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Research Article

The effects of *Benthosema pterotum* gelatin replacement on physicochemical, textural and sensory properties of pastille

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Abstract

There is an on-growing demand for alternative sources of commercial gelatin. The by-products generated by fish-processing industries are a potential source for the production of halal gelatin in different industries such as food products. In this study, pastille was formulated with different concentrations of fish (*Benthosema pterotum*) (FG) and bovine (BG) gelatins. Different quality attributes of gelatin including humidity, ash, protein, water activity, sensory, texture and color were investigated. Statistical analysis was done by the ANOVA and LSD test was applied for mean comparison of the samples at $p<0.05$. Data analysis was carried out by SAS software (Version 9.8). The findings showed that total ash, springiness, elasticity, humidity, water activity and adhesiveness significantly increased with an increase in the *Benthosema pterotum* gelatin concentration in the pastille samples ($p<0.05$). However, the best pastille texture was observed in 50% BG 50% FG and for other parameters in samples with 100% BG ($p<0.05$). According to the results of sensory evaluation and color characteristics of treatments, it can be concluded that 25% *Benthosema pterotum* gelatin can be added to the bovine gelatin in the pastille formulation. The fish gelatin can be applied as cost-efficient source and also as quality improver in the production of pastilles.

Keywords: *Benthosema pterotum* fish, Halal gelatin, Pastille, Physicochemical properties, Sensory evaluation, Texture

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Introduction

Gelatin is the most widely-used colloidal protein constituent and is produced from different collagen resources. In fish processing, the obtained residue after filleting consists of large part skin and bones with high quantities of this protein (Da Trindade Alfaro *et al*., 2015). The solid product is one of the functional products in food, pharmaceutical, photographic and other products (Jayathilakan *et al.*, 2012).According to FAO (2019), the global gelatin market demand was 627.4 kilotons in 2020 and is projected to expand at a volume-based Compound Annual Growth Rate (CAGR) of 5.9% from 2020 to 2027.

Due to the recent health problems associated with Bovine Spongiform Encephalopathy (BSE) and religious beliefs on pig gelatin, there is a great tendency to use fish gelatin (Da Trindade Alfaro *et al*., 2015) as halal source. Different fish species vary greatly in the amino acid composition of collagen (Da Trindade Alfaro *et al*., 2015). However, the extraction process is very important because it determines the molecular weight distribution of gelatin. Fish gelatin has similar characteristics to meat gelatin but it has a low melting point and poor gel strength (Gómez-Guillén *et al.*, 2007). Therefore, fish gelatin can be an alternative to mammalian gelatin in modern food technologies (Ratnasari *et al.*, 2014, Zakaria, and Bakar, 2015, Fan *et. al*, 2017, Silva *et al.*, 2017).

The quality control of different products is important, given that besides the source and species and the properties of fish gelatin strongly depend on the conservation of raw materials (Da Trindade Alfaro *et al*., 2015). The advantages of using commercial gelatin in food industries include melting at body temperature along with a rapid release of flavor, unique texture, elasticity and brightness, easy digestion, preventive activity against arthritis and osteoporosis and being rich in protein. However, the disadvantages of using gelatin in food industries include low heat stability, low jelly temperature, slow jellification, high-temperature dissolution, bovine spongiform encephalopathy (BSE), animal source and religious limitations (Karim and Bhat, 2008).

Choobkar *et al.* (2018) studied the effect of replacement of cow's gelatin by freshwater fish skin gelatin (*Cyprinus carpio*) on some mineral contents and color parameters of a functional pastille. The results showed that treatment with 75% fish gelatin had the highest level of humidity, protein, phosphor, iron, ash and zinc, so the fish gelatin can be a valuable nutritional source especially in children's favorite junk foods.

On the other hand, many attempts have been made to use small marine fish and by-products in the fishery industry (Moosavi-Nasab *et al.*, 2018). A class of marine fish abundantly hunted from the Oman Sea is *Benthosema pterotum* (Nolsøe and Undeland, 2009). *Benthosema pterotum* (Alcock, 1890) is a small fish from Myctophidae family that is found all around the world (Chai *et al.*, 2012). Studies have estimated that there are large and intact resources of this fish in the Oman Sea, which is about 1-4 million tons in Iranian waters (Valinassab *et al.*, 2007).

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Chai *et al.* (2016) conducted a study on the peptides prepared from the hydrolysis of *Benthosema pterotum* and showed that the proteins prepared had high antioxidant properties and affected the immune system through blood circulation (Chai *et al.*, 2016). Since *Benthosema pterotum* can be a rich and cost-efficient source and worth extracting of gelatin, the present study was performed to investigate the production of pastille from *Benthosema pterotum* gelatin in combination with bovine gelatin as well as its physicochemical, textural and sensory properties as an alternative source of bovine gelatin.

Materials and methods

Ingredients

The ingredients used in this study consisted of bovine gelatin (Framand, Iran), sugar, distilled water, hydrochloric acid, potassium sulfate, sodium hydroxide, copper sulfate, selenium oxide, sulfuric acid, and boric acid. All chemicals in this study were purchased from Merck & Co., Inc. and the source of *Benthosema pterotum* was prepared from the Persian Gulf. The samples were transported to the laboratory by ice packaging at an appropriate temperature (-4°C).

