since scales is one of the waste products of the fish processing industry worldwide, this study will help reduce these wastes through recovery of marketable intermediate products from fish scales, which in this case is gelatin. This in turn can help the fishing communities in the country by providing additional revenue or profit to fishermen especially during fishing season when there is an oversupply of fish, hence fish waste products. This can be achieved by educating the fishermen on how to process these waste products into intermediate products such as gelatin. In this study, the physical characteristics of gelatin extracted from milkfish scales and the organoleptic indices of the marshmallow produced from fish gelatin were compared to the commercially-available bovine gelatin.

Materials and Methods

Collection of Samples

Milkfish scales were collected from the Science City of Muñoz Public Market, Science City of Muñoz, Nueva Ecija, Philippines. The collected samples were transported in iced condition. Samples were washed with distilled water, and kept frozen at -20°C until further processing and analysis.

Extraction of Fish Gelatin

The procedure for the extraction of gelatin was based on the study of Gudmundsson and Hafsteinsson (1997) with some modifications. Tap water was used to wash off the acid and alkali during the pre-treatment process; however, distilled water was used in extraction. A total of 400 g of samples were treated with 0.2% of food grade NaHCO3 (Arm & Hammer®) solution (w/v) at 1:7 ratio for 40 minutes. After treating the samples with NaHCO3, the samples were washed with tap water. The samples were then treated with 0.2% of food grade H3PO4 (NECO Philippines, Inc.®) solution (w/v) at 1:7 ratio for 40 minutes. Then, the samples were washed with tap water. This treatment was repeated three times. Finally, the samples were treated with 1% of food grade citric acid (NECO Philippines, Inc.®) solution (w/v) for 40 minutes. The samples were washed with distilled water. This process was also repeated three times. After that, the samples were extracted with distilled water at a ratio of 1:7 on a water bath at 60°C for 8 hours without stirring. After 8 hours, the samples were filtered. The filtrates were then heated on a water bath at 60°C for 8 hrs. to reduce the sample volume to 1/3 of the original volume. The clear extract that was obtained was filtered using a strainer, followed by oven drying and made into powder by pestle and mortar and packed in an airtight container. All samples were measured in triplicates.

Characterization of Fish Gelatin

The physical properties of the fish gelatin were determined: gelatin yield, gel strength, color, clarity, and pH. These characteristics of fish gelatin except for the yield were compared to commercially-available bovine gelatin.

The yield of gelatin was calculated based on the ratio of weight of dried gelatin to the total wet weight of fresh scales using the following formula (Mahmoodani et al., 2014):

$$\text{Yield of gelatin} = \frac{\text{Weight of vacuum oven dried gelatin}}{\text{Wet weight of fish waste}} \text{ x } 100$$

Gel strength was determined on a 6.67% gel (w/v), prepared by dissolving the dry gelatin in distilled water at 60°C, and cooling the solution in a refrigerator at 7°C for 16 hours. This is the standard gelatin concentration set by the Gelatin Manufacturers Institute of America. Inc. (2019). Gel strength was determined using Brookfield CT3 Texture Analyzer (AMETEK Brookfield, MA, USA) with a load cell of 5kN, cross-head speed 1 mm/s, and equipped with a 1.27 cm diameter flat-faced cylindrical plunger. The maximum force (g), was determined when the plunger had penetrated 4mm into the gelatin gels. To measure the color of the gelatin solutions, the samples were heated in a 65°C water bath for 10–15 minutes. The samples were then removed from the water bath and set aside until the temperature decreased to 45°C. The T60 UV-Vis Spectrophotometer (PG Instruments Limited, FB, UK) was calibrated to 100% transmittance with distilled water as blank at 450 nm. An aliquot of the sample was transferred to 1-cm cuvette. Percent transmittance was recorded at 450 nm (Gelatin Manufacturers Institute of America, 2019).

The clarity of gelatin was determined using a spectrophotometer. Briefly, a 6.67% gelatin solution was prepared, covered and let stand for 1–3 hours at room temperature. The sample was placed in a 45°C water bath and held until sample temperature is 45±1°C. Afterwards, the spectrophotometer was calibrated to 100% transmittance with deionized water blank at 620 nm according to the manufacturer's instructions. An aliquot of the sample solution was transferred to the cuvette and the absorbance value was recorded at 620 nm.

