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Increasing calcium and dietary fiber of Mu Yo using fish bone and Konjac flour

Nachayut Chanshotikul[1], Natcharee Jirukkakul[1], Nipaporn Sengkhamparn[1], Bung-Orn Hemung[1,*]

[1] School of Agro-Industry, Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai, Thailand, 43000
*Corresponding author: bunghe@kku.ac.th, hemung3@gmail.com

Abstract

Fish bone powder (FBP) and konjac flour (KJF) are considered as the good sources of natural calcium and dietary fiber. They have a potential to improve nutritional qualities of Mu Yo, emulsion pork sausage. Then, the aim of this research was to increase calcium and dietary fiber in Mu Yo with FBP and KJF supplements. KJF and FBP were incorporated separately at 0.5% into Mu Yo and the sample quality was compared with the control as well as the synergistic effects (0.5% KJF and 0.5% FBP). A reduction of moisture content was found when both KJF and FBP were added. Total ash content was increased significantly by FBP addition. Consequently, the total calcium content in Mu Yo increased from 5.5 to be 191 mg per serving size (100 g). Thus, this product can be classified as high calcium product. The crude fiber and total dietary fiber were also increased ($P < 0.05$) when KJF was added regardless of FBP addition. Based on texture profile analysis, addition of KJF resulted in an increase hardness and adhesiveness of product. Yellowness and redness of product were increased with addition of FBP, while a reduction of whiteness was found slightly. The integrity of emulsion gel was neither affected by KJF nor FBP as assessed by scanning electron microscopy. Sensory evaluation revealed that there is no different preference among the samples.

Keywords: Fish bone powder, Konjac flour, Mu Yo, Calcium, Fiber

1. Introduction

Mu Yo is the traditional sausage and classified as emulsion sausage based on technical processing. It is originated in Vietnam and has been widely consumed popularly in several areas throughout Thailand, including the North eastern part [1]. Pork and back fat are used as the main raw materials and they are processed into emulsion batter prior heating to stabilize the emulsion into the gel. This processing technology provides an elastic texture, which is the desirable attribute for general consumer. The unique flavor of Mu Yo varied from one recipe to another recipe depending on added seasoning such as salt, nitrite compounds, sugar, flour, sauce, phosphate and pepper. Based on the main ingredients, Mu Yo is rich in fat and protein. However, the lack of dietary fiber might be the limitation of product for promoting health benefit. Although addition of phosphate could stabilize emulsion gel and increase water holding capacity, phosphate may inhibit calcium absorption. The functionality of phosphate and its health effect are compromised for Mu Yo processors. Thus, fortification of natural calcium synergist with a reduction of phosphate might be the alternative choice for manufacturing healthier Mu Yo. However, fortification of calcium into Mu Yo has not been investigated, while the development of Mu Yo packaging and exposure to nitrite from Mu Yo consumption have been performed [2-3].

Tilapia fish bone is currently generated as by products from tilapia processing such as frozen fillet, dried fillet, and smoke fillet. This type of by product could be estimated approximately 10-15% of total weight. An appropriate handling is required to meet the environmental standard, resulting in the burden budget. Fish bone is currently sold as a cheap ingredient for animal feed production. However, another application of fish bone should be applied for higher potential utilization since it is considered as the natural source of important mineral especially calcium [4-5]. Recovery of tilapia bone for being the calcium supplement has been reported [6-7]. High ash content of tilapia bone powder was prepared successfully using an alkaline treatment [8]. Calcium from tilapia bone powder could increase gelling properties of fish sausage when acetic acid was used to extract [9]. Moreover, direct fortification of fish bone powder into protein gel was performed to improve textural properties although the particle size of bone was affected [10-11]. However, nutritional improvement regarding addition of fish bone has not been concerned. Moreover, fortification of tilapia bone powder into Mu Yo in order to increase natural calcium has not been investigated.

Konjac plant (*Amorphophallus konjac*) is in the family of Araceae. It is herbaceous herb and grows in mountain or hilly area of subtropical regions, especially in South East Asia. It is also found in North Eastern part of Thailand including Loei, Nong Khai and Bueng Kan provinces. The tubers of konjac can be used to prepare konjac flour and glucomannan is reported to be the main constituent [12]. Konjac glucomanan is considered as the polysaccharide providing several benefits for health. The cholesterol and sugar level could be lowered by konjac glucomanan [13]. It could not be digested by enzymes in digestive tract of human, promoting the activity in intestine and prevent the constipation. Konjac glucomanan and flour have been used as food additives since it could generate several functionalities such as water absorber, stabilizer, gelling agent, thickening agent and film forming agent [12-13]. Direct incorporation of konjac flour into Mu Yo would be the way to increase dietary fiber, providing healthier Thai traditional meat products. However, incorporation of konjac flour into Mu Yo has not been investigated.