Fish gelatin preparation

Given the small size of *Benthosema pterotum* all, different parts of the fish were used for the extraction of gelatin. Extraction of gelatin from the *Benthosema pterotum* was performed in six stages included: (1) The fish (mean weight :2g and mean length: 3cm) were rinsed and kept at -20°C; (2) the samples were mixed with 0.55 N sodium hydroxide solution for 90 min in a 5:1 ratio (v/w) at ambient temperature; (3) the samples were washed with 0.1 N hydrochloric acid for 90 min in a 5:1 ratio (v/w) at ambient temperature; (4) the samples were washed with water in a 5:1 ratio (v/w) until the pH reached a neutral rate; (5) the fish residues were isolated by centrifuge (FB50, Behsan, Iran) for 15 min at 4000 rpm; (6) the samples were dehydrated and dried by a rotary evaporator (Buchi, Switzerland) and vacuum incubator (Shimaz, Iran) with mild evaporation and high suction (Ratnasari *et al.*, 2014; Choobkar *et al.*, 2018). Gel strength (g Bloom) of the gelatin was 75g due the using the small whole fish (GMIA standard of gelatin is 50-300 g).

Pastille preparation

To produce pastille, 7.5% gelatin, 20% sugar, 62.5% distilled water, and 10% additives were used (Abbasi *et al*., 2011). First, gelatin with different ratios of fish and bovine gelatins (0, 25, 50, 75 and 100% fish gelatin in the mixture) was dissolved in distilled water and stirred by a magnetic stirrer at 60°C.

To remove the air bubbles and clarify the solution, the obtained mixture was placed in a hot water bath at 70 °C. Then, sugar was added to the gelatin solution and the sugar-gelatin solution was poured in a cast and placed in an oven at 37°C for 24 h. Then, it was exposed to ambient temperature for 24 h. After 24-h storage at room temperature, required tests were performed (Boran *et al.*, 2010). The tests were done according to the standard edible gelatin tests defined by the Gelatin

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Manufacturers Institute of America (GMIA, 2013).

Color measurement

The color of the samples was measured instrumentally using a Hunter lab colorimeter (Hunter Lab, Color Flex, USA). The results were expressed in accordance with the CIELAB uniform color system in terms of L^*, lightness (values increase from 0 to 100%); a^*, redness to greenness (positive to negative values, respectively); b^*, yellowness to blueness (positive to negative values, respectively). The total color difference (ΔE^*) and color intensity (chroma:C^*) between the control and enriched samples were calculated by $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ and $C^* = [(a^*)^2 + (b^*)^2]^{0.5}$ respectively (Khosravi Darani *et al.*, 2017).

Humidity and total ash measurement

To perform this test, a specific amount of each sample was placed on the washed and dried glass plates and then the plates were placed in a hot oven at 105 °C for 1 h. Then, they were placed in a desiccator for 15 min and weighed afterward. Finally, the humidity percentage of the samples was calculated by the weight difference.

Furthermore, an electric furnace at 550°C was used to measure the ash content of the samples.

Protein (nitrogen) measurement

In order to determine the protein content, the Kjeldahl method was used and three stages including digestion, distillation and titration. For this purpose, nitrogen content in the treatments was determined and then the protein content was obtained (GMIA, 2013) from equation 1:

$$\text{Protein\%} = ((V \times N \times A \times P)/m) \times 100 \quad (1)$$

Where, V is the volume of hydrochloric acid, N is the acid normality, A is the mass value of nitrogen equal to 0.014, P is the protein factor equal to 6.25, and m is the weight of the sample (GMIA, 2013).

Water activity measurement

To determine the water activity of samples, equal weights of each sample were crushed, poured onto the small plates and put in an Active Water (AW) machine (AW SPRINT TH 500). When the vapour pressure reached a fixed level, the figure indicated in the machine shows the water activity of the samples (GMIA, 2013).

Sensory evaluation

For sensory evaluation of the pastille samples, some sensory features such as color, flavor, chewiness, gumminess, texture and total acceptance were evaluated by ten semi-trained evaluators. A five-point hedonic test was used to classify the samples into bad, poor, average, good, and very good categories (Korus *et al.*, 2009).

Evaluation of texture properties

The texture of each sample was assessed (thickness 2 cm, diameter 2 cm) by TPA test (Brookfield Texture Analyzer, Probe: TA5, Test speed: 2 mm/s and force: 50 N) in two reciprocating cycles using a flat-plate cylindrical probe with a diameter of 3.5 cm.

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Statistical analysis

The study was conducted with five treatments in triplicate in a completely randomized design. Having confirmed the normality of data distribution, statistical analysis was performed by ANOVA and LSD test for comparison of the means at 95% level (p<0.05). Data analysis was carried out by SAS (Version 9.8) software.

Results

Evaluation of color parameters (L, a*, b*)*

Fig. 1 illustrates the comparison of the mean values of color indices (L*, a*, b*) in the treatments indicating that treatment with 100% fish gelatin had significantly the lowest brightness index (24.2) compared with the other treatments (p<0.05). As shown in Fig. 1, the green color of treatments is reduced with an increase in the percentage of fish gelatin. The minimum level of index a* with respect to redness was found for the treatment without fish gelatin indicating that the pastille containing 100% bovine gelatin had more tendency to be green in color than red. Fig. 2 shows the images of the prepared pastille samples.