After the 12-hour extraction of the gelatin solutions, the samples were filtered and the pH was measured using Ohaus Starter 3100 Benchtop pH meter (Ohaus Corporation, NJ, USA).

Product Development and Sensory Evaluation

The extracted gelatin was used to make marshmallows following the protocol described by Brown (2019) with modifications. Briefly, 3 tbsp gelatin was bloomed in ½ cup of cold water. In a small saucepan, ½ cup of cold water was combined with 1 cup light corn syrup (Peotraco®) and a pinch of sea salt. The mixture was cooked over medium heat. Once it boiled, a candy thermometer was clipped on the side of the pan and the solution was cooked for 8 minutes until it reached 240°F. The solution was immediately removed from the heat and carefully poured into the gelatin mixture while the mixer was turned on at low speed. Once all the syrup had been added, the speed was increased to high. One (1) tsp of vanilla extract (McCormick®) was added towards the end of mixing. After 5 minutes, the mixture was poured into a greased pan. The marshmallow was cured overnight then sprinkled with confectioner's powdered sugar (Peotraco®) and cut into small cubes. Marshmallows were also made using the commercially-available bovine gelatin (Spices and Food Mix House®) following the abovementioned procedure.

The marshmallows were subjected to sensory evaluation. A questionnaire developed by the faculty members and research staff of the College of Home Science and Industry was used to evaluate the acceptability and determine if an attribute's intensity is at an optimal level (Appendix A). The indices of organoleptic characteristics such as color, aroma, texture, taste, shape, sweetness, and aftertaste were measured. Purchase intention was also determined. A total of 50 randomly selected respondents from the University Science High School and College of Veterinary Science and Medicine were recruited in the study to evaluate the samples in terms of overall opinion, degree of liking per attribute or index, and intention to purchase. The appropriateness of the level of a specific attribute and the consumer's preference were measured using the just about right (JAR) scale and the nine (9)-point hedonic scale.

Statistical Analysis

The data on physical properties of fish and bovine gelatin were analyzed using single-factor ANOVA while the mean scores of the different attributes were analyzed using one-way ANOVA in the SPSS Statistics 23 software. The graphs for the JAR scale were made using Crosstab and Microsoft Excel.

Results and Discussion

Physical Properties of Fish and Bovine Gelatin

Gelatin was successfully extracted from milkfish scales at home using the method of Gudmundsson & Hafsteinsson (1997) with some modifications; sodium hydroxide was replaced by sodium bicarbonate (baking soda) and food-grade phosphoric acid was used in place of sulfuric acid. The gelatin yield was calculated based on the ratio of the weight of dried gelatin to the total weight of fresh scales on wet basis. Out of the 400 grams of milkfish scales used, 8.7% of gelatin was extracted.

This is higher than the gelatin extracted from other fish species: sole (8.3%), megrim (7.4%), cod (7.2%), hake (6.5%) (Gómez-Guillén et al., 2001), bigeye snapper skins (6.5%) (Jongjareonrak et al., 2006), Nile perch bone (2.4%) (Muyonga et al., 2004), and tuna fins (1.25%) (Aewsiri et al., 2010). However, this yield is lower than in farmed Amur sturgeon (24.11%) (Nikoo et al., 2014), Brownbanded bamboo shark (19.06-22.81%) (Kittiphattanabawon et al., 2005), freshwater carp (13.5%) (Chandra & Shamasundar, 2015), black tilapia (11.5-18.3%) (Tan et al., 2019), and Atlantic salmon skin (11.3%) (Arnesen and Gildberg, 2007). In these studies, it was highlighted that the yield and strength of gelatin decreases as the acid concentration and pre-treatment time increases. In addition, the purpose of mild acid pretreatment is to achieve adequate swelling and disruption of the non-covalent intra- and inter-molecular bonds while alkali pretreatment, through slight hydrolysis of the polar regions, also causes swelling, reduces protein molecular size, and converts noncollagenous components into a more soluble product (Monsur et al., 2014; See et al., 2015). Likewise, the higher the extraction temperature, the higher the gelatin yield. This is exemplified in the study conducted by Tan et al. (2019) wherein gelatin yield significantly increased from 11.52% to 18.27% when the extraction temperature was increased from 45˚C to 75˚C. Increasing the extraction temperature means more energy was applied to the extraction system and more collagen could be extracted, hence the increase in gelatin yield (Sinthusamran et al., 2014). This just proves that pre-treatment methods or extraction conditions, specifically the concentrations of acids and bases as well as the extraction temperature, greatly affect collagen hydrolysis, which in turn influence the gelatin yield.