Therefore, the aim of this study was to develop nutritional value of Mu Yo by incorporating fish bone powder as the natural source of calcium and konjac flour as the source of dietary fiber. Then, this product could be high nutritional value product for consumer.

2. Material and Methods

2.1 Materials

Konjac bulk (1.7 kg) was obtained from the rural forest at Sanghom sub district, Nong Khai province. After peeling, it was cut into strip (2×2×50 mm) prior drying at 100 ºC for 24 h. Dried samples were ground using hammer mill (Retsch, Haan, Germany) with the sieve of 0.5 mm and konjac flour was kept in vacuum packaging and stored at room temperature.

Tilapia frame was brought from local market (Jaeng Sawang Market), Muang Nong Khai, Nong Khai province, Thailand. Tilapia bone powder was prepared according to the method described previously [8]. The powder was kept under vacuum condition at room temperature until used.

Pork ham was brought from the same market after slaughtering not over 3 h and frozen back fat of pig was used. Both of ham and back fat were ground separately through the meat grinder (Champ, Kent, U.K.) with perforation of 3 mm. The ingredients in Mu Yo recipe were of food grade. Chemicals for analysis were of analytical grade.

2.2 Mu Yo Preparation

Mu Yo was manufactured as the manner of an emulsion sausage. To formulate the control emulsion, pork mince, back fat, ice, salt, sugar, phosphate, oyster sauce, and tapioca flour were controlled at 55, 17, 20, 1.8, 1.0, 0.1, 1.6, and 2.0%, respectively. KJF or FBP were added separately at 0.5% in order to study their effect on Mu Yo quality. In addition, synergistic effects of KJF and FBP were also evaluated at 0.5%. These ingredients were added up to the control recipe. To prepare emulsion, pork mince was chopped with a little amount of ice for 1 min. Then powder ingredients were added prior chopping continuously for 2 min. Then, back fat and liquid ingredients were added before being chopped more for 2 min until the sticky and smooth batter were obtained. The batter was stuffed into casing (25 mm diameter) prior incubating at 40 º C for 30 min followed by cooking at 80 º C for 30 min. The cooked Mu Yo was cooled immediately in ice-water for 10 min before keeping in cold room until analysis.

2.3 Proximate Analysis

Chemical analysis of Mu Yo was based on AOAC methods [14]. Moisture content was by oven drying. Protein was estimated by Kjeldahl method using the conversion factor of 6.25. Crude fat content was performed using Soxlet method and dry ashing was applied for total ash determination

2.4 Fiber Analysis

After lipid extraction, the crude fiber content was determined by acid and alkali digestion using raw fiber extractors. Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) contents were analyzed by an enzymatic–gravimetric procedure using TDF-100 test kit (Sigma chemical company). The sample was sequentially enzymatic digested using heat-stable α-amylase, protease and amyloglucosidase to remove protein. After filtration, the retentate residue after being washed with distilled water was defined as IDF. While the filtrate after being washed precipitation with 95% ethanol was considered as SDF. Both SDF and IDF residues are corrected for protein and ash contents for the final values. The total dietary fiber was a summation of SDF and IDF.

2.5 Total Calcium Determination

Total calcium ion of dried Mu Yo sample was digested with nitric acid before analyzing the calcium ion using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer, Norwalk, CT. USA). The emission was detected at 317.933 nm.

2.6 Color Measurement

The samples were incubated at room temperature for at least 4 h and cut into cubic (1×1×1 cm). The color of the cross-sectional area was measured using colorimeter (Color meter, JS555, China). The color value (*L, a, b*) were reported as average value from 5 measurements.