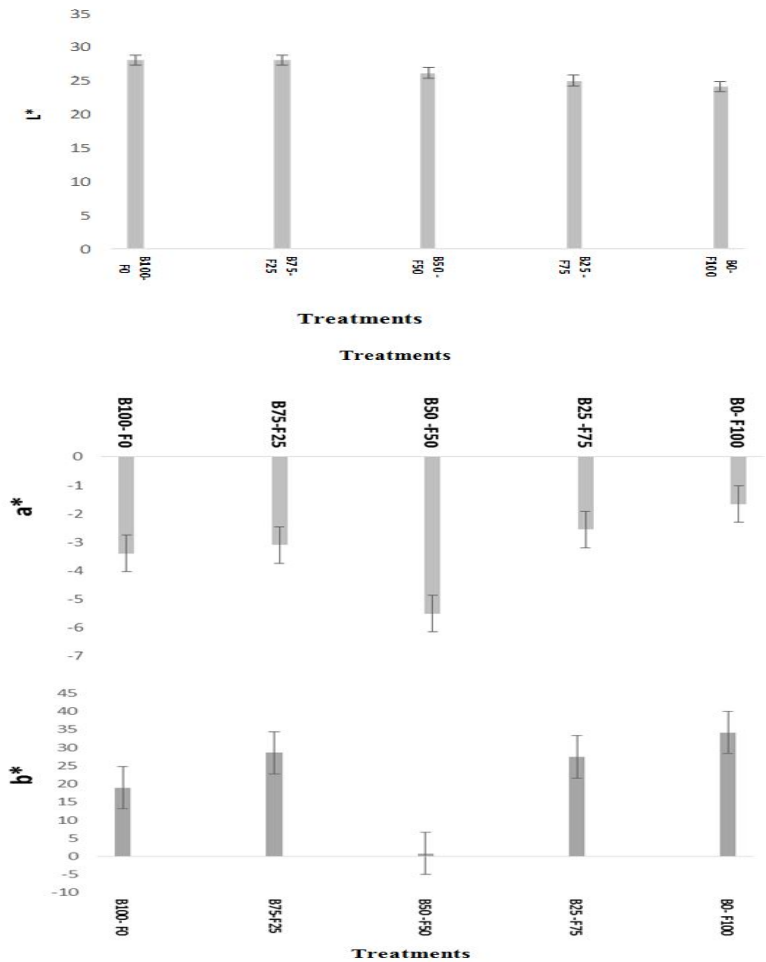

Figure 1: Comparison of mean color parameters (L*, a*, b*) in different treatments. "B" represents the bovine gelatin percentage and "F" shows the fish gelatin percentage. Treatments with at least one common letter are not significantly different at 5% probability level (p<0.05).

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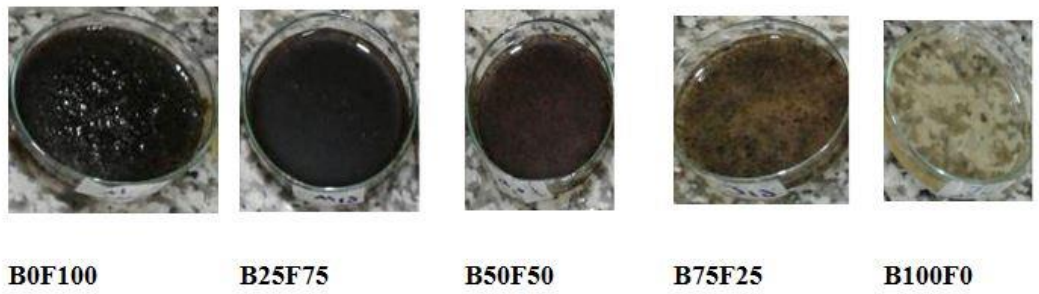

Figure 2: Treatment samples (B: bovine gelatin, F: fish gelatin).

Humidity evaluation

The mean humidity in different treatments is shown in Fig. 2. The highest level of humidity was reported for treatment with 100% fish gelatin (27.98%) and the lowest level of humidity was found for treatment with 100% bovine gelatin (16.72%).

Total ash content

Fig. 3 illustrates the mean ash values of the treatments. As shown, treatment with 100% fish gelatin had the highest amount of ash (1.35%) indicating a significant difference from the other treatments (p<0.05). The lowest amount of ash (0.9%) belonged to the treatment 100% bovine gelatin, showing a significant difference from the other treatments except for the sample containing 75% bovine gelatin and 25% fish gelatin (p<0.01).

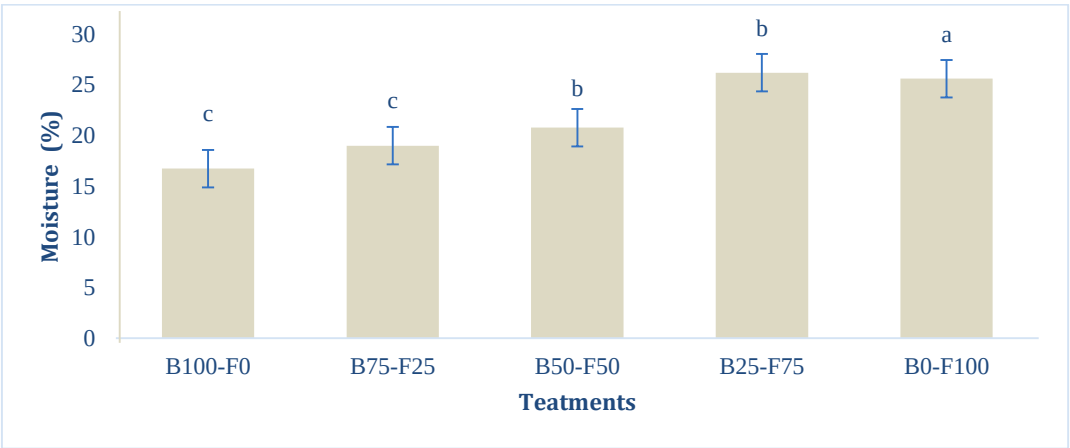

Figure 3: Comparison of humidity in the treatments. "B" represents the bovine gelatin percentage and "F" shows the fish gelatin percentage. Treatments with at least one common letter are not significantly different at 5% probability level (p>0.05).

Protein (nitrogen) measurement

As shown in Fig. 4, the percentage of protein is reduced with increase in the percentage of fish gelatin in different treatments. There was a significant difference between treatment with 100% fish gelatin and also 100% bovine gelatin (p<0.05). The lowest percentage of protein was found for 100% fish gelatin (36.13%) and the highest percentage was reported for 100% bovine gelatin (40.43%).