Table I

Physical Properties of Gelatin Extracted from Milkfish Scales and Commercial Bovine Gelatin

Gelatin Source	Gel Strength (g)	Color	Clarity	pH
Milkfish scales (6.67% w/v)	505.17±43.8[a]	0.54±0.03[a]	0.34±0.002[a]	5.25±0.09[b]
Bovine (6.67% w/v)	648.0±101.4[a]	0.09±0.02[b]	0.02±0.01[b]	5.68±0.02[a]

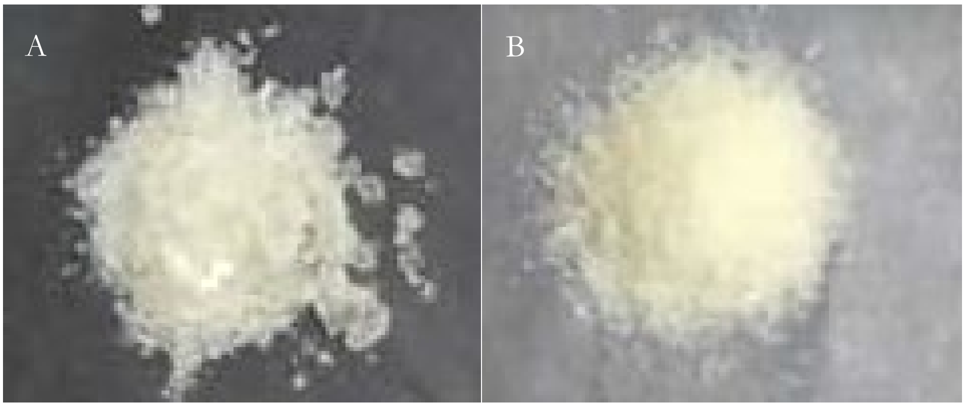
Results are presented as mean± standard deviation of n=3. Values within a column with different superscripts are significantly different *($P<0.05$)*.

The Bloom value of extracted gelatin from milkfish scales was 505 g, which was comparable to that of bovine gelatin (Table 1) but higher than those obtained in other studies using either fish skin (See et al., 2015; Monsur et al., 2014; Nikoo et al., 2014; Cho et al., 2005; Jamilah & Harvinder, 2002; Jongjareonrak et al., 2006; Muyonga et al., 2004) or bones (Arpi et al., 2018; Zhang et al., 2011; Aewsiri et al., 2010; Gómez-Guillén et al., 2001; Muyonga et al., 2004). The gel strength of gelatin extracted from fish by-products varies greatly with the highest Bloom value recorded in black tilapia (*Oreochromis mossambicus)* with 850 g (Tan et al., 2019) and the lowest value in Nile tilapia (*O. niloticus)* with 2.83 g (Arpi et al., 2018). This once again emphasizes the effect of extraction conditions on gel strength. Nevertheless, the high gel strength obtained in this study could mean that the amount of gelatin needed may be lower in food or industrial applications compared to other fish gelatin sources.

The color, clarity, and pH of fish gelatin were significantly different than in bovine gelatin. The color of gelatin derived from milkfish scales was crystal-white while it was off-white in bovine gelatin (Fig. 1). This white appearance may be due to the use of citric acid as swelling agent which was also observed by Gudmundsson and Hafsteinsson (1997) in codfish skin and Alfaro et al. (2013) in wami tilapia skin. Clarity or turbidity is influenced by filtration process which eliminates particles that precipitate as a result of concentration. A linear relationship exists between absorbance and concentration of solutions, i.e., the higher the absorbance or the closer it is to 1.0, the higher the concentration. These two events could explain the lower absorbance seen in bovine gelatin and higher absorbance of fish gelatin. Thus, it is recommended that further filtration of fish gelatin be done to improve its clarity. Fish gelatin had a slightly lower pH (5.25) than bovine gelatin (5.68). Likewise, the pH of fish and bovine gelatin was acidic contrary to porcine gelatin which was basic (7.5). This corroborates with studies in rohu and carp (Ninan et al., 2014; Chandra & Shamasundar, 2015), as well as in tilapia and golden threadfin bream (Alfaro et al., 2013; Maki & Annaka, 2020).