2.7 Texture Profile Analysis

In order to prepare sample for texture profile analysis (TPA), the obtained batter from each treatment was filled in the conical tube (50 mL) for 40 g/each. Then, the samples in the tube were centrifuged by setting the centrifuge at 2,000×g. However, when the speed reached 2,000×g, the centrifugation was stopped immediately. This allowed the batter to pack homogenously and also to remove air bubble, trapped inside the batter. Then, the samples were incubated at 40 and 80 ^{o}C for 30 min in each step. After being cooled in ice-water, the samples were kept in the cold room overnight and brought to room temperature prior sample cutting for TPA analysis. Mu Yo was cut into 1×1×1.5 cm. Texture profile analysis (TPA) was performed using the texture analyzer (TA-XTS, Micro Stable Systems, Godalming, UK) equipped with a cylindrical probe with diameter of 25 mm. The sample was pressed twice for 75% of sample height and the probe speed was controlled at 5 mm/s for either before and after pressing. The deformation and force were recorded to calculate each texture parameter.

2.8 Microstructure

Mu Yo samples were cut into small pieces before being fixed in glutaraldehyde (2.5%) and post fixed with osmium tetraoxide (1%). The fixed gels were dehydrated by serial dilution of ethanol from 50-100% followed by acetone solution (100%). The dried pieces of gel were analyzed for their microstructure by gold coating (Joel, JEC-110E, Tokyo, Japan). Microstructure was analyzed by scanning electron microscopy (SEM) at 2,000 magnification using Neoscope JCM 5000 (Nikon, Tokyo, Japan).

2.9 Sensory Evaluation

Consumer acceptance toward each Mu Yo recipe was evaluated by untrained panelist (30 panelists). The 9 point hedonic scale was used to evaluate the preference regarding an appearance, color, texture, flavor, and overall acceptance.

2.10 Statistical Analysis

The mean values were subjected for analysis of variance. The SPSS 16.0 (SPSS Inc., Chicago, Il, USA) was used to determine the difference between mean values at $p < 0.05$.

3. Results and Discussion

3.1 Chemical Composition

Chemical compositions of Mu Yo are shown in Table 1. The results clearly showed that moisture content of samples incorporated with FBP and KJF was reduced significantly. A reduction of moisture content in those samples was from the higher solid in the recipe up on addition of either FBP or KJF. Thus, the ratio of water to solid would be lowered significantly. Crude fat content in Mu Yo with KJF or FBP was reduced slightly. It can be noticed that total ash content increased clearly when FBP was added regardless the present of KJF. The results suggested that FBP have a potential ingredient for improving mineral content in Mu Yo.

Table 1 Chemical composition of Mu Yo as affected by KJF and FBP

Composition (% wet basis)	Treatment CTR	KJF	FBP	KJF+FBP
Moisture	65.67±0.24 [a]	65.50±0.26 [a]	65.53±0.3 [a]	63.75±0.07 [b]
Crude fat	14.95±1.03 [bc]	14.27±1.40 [bc]	13.93±1.03[c]	15.60±0.40 [a]
Crude protein	20.27±0.40 [b]	20.40±0.29 [b]	21.00±0.29 [a]	20.41±0.12 [b]
Total ash	2.00±0.04 [b]	2.14±0.16 [b]	3.03±0.10 [a]	3.11±0.09 [a]

*Different letters in the same row indicates the statistically different ($p < 0.05$)
** CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

3.2 Dietary Fiber Content

Dietary fiber has been known to be related with health. It could maintain the function of the gastrointestinal system and reduce the risks of colon cancer and coronary heart disease [15]. Glucomannan in KJF is classified as a soluble dietary fiber [12]. Therefore, the total dietary fiber (TDF) and crude fiber content of samples were expected to be increased up on fortification of KJF into the Mu Yo. The results showed clearly that fortification of KJF and KJF along with FBP resulted in an increase the crude fiber content when compared to a control sample (Table 2). The amount of TDF was found in the same tendency with crude fiber. Concurrently, the crude fiber content in FBP—fortified Mu Yo was compared to the control sample. However, KJF-fortified Mu Yo exhibited the highest content of crude fiber.

It can be seen that TDF of all samples were higher than the crude fiber content (Table 2). Determination of crude fiber was obtained by hot acid and alkali digestion. Hence, only cellulose and lignin were obtained. However, dietary fiber majorly consists of cellulose, hemicellulose, pectin, lignin, and hydrocolloids (gums, mucilages, and algal polysaccharides) [16]. To obtain the information regarding the amount of TDF, specific enzymes (α-amylase, protease, and amyloglucosidase) are required to digest those fibers prior gravimetric measurement. Thus, the TDF would be observed at the higher amount than the crude fiber for all samples. TDF and IDF contents of sample were highest when KJF was fortified along with FBP. This might be a result from the electrostatic interactions between acetyl group (negative charge) from KJF and calcium ion (positive charge) from FBP. Those complexes may be tolerance to enzymatic digestion, providing higher IDF. The cross-linking of low acyl gellan gum by divalent cation has been reported [17].