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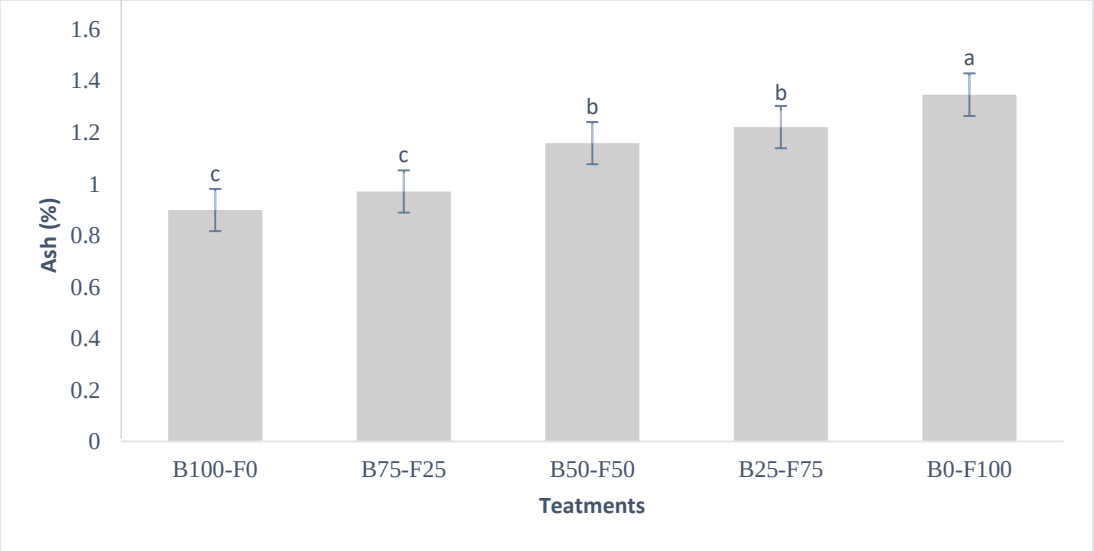

Figure 4: Comparison of ash content in the treatments. "B" represents the bovine gelatin percentage and "F" shows the fish gelatin percentage.Treatments with at least one common letter are not significantly different at 5% probability level (p>0.05).

Water activity evaluation

Fig. 5 indicates the results of the analysis of water activity in different treatments.

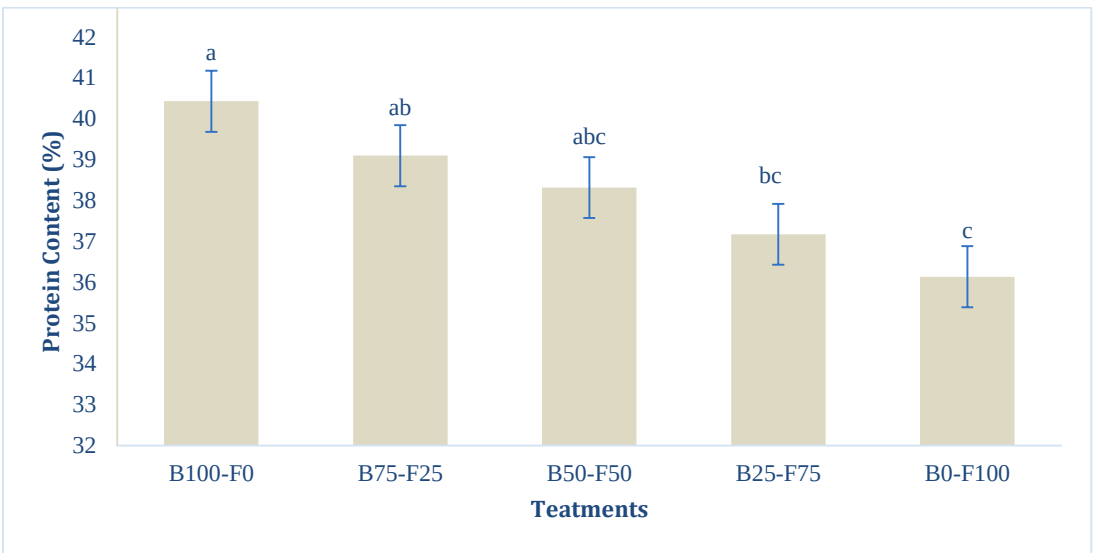

Figure 5: Comparison of protein content in the treatments. "B" represents the bovine gelatin percentage and "F" shows the fish gelatin percentage. Treatments with at least one common letter are not significantly different at 5% probability level (p>0.05).

As indicated above the lowest amount of water activity was reported for treatment with 100% bovine gelatin (0.73). The water activity increases with an increase in the amount of fish gelatin. Therefore, the highest water activity (0.84) was observed in the sample with 100% fish gelatin.

Texture evaluation

The results of sensory evaluation of texture are presented in Table 1.

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Table 1: Mean sensory scores of texture analysis in the study treatments.

Treatment*	Aroma	Flavor and taste	Texture	Color	Total acceptance
B100-F0	5±0.18[a]	4.5±0.43 [a]	4±0.17 [ab]	5±0.36 [a]	5±0.22 [a]
B75-F25	5±0.40[a a]	4±0.52 [a]	4±0.09 [ab]	5±0.27 [a]	5±0.18 [a]
B50-F50	4±0.33 [ab]	4±0.25 [a]	5±0.22 [a]	4±0.61 [a]	4±0.16 [ab]
B25-F75	4±0.01 [ab]	3±0.28[a]	4±0.48 [ab]	4±0.21 [a]	4±0.42[ab]
B0-F100	3±0.21 [b]	3±0.17 [a]	3±0.58 [b]	4±0.34 [a]	3±0.37 ±0.08 [b]

*B represents the percentage of bovine gelatin and F indicates the percentage of fish gelatin. Treatments with at least one common letter show no statistically significant difference at 5% probability level.