Figure 1

The Color of Dried Gelatin from Fish (A) and Bovine (B). Note the White Color of Fish Gelatin Compared to the Off-White Color of Gelatin Derived from Bovine

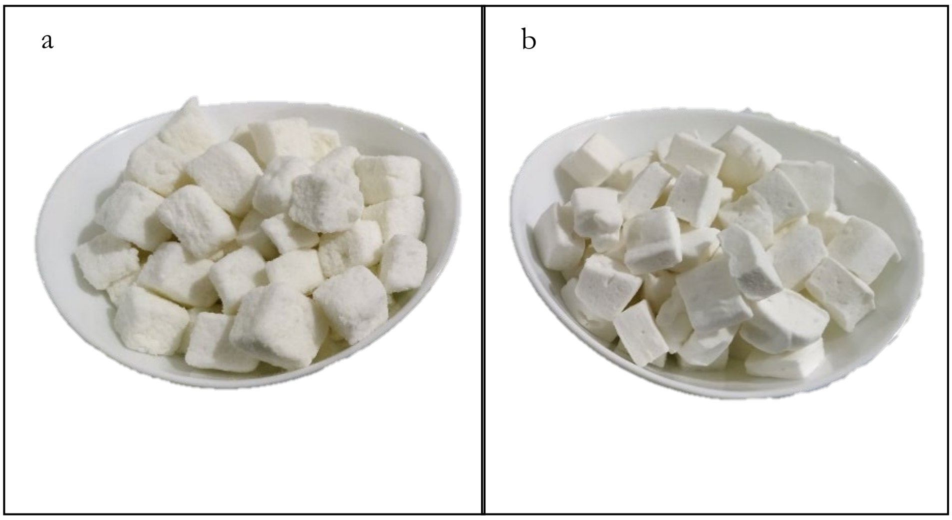


Product Development and Sensory Evaluation

Marshmallows were successfully made using the gelatin extracted from milkfish scales and commercially available bovine gelatin (Figure 2). The same set of ingredients were used in both marshmallows except for the type of gelatin. The indices of organoleptic characteristics such as color, aroma, texture, taste, sweetness, and aftertaste, as well as the overall opinion and intention to purchase were evaluated by 50 randomly-selected respondents or panelists from the University Science High School and College of Veterinary Science and Medicine, Central Luzon State University.

Figure 2

Marshmallows Made from Fish (A) and Bovine (B) Gelatin



Based on the result of the survey conducted, marshmallows made from bovine gelatin had the highest mean score in all the attributes except aftertaste (Table 2). However, the samples were still comparable since there was no significant difference between the two marshmallows. The degree of liking falls to like moderately to like extremely. When panelists were asked how likely they will buy the marshmallows if they were available in the market, most of them said that they will probably buy the products.

Table 2

Mean Scores of Organoleptic Indices and Purchase Intention for Marshmallows Made from Fish and Bovine Gelatin

Marshmallow	Overall opinion	Color	Aroma	Texture	Taste	Sweet-ness	After taste	Purchase Intention*
Fish Gelatin	11.95 ±2.29^{a}	12.42 ±1.79^{a}	11.76 ±2.11^{a}	11.78 ±2.92^{a}	12.02 ±2.62^{a}	11.78 ±2.75^{a}	11.57 ±2.78^{a}	4.06 ±0.87^{a}
Bovine Gelatin	12.42 ±2.09^{a}	12.51 ±1.94^{a}	11.89 ±1.99^{a}	12.51 ±1.82^{a}	12.37 ±2.20^{a}	12.08 ±2.45^{a}	11.18 ±2.70^{a}	4.34 ±0.80^{a}

Results are presented as mean± standard deviation of *n*=50. Values within a column with different superscripts are significantly different (*P<0.05*). Ranges of scores: 0-1.8= dislike extremely- dislike very much, 1.8- 3.8= dislike very much – dislike moderately; 3.8-5.6= dislike moderately- dislike slightly, 5.6-7.5 =dislike slightly-neither like nor dislike, 7.5-9.4= neither like nor dislike- light slightly, 9.4-11.3= like slightly- like moderately, 11.3-13.1=like moderately- like very much, 13.1-15= like very much- like extremely.