Based on the results of fiber content, fortification of KJF along with FBP provided the benefit for health due to highest IDF and TDF contents. Beside, the IDF has been reported to shorten bowel transit time, increases fecal bulk, and renders feces softer [18]. Foods containing high content of dietary fiber have been recommended by the diabetes and nutritional associations [12].

Table 2 Insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total dietary fiber (TDF) and crude fiber content of Mu Yo as affected by KJF and FBP (% dry basis)

Treatment	Type of fiber			
	IDF	SDF	TDF	Crude Fiber
CTR	9.00 ± 0.47 [b]	0.11 ± 0.03 [ab]	9.10 ± 0.47 [b]	0.39 ± 0.12 [bc]
KJF	9.66 ± 0.19 [ab]	0.05 ± 0.02 [c]	9.71 ± 0.18 [ab]	0.71 ± 0.17 [a]
FBP	7.55 ± 0.68 [c]	0.07 ± 0.02 [bc]	7.62 ± 0.69 [c]	0.34 ± 0.09 [c]
KJF+FBP	10.24 ± 0.32[a]	0.12 ± 0.03 [a]	10.36 ± 0.32 [a]	0.59 ± 0.09 [ab]

*Different letters in the same column indicates the statistically different ($p < 0.05$)
**CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

3.3 Total Calcium Content

Total calcium content in Mu Yo without FBP was about 161 mg/kg of dried sample. When FBP was added at 0.5%, total calcium in the product increased sharply for approximately 30 fold and the total calcium content was found at about 5,568 mg/kg of dried sample (Table 3). This result strongly confirmed that FBP could be an ingredient to increase mineral especially calcium. The amount of calcium could be calculated to be 191 and 161 mg/serving size (100 g). It has been recorded that food product containing calcium per serving size higher than 20% (160 mg) of recommended daily intake (800 mg) [19]. Therefore, this Mu Yo could be classified as a high calcium-product when FBP was added at 0.5%. Calcium bioavailability from bone extract was evaluated in vivo and it could improve bone metabolism of rats [20]. Consumption of salmon and cod bone powder resulted in ready absorption of calcium in young human [21]. The bioavailability of calcium extracted from chicken bone up on fortified in food product was reported after in vitro experiment [22]. Based on this information, calcium fortification of Mu Yo using natural ingredient, fish bone powder, was an alternative strategy to produce healthy product.

Table 3 Total calcium content of Mu Yo as affected by KJF and FBP

Type	Treatment			
	CTR	KJF	FBP	KJF+FBP
Total calcium (mg/kg of dried sample)	161	162	5,568	4,493
Total calcium per 100 g (mg)	5.53	5.61	191	162

*CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

3.4 Color of Mu Yo

The color of Mu Yo was evaluated although it may not be the critical attribute for grading the Mu Yo. The results indicated that Mu Yo became whiter when FBP were added (Table 4). This may be because of the natural white powder of FBP. High value of whiteness was found in tilapia bone powder [8]. However, addition of fish bone for the white protein gel, surimi gel, resulted in a reduction of whiteness [10]. This suggested that addition of fish bone into surimi gel may be limited since whiteness is important for determining product quality. Yellowness and redness of Mu Yo increased significantly when FBP was fortified. This may possibly be due to the browning pigments from protein or lipid oxidation as reported previously [8]. Fortunately, higher in redness and yellowness may not be the defect since darker color is acceptable for Mu Yo. Therefore, both FBP and KJF may not negatively affect the consumer acceptability.