As shown in Table 1, treatment with 100% bovine gelatin was found to have the highest scores for most parameters including aroma, flavor, taste, color, and total acceptance. Higher scores for the texture of treatments were reported only for 50% bovine gelatin and 50% fish gelatin. Treatment with 100% fish gelatin had the lowest score among the treatments. Further, treatment with 25% fish gelatin had many similar characteristics to treatment with 100% bovine gelatin.

Instrumental evaluation of texture

Fig. 6 shows an example of a graph containing textural parameters. Table 2 shows the different textural parameters of pastille in different treatments.

The highest hardness is related to 100% bovine gelatin (54.84 N) and the lowest hardness is found on 100% fish gelatin (21.62 N) (p<0.05). As indicated in Table 2, the hardness was reduced by adding 25% fish gelatin indicating no significant difference between the two treatments (p>0.05).

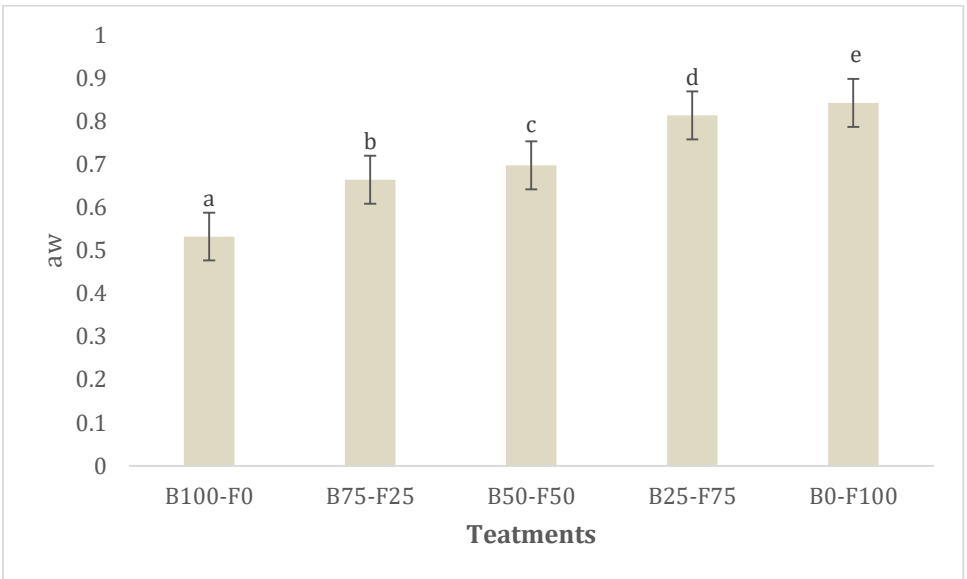

Figure 6: Comparison of water activity in the treatments. "B" shows the percentage of bovine gelatin and "F" indicates the percentage of fish gelatin. Treatments with at least one common letter show no statistically significant difference at 5% probability level (p>0.05).

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Table 2: Comparison of mean textural parameters of different pastille formulas containing bovine gelatin and fish gelatin.

Treat-ment	Hardness (N)	Adhesiveness (N)	Cohesive-ness	Elasticity	Springi-ness	Stringiness (mm)	Fractura-bility (N)	Gummi ness (N)	Chewiness
100-F0	54.84±0.08^{a}	3.79±0.04^{c}	0.95±0.07^{a}	0.48±0.02^{d}	0.99±0.03^{a}	1.54±0.09^{a}	54.84±0.06^{a}	45.24±0.04^{a}	45.93±0.03^{a}
B75-F25	47.50±0.05^{a}	4.5±0.03^{bc}	0.86±0.10^{b}	0.55±0.03^{c}	0.997±0.04^{ab}	1.42±0.02^{ab}	47.50±0.09^{ab}	40.16±0.02^{ab}	42.77±0.03^{a}
B50-F50	35.54±0.03^{b}	6.60±0.03^{abc}	0.81±0.05^{c}	0.68±0.04^{b}	1.005±0.03^{abc}	1.21±0.15^{b}	35.65±0.04^{ab}	32.04±0.06^{bc}	37.05±0.12^{b}
B25-F75	26.65±0.06^{bc}	7.37±0.08^{ab}	0.78±0.02^{c}	0.78±0.03^{a}	1.01±0.08^{bc}	0.79±0.12^{c}	26.65±0.08^{b}	22.96±0.08^{cd}	23.15±0.11^{c}
B0-F100	21.62±0.01^{c}	7.73±0.01^{a}	0.71±0.01^{d}	0.82±0.02^{a}	1.02±0.05^{c}	0.77±0.11^{c}	21.62±0.04^{b}	20.58±0.03^{d}	21±0.02^{c}

B represents the percentage of bovine gelatin and F indicates the percentage of fish gelatin.
Data with different letters in each column are statistically significant (p<0.05).

The lowest adhesiveness was found on treatment with 100% bovine gelatin (3.79 mJ). Further, the adhesiveness of the treatments increased by using fish gelatin to their formulation as adding 25% fish gelatin which enhanced the adhesiveness. However, no significant difference was observed between two treatments. Moreover, the highest adhesiveness was reported for 100% fish gelatin (7.73 mB), which was twice the amount of adhesiveness in treatment with 100% bovine gelatin and was significantly different (p<0.05).