*Purchase Intention- 1= I will definitely not buy, 2= I will probably buy, 3= I am not sure if I will buy or not buy, 4= I will probably buy, 5= I will definitely buy

Just about right (JAR) scale was also used in this study to determine if an attribute's intensity was at an optimal level or if there was a need to improve a certain attribute. This was done using a 5-point scale consisting of the following: (1) much too weak, (2) too weak, (3) just right, (4) too strong and (5) much too strong. A score of 70% and above indicated that the attribute was at an optimal level. The results obtained for the JAR scales are shown in Figures 3–8. JAR scaling showed that more than 70% of respondents or panelists perceived all the attributes of both marshmallows at optimal levels except for the sweetness. Only 52% of the panelists considered the sweetness of marshmallows produced from fish gelatin as "just about right," while 34% and 10% perceived their sweetness as too strong and too much strong, respectively. This could be explained by the amount of sugar and cornstarch left in these marshmallows upon coating due to their softer texture as compared to the marshmallows from bovine gelatin. Hence, it is recommended to adjust the sweetness level of the marshmallows from fish gelatin by reducing its sugar and cornstarch coating.

Figure 3.

Just About Right (JAR) Scale Percentages of Responses on the Color of Marshmallows Produced from Fish and Bovine Gelatin. Note that 90% and 96% of the Panelists Perceived the Color of Marshmallows from Fish and Bovine Gelatin, Respectively as "Just About Right" or at Optimal Levels (N=50)

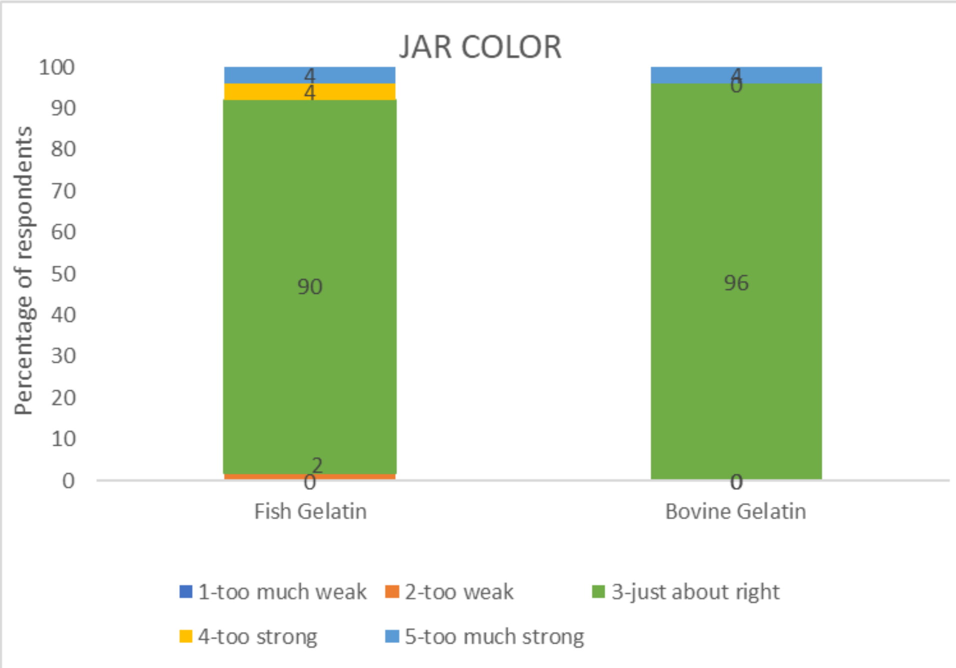


Figure 4

Just About Right (JAR) Scale Percentages of Responses on the Aroma of Marshmallows Produced from Fish and Bovine Gelatin. Note that 76% and 82% of the Panelists Perceived the Color of Marshmallows from Fish and Bovine Gelatin, Respectively as "Just About Right" or at Optimal Levels (N=50)