Table 4 Color values of Mu Yo as affected by KJF and FBP

Treatment	Color value		
	L	*a*	*b*
CTR	65.36 ± 0.21^{d}	2.37 ± 0.06^{b}	11.26 ± 0.06^{b}
KJF	66.34 ± 0.37^{c}	2.29 ± 0.09^{b}	10.98 ± 0.34^{b}
FBP	68.27 ± 0.48^{a}	2.92 ± 0.10^{a}	11.76 ± 0.28^{a}
KJF+FBP	67.43 ± 0.48^{b}	2.33 ± 0.06^{b}	11.33 ± 0.07^{b}

*Different letters in the same column indicates the statistically different ($p < 0.05$)
**CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

3.5 Textural Properties

The results in Table 5 revealed that hardness of Mu Yo with FBP was comparable to the control, suggesting the ineffective ingredient to improve gel texture. Our finding was disagreement with previous data [10]. They found that hardness of fish protein gel increased significantly by addition of fish bone powder. Such improvement was associated with activation of endogenous enzyme, transglutaminase (TG), by calcium ion released from fish bone. TG normally catalyzes protein cross-linking via an acyl transfer reaction between glutamine-bound proteins and lysine-bound proteins. This reaction is dependent with the present of calcium ion and optimal concentration for each TG is varied [23]. An endogenous TG can be found normally in muscle of fishery, which either is vertebrate or invertebrates [24]. Purify fish TG induced viscoelastic properties of fish mince [25].

Table 5 Texture profile analysis of Mu Yo as affected by KJF and FBP

Textural attributes	Treatment			
	CTR	KJF	FBP	KJF+FBP
Hardness (g)	630^{b}	752^{a}	717^{ab}	763^{a}
Adhesiveness (g.s)	2.83^{b}	6.67^{a}	4.33^{b}	3.83^{b}
Gumminess	360.51	513.52	427.01	448.56
Springiness (mm)	0.51	0.64	0.55	0.55
Cohesiveness	0.53	0.68	0.59	0.58

*Different letters in the same row indicates the statistically different ($p < 0.05$)
**CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

Based on our preliminary results, TG activity in pork mince was not observed (data not show). FBP could not be gel enhancer for this system might be due to the insufficient TG in pork. However, an increase in gel hardness of Mu Yo up on addition of KJF was found regardless the addition of FBP. Such improvement of gel hardness may be because of the water holding capacity and gelling ability of KJF. Rehydration of KJF until gel forming was used as the fat replacer for Bologna sausage [26]. The KJF gel might trap water molecules in three dimensional structure of protein gel, providing harder texture. The adhesiveness of sample was highest when KJF was incorporated, while that for other samples were similar. Thus, addition of KJF could improve not only dietary fiber content but also textural properties.

3.6 Microstructure

The microstructures of Mu Yo showed clearly the fat globule, suggesting the emulsion structure in all samples (Figure 1). This is quite different from that of protein gel, surimi gel, as reported previously [11]. The empty hold on structures of sample with KJF might be that for water, trapping in swollen KJF (Figure 1B).