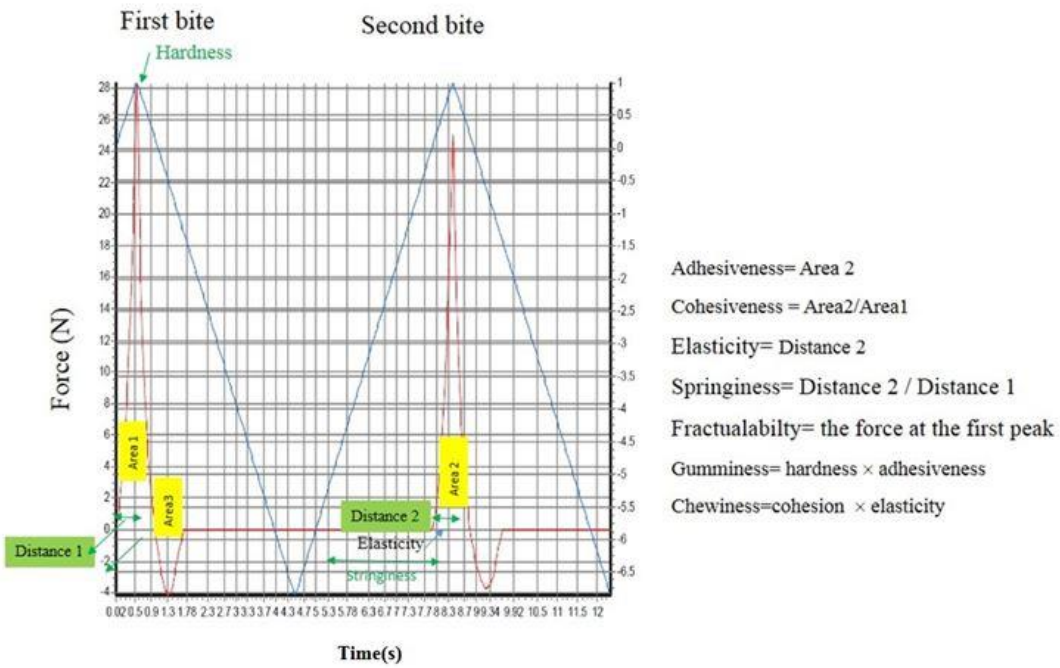

Figure 7: TPA Graph related to treatment of 50% bovine gelatin and 50% fish gelatin containing different tissue parameters.

The highest cohesiveness was reported for treatment with 100% bovine gelatin (0.95). The lowest cohesiveness, however, was found on treatment with 100% fish gelatin (0.71).

The results of the elasticity of treatments are presented in Table 2. The highest levels of elasticity were found on 100% fish gelatin (0.82). However, the lowest level of elasticity was reported for 100% bovine gelatin (0.48). The elasticity of

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treatment increased to 0.55 by adding 25% fish gelatin. However, the elasticity of pastille was significantly higher when using higher percentages of fish gelatin. Treatment with 100% fish gelatin showed the highest elasticity.

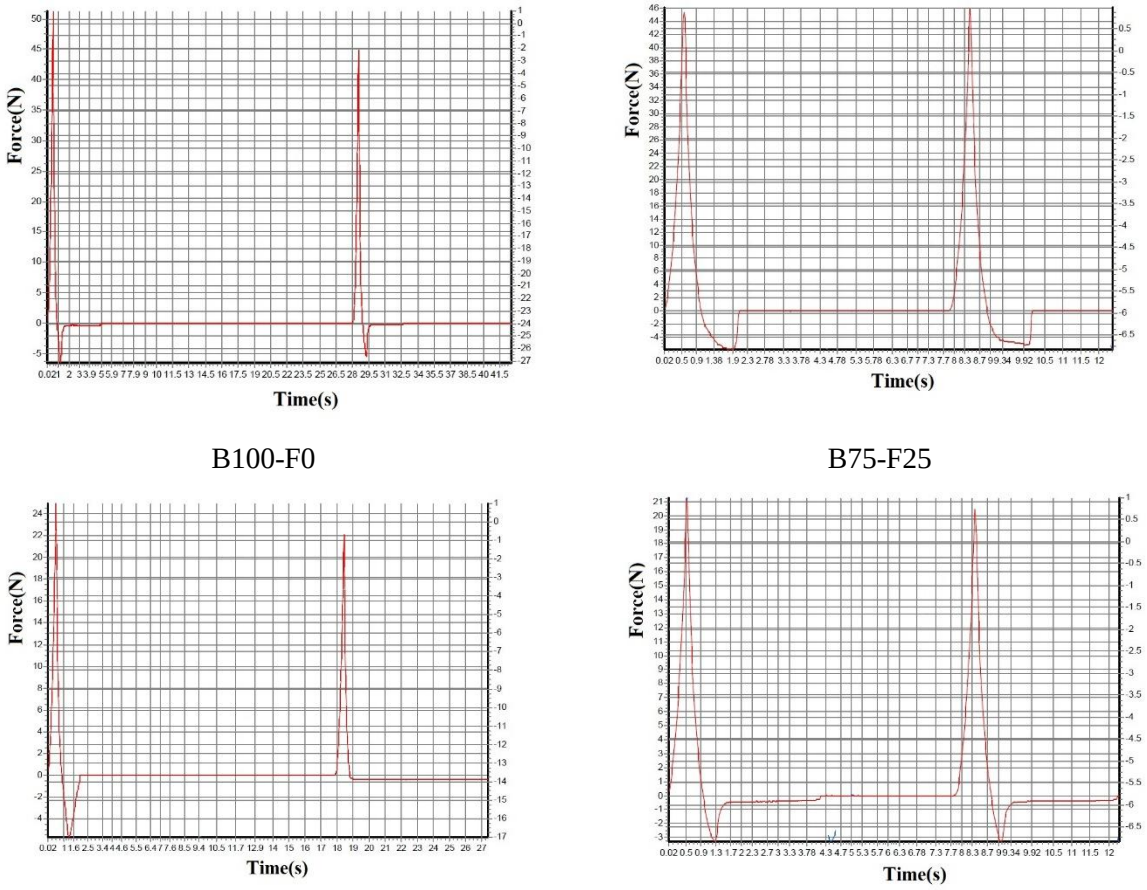

Figure 8: TPA graphs of different bovine gelatin percentage in pastille. *B represents the percentage of bovine gelatin and F indicates the percentage of fish gelatin.