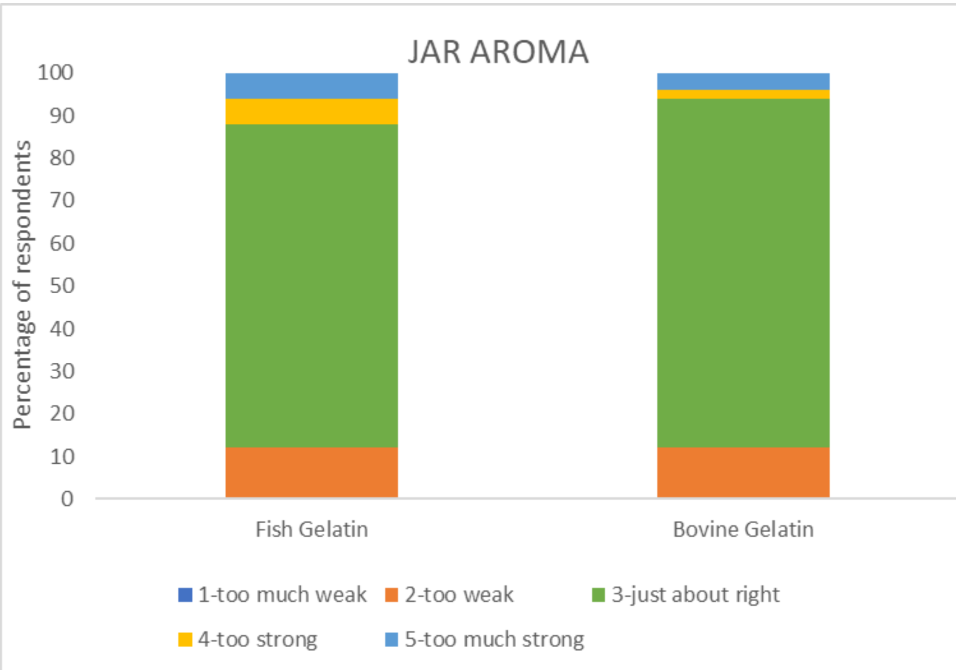


Figure 5

Just About Right (JAR) Scale Percentages of Responses on the Texture of Marshmallows Produced from Fish and Bovine Gelatin. Note that 72% and 84% of the Panelists Perceived the Texture of Marshmallows from Fish and Bovine Gelatin, Respectively as "Just About Right" or at Optimal Levels (N=50)