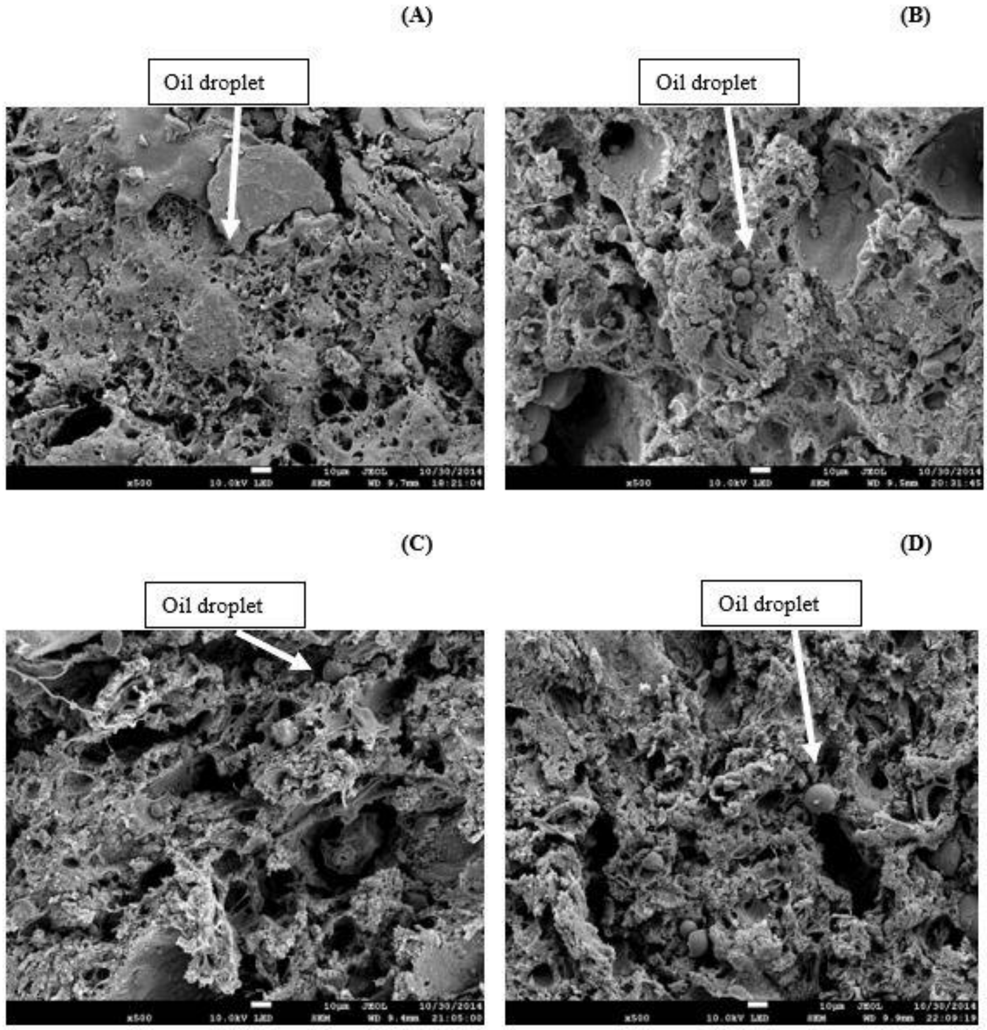

Figure 1 Microstructure of control Mu Yo **(A)**, with KJF **(B)**, with FBP **(C)**, and with KJF and FBP **(D)** at magnification of 500. CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

The high ability to hold water is considered as the important functionality of glucomannan in KJF [13]. The ability to hold water of sample with KJF was observed regardless of FBP as evidenced by observing similar microstructure in Figure 1D. It has been reported that hardness of emulsion gel increased with an increasing water holding capacity. The SEM structure would likely support the observation of increasing the hardness of sample with either KJF alone or KJF with FBP (Table 4). The porous structure of control sample was similar to that of sample with FBP (Figure 1A and C). This suggested addition of FBP did not affect the continuity of an emulsion gel, while it induced the discontinuous gel network in fish protein gel [11]. Based on the SEM, fortification of KJF and FBP would not play the negative role on the overall structure of an emulsion gel. Therefore, the consumer perception would not likely be affected.

3.7 Consumer Acceptability

The results from a hedonic test revealed that all products were accepted regardless the addition either KJF or FBP. This was evidenced by the overall acceptance score, which was found at 5.95 from the maximum score of 9 (Table 6). The preference for each sensory attribute was also evaluated individually. Although the color preference toward Mu Yo seemed to be decreased when KJF and FBP were added, statistical significance was not observed. These results inferred that fortification of KJF and FBP did not play the negative role on consumer preference. Therefore, they could be the ingredient for improving nutritional values by increasing calcium and dietary contents for an acceptable Mu Yo.

Table 6 Effects of KJF and FBP on consumer preference of Mu Yo

	Formula			
Sensory attributes	CTR	KJF	FBP	KJF+FBP
Color	6.18±1.32	5.88±1.72	6.0±1.71	5.92±1.50
Odor	5.76±1.81	6.04±1.41	5.64±1.80	5.80±1.84
Texture	5.36±1.97	5.80±1.70	5.30±1.93	5.20±1.78
Flavor	5.86±1.83	5.90±1.74	5.64±1.86	5.72±1.92
Overall acceptance	5.98±1.73	6.18±1.64	5.76±1.81	5.90±1.63

* CTR = control Mu Yo; KJF = Mu Yo with konjac flour powder (0.5%); FBP = Mu Yo with fish bone powder (0.5%); KJF+FBP = Mu Yo with konjac flour powder (0.5%) and fish bone powder (0.5%).

4. Conclusion

FBP and KJF could be natural ingredient to increase the dietary fiber and calcium contents in Mu Yo. Gel hardness of Mu Yo was improved significantly by incorporation of KJF. The darker product could be obtained after addition of either KJF or FBP, while negative effect for color perception was not found. Structural integrity of emulsion gel was affected neither by KJF nor FBP, concomitantly with the similar overall acceptability. Therefore, konjac flour and fish bone powder exhibited the potential as natural ingredients for healthy Mu Yo production by increasing calcium and dietary fiber.

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