Springiness is a textural parameter related to the elasticity of the food samples. Springiness in the texture profile refers to their original state over the time between the end of the first bite and the start of the second bite (Szczesniak, 2002). The springiness level was elevated by increasing the percentage of fish gelatin. The lowest amount of springiness was related to 100% bovine gelatin (0.99 mm) and the highest level was found on 100% fish gelatin (1.02 mm) indicating a significant difference (p<0.05).

The length of stringiness of the foods depends on their intramolecular adhesiveness (cohesiveness) (Skrede, 1985). On the other hand, the length of stringiness is decreased with a rise in the elasticity of the treatments. This can be attributed to the tendency of the material to return to its original state and consequently its resistance against the length increase (Schreiber and Gareis, 2007). The highest work done on stringiness and the highest length of stringiness were found on treatment with 100% bovine gelatin. The

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work done and length of stringiness was reduced with an increase in the percentage of gelatin in the formulation of treatments. The stringiness was reduced by adding 25% fish gelatin. The lowest rate of stringiness was found on 100% fish gelatin. There was a significant difference between the stringiness rate of the samples (p<0.05).

The highest fracturability was related to 100% bovine gelatin. As shown in Table 2, the fracturability rate was reduced by adding fish gelatin (p>0.05).

As shown in Table 9, the highest level of gumminess was found on 100% bovine gelatin (45.24 N) indicating a significant difference compared with the other treatments (p<0.05). By increasing the percentage of fish gelatin in the formulation of treatments, the gumminess of pastille texture reduced significantly. By increasing the percentage of fish gelatin to 25%, no significant difference was observed between this treatment and treatment with 100% bovine gelatin, and gumminess of samples decreased on 100% fish gelatin (p<0.05).

As indicated in Table 2, bovine gelatin had a significantly greater effect than fish gelatin on the chewiness of pastille; i.e. it increased the force required for chewing the product (Shafiur Rahman and Al-Mahrouqi, 2009).

By increasing the percentage of fish gelatin in the formulation, the lowest rate of chewiness was reported on treatment 100% fish gelatin (21 mB). In terms of chewiness, there was a significant difference between 100% fish gelatin and 100% bovine gelatin (p<0.05). Yet, there was a non-significant difference in chewiness between treatment 100% bovine gelatin and treatment 25% fish gelatin.

Discussion

Evaluation of color parameters (L, a*, b*)*

Color is one of the most important indices in formulation of food products that changes the visual quality making them attractive (Schreiber and Gareis, 2007). The color of food products is shown by L*, a*, and b* indices. The lightness index (L*) varies from 0 (black) to 100 (white), a* varies from -120 (pure green) to +120 (pure red), and b* varies from -120 (pure blue) to +120 (pure yellow) (Khosravi-Darani *et al.*, 2017).

The color of the fish body is related to the chromatophores which are mostly found in the dermis and are rarely found in the epidermis. These cells have black, yellow, orange, and red pigments. Other colors observed in the body of fish are developed by a combination of the above colors (Skold *et al.*, 2016). In other words, the presence of fish gelatin causes higher redness in pastille formulation .Therefore, the type of gelatin has a significant effect on the color profile (Skrede, 1985) of pastille samples.

The analysis of index b* which specifies the closeness of the color of samples to blue and yellow shows that the color of treatments tends to be yellow with an increase in the percentage of fish gelatin. The high yellowness of treatments with fish gelatin can be attributed to the presence of carotenoid pigments in the fish texture, which changes the color of treatments to yellow (+b) (Schreiber and Gareis, 2007).

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Humidity evaluation

Humidity is one of the most important factors affecting the quality of food products. It is indicative of the amount of free water in the samples which is extracted by drying. Hydrocolloids generally affect the humidity level of food products by creating hydrogen bonds (Shafiur Rahman and Al-Mahrouqi, 2009). Generally, the water absorption capacity varies in different foods depending on the type of amino acids, spatial arrangement of protein, hydrophilic and hydrophobic rates and presence of hydrophilic carbohydrates (Lewicki, 2004; Choobkar *et al.,* 2018). Some other researchers believe that water absorption and consequently humidity are reduced by increasing the fat content of the sample since fat covers the areas that can bond to the water. The hydrocolloid compounds such as gelatin, given their functional characteristics, are used in the formulation of food products as stabilizers and for maintaining the humidity (Shafiur Rahman and Al-Mahrouqi, 2009). Also, Choobkar *et al* showed that the humidity rate in the pastille prepared from bovine gelatin was less than the pastille prepared from *Cyprinus carpio* gelatin (Choobkar *et al.*, 2018).

Total ash content

The amount of ash increased as the percentage of fish gelatin in the samples increased. This increase can be attributed to the presence of fish scale and fin and even using of whole fish in the study. The ash content of food products depends on the amount of their minerals, proteins, polysaccharides, and salt (Da Trindade Alfaro *et al*., 2015). Gelatin mainly consists of protein and moisture. The presence of ash, lipid and other impurities in very low amounts is highly important for the quality of gelatin. In general, the ash content and impurities are higher in the fish gelatin treatments than the industrial gelatin treatments (bovine gelatin) (Choobkar *et al.,* 2018).