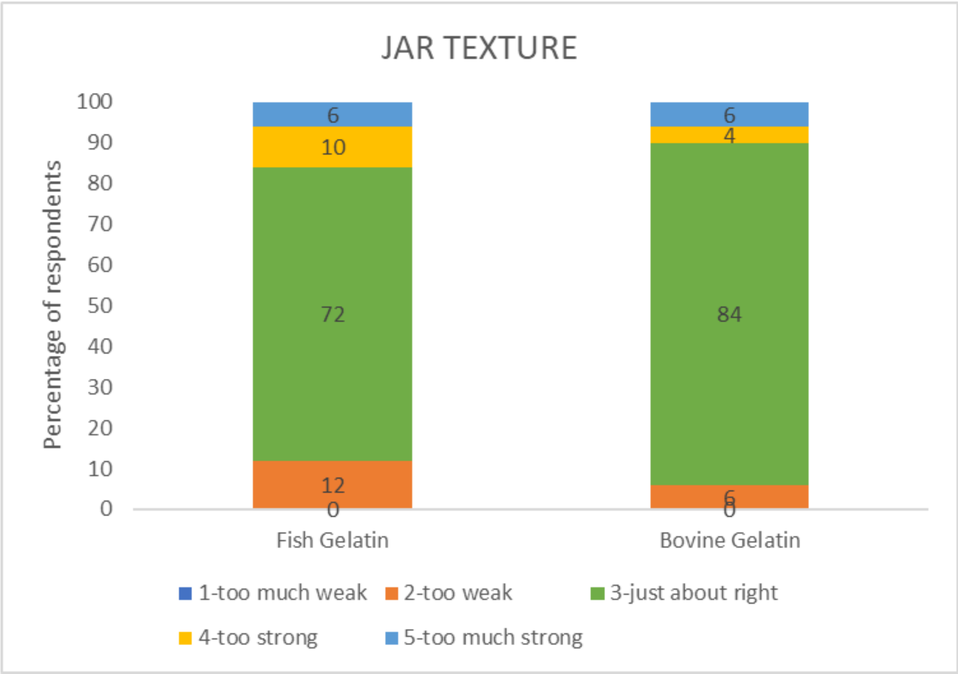


Figure 6

Just About Right (JAR) Scale Percentages of Responses on the Taste of Marshmallows Produced from Fish and Bovine Gelatin. Note that 76% of Respondents Perceived the Taste of Both Marshmallows as "Just About Right" or at Optimal Levels (n=50)

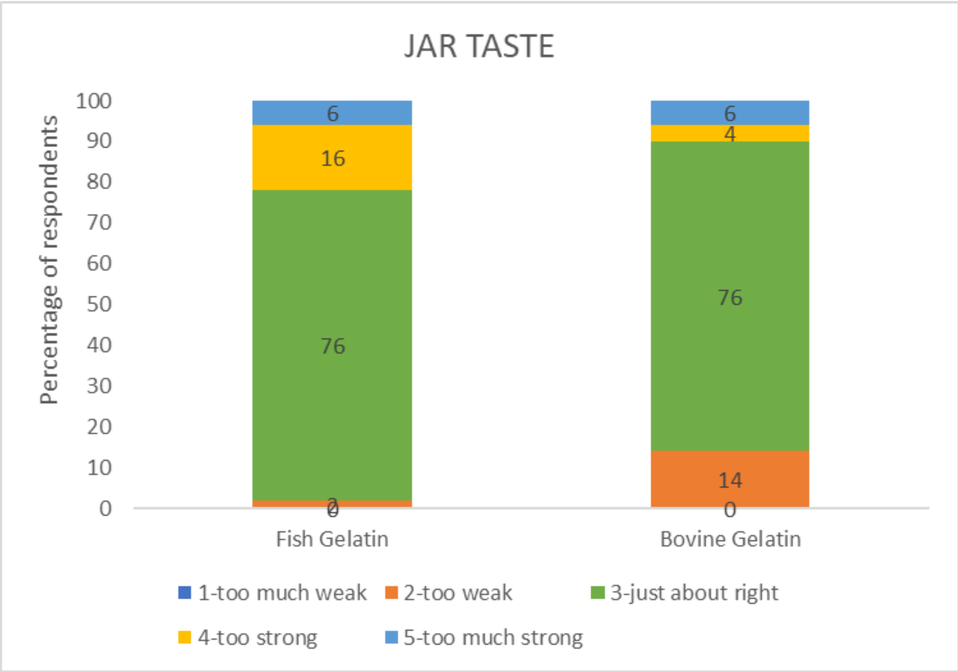


Figure 7

Just About Right (JAR) Scale Percentages of Responses on the Sweetness of Marshmallows Produced from Fish and Bovine Gelatin. Note that 34% and 10% of the Panelists Perceived the Sweetness of Marshmallow from Fish Gelatin as Too Strong or Too Much Strong (N=50)