Protein (nitrogen) measurement

The lowest percentage of protein was found for 100% fish gelatin (36.13%) and the highest percentage was reported for 100% bovine gelatin (40.43%). Since the amounts of proline and hydroxyproline are lower in fish gelatin than the bovine gelatin, the qualitative features of fish gelatin differ from bovine gelatin. For instance, low amount of proline and hydroxyproline have less gel strength than bovine gelatin. However, these gelatins specifically have good film-formation and emulsification properties (Haug *et al.,* 2004). Some other studies have also shown many differences between the protein content of bovine gelatin treatment and fish gelatin treatment, including *Cyprinus carpio* (Asgarzadeh *et al*., 2020).

Water activity evaluation

Water activity is defined as the amount of free water in the material for hydration (Lewicki, 2004)**.** The values of water activity parameters vary from zero to one. If the water activity of a material is equal to one, it is indicative of completely pure water and if it is equal to zero, it shows the absence of free water which can be due to the presence of high levels of mineral. The water activity increases with an increase in the amount of fish gelatin. Lewicki **(**2004) showed that the higher intensity of hydrocolloids and therefore higher water

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bonding resulted in lower water activity (Lewicki, 2004).

Water absorption should be considered as the most significant physical feature of compounds with protein structures like gelatin. This phenomenon not only affects the physical structure and processing features of the food containing protein but also it is highly important for food spoilage (Lewicki, 2004). The water activity of a food is different from its humidity and the percentage of humidity increased with an increase in the amount of fish gelatin in the formulation (Choobkar *et al.,* 2018).

Instrumental evaluation of texture

The instrumental analysis of data can provide a more precise understanding of the texture. The production and analysis of data by machines is called Texture Profile Analysis (TPA). Texture profile analysis has been used for many years as a suitable method for measuring the textural properties of food and the parameters obtained from its curves are well correlated with sensory data.

Hardness is the resistance of the food to the application of the applied pressure force. The hardness of foods depends on the applied force. With a rise in fish gelatin ratio in pastille formulation, the treatment resistance against the force applied during biting is reduced which can indicate the hardness of the pastille prepared with bovine gelatin. The humidity and water of samples increased with an increase in fish gelatin which could be a factor affecting the softening of the texture and reduction of the hardness. The type of hydrocolloid and level of hardness plays a key role in the release and perception of taste (Chandra and Shamasundar, 2015a).

Cohesiveness is the internal strength of a food structure and its extent depends on the extent of the molecular interactions of the formulation components. In food products, adhesiveness is defined as the negative force used in the first bite which indicates the work required for overcoming the tensile forces between the surface of foods and surface of other materials that the foods have contact with (Azari Anpar *et al.,* 2017; Azari Anpar *et al*., 2021). The higher amount of water in gelatin leads to the higher free points in protein molecule (Shafiur Rahman and Al-Mahrouqi**,** 2009; Chandra and Shamasundar, 2015b). Cohesiveness is defined as the strength of structural bonds within a food product created due to intermolecular interactions which cause the structure of the food. Cohesiveness determines the deformability of material before breakdown. Since fish gelatin has a lower hardness than bovine gelatin, the cohesiveness of the treatments is reduced by increasing the percentage of fish gelatin (Chandra and Shamasundar, 2015a).

Elasticity is the ability of a material to return from deformation to its original state. In other words, elasticity is the elastic recovery of a material (Szczesniak, 2002). Elasticity is related to the network formed within gelatin. By increasing the fish gelatin in formulations, the sample gel induced less strain against the tension caused by the probe of texture profile analysis. In fact, the elastic range of the samples was elevated by increasing fish gelatin. It has been reported that higher

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interaction between protein chains reduces the solubility and causes the formation of elastic gel (Chandra and Shamasundar, 2015a).

Fracturability is a force with which a sample crumbles cracks or shatters. Fracturability is the result of a high degree of hardness and low degree of adhesiveness (Szczesniak, 2002). Since pastille is not a brittle food, the force required for their breakdown is reduced with a rise in the percentage of fish gelatin and consequently a reduction in the hardness of treatments (Chandra and Shamasundar, 2015a). Fracturability of treatments was decreased with an increase in the percentage of fish gelatin.

Gumminess is the result of hardness and cohesiveness (Boran *et al.*, 2010). On the other hand, gumminess is increased when the hardness of the sample increased (Shafiur Rahman and Al-Mahrouqi, 2009**)**. Chewiness is defined as the length of time or the number of chews required masticating a solid food to a state pending for swallowing. Chewiness is a product of hardness, cohesiveness and springiness (Szczesniak *et al.*, 2002).

In the present study, pastille with different concentrations of *Benthosema pterotum* gelatin and bovine gelatin was prepared. The findings showed that the bovine gelatin treatment had better color properties than the other treatments. Due to impurities in the extraction of fish gelatin, treatment 100% fish gelatin had the highest percentage of ash among all treatments and treatment 100% bovine gelatin had the highest percentage of protein. Thus, treatment with 100% bovine gelatin had the highest score on flavor and taste, aroma, color and total acceptance parameters. Nonetheless, by adding 25% fish gelatin to the pastille formulation, similar characteristics were found on the pastille prepared with bovine gelatin with respect to the sensory features of the texture and it can increased the food value of the junk food.

However, the quality of *Benthosema pterotum* gelatin and the prepared pastille can be promoted by improving the extraction method and reducing the waste. Improving the functional properties of fish gelatin, especially the rheological properties, would broaden its range of applications. The fish gelatins with lower rheological properties can be applied in various products, which do not require high gel strength and high melting point and also can be used as improver and to enrich pastilles.

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