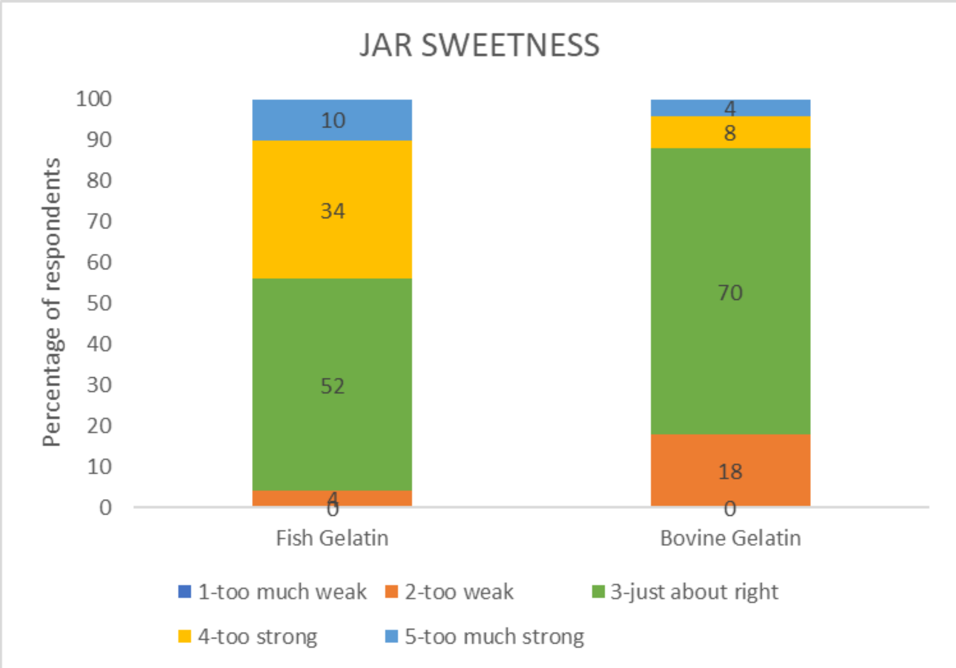
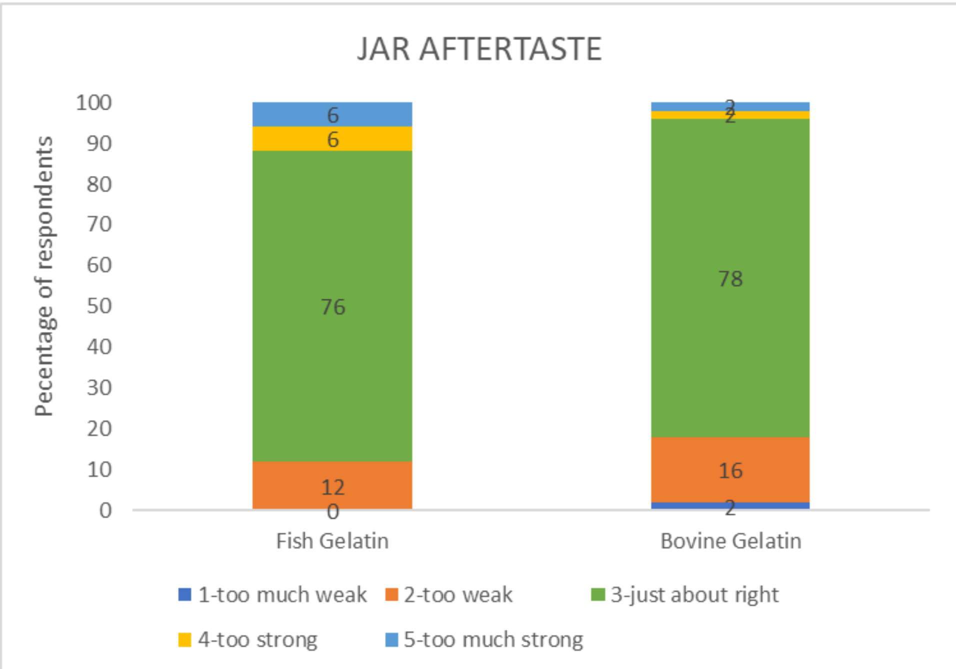


Figure 8

Just About Right (JAR) Scale Percentages of Responses on the Taste of Marshmallows Produced from Fish and Bovine Gelatin. Note that 76% and 78% of the Panelists Perceived the Aftertaste of Marshmallows from Fish and Bovine Gelatin, Respectively As "Just About Right" or at Optimal Levels (N=50)



Gelatin was successfully extracted from milkfish scales using the method of Gudmundsson and Hafsteinsson (1997) with some modifications: sodium hydroxide was replaced by food grade sodium bicarbonate (baking soda) and food grade phosphoric acid was used in place of sulfuric acid. Milkfish gelatin is comparable to bovine gelatin in terms of strength but they differ in terms of color, clarity, and pH. Marshmallows were successfully developed using milkfish gelatin and is comparable to marshmallows from bovine gelatin in terms of color, aroma, texture, taste, sweetness, and aftertaste. Overall, the marshmallows developed in this study fall within acceptable limits using both the nine-point hedonic and 5-point JAR scales. This simply implies the great potential of milkfish scales as an alternative source of gelatin and has proven its application in the food industry.

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