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Effects of Tilapia Bone Calcium on Qualities of Tilapia Sausage

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ABSTRACT

Tilapia bone powder was extracted using acetic acid (0.25 M) at a ratio of 1:50 (weight per volume) and stirred for 48 hr at room temperature (approximately 28°C). The calcium extract was adjusted to neutral pH. The calcium content in the extract was 2,376 $mg.L^{-1}$, assessed by inductively coupled plasma-optical emission spectrometry. The calcium extract was added to fish sausage by replacing the ice content in the recipe with the frozen calcium extract. The sausage quality was compared to the control (without adding calcium). Proximate analysis showed that the addition of calcium resulted in moisture reduction while the ash contents increased by 1.42%. The protein and fat contents as well as the pH value were not affected ($P > 0.05$). The lightness, assessed by colorimeter, increased slightly ($P < 0.05$). Texture profile analysis revealed that the hardness and gumminess of the fish sausage increased upon adding the calcium extract. The sensory evaluation also indicated that the textural attribute of the fish sausage with calcium extract exhibited higher overall acceptance than the control.

Keywords: calcium, fish bone, fish sausage, tilapia, textural properties

INTRODUCTION

Fish are an important source of protein for human consumption and globally approximately 148 million t were harvested in 2010 (Yin and Park, 2014). Tilapia is one of the fresh water commercial fish species and it is produced using aquaculture around the world (Hemung, 2013). Tilapia is generally processed into a frozen fillet, which can also generate by-products accounting for up to 15% of the fish weight, with fish bone being the major by-product (Toppe *et al*., 2007). The fish bone is currently discarded or processed into bone meal for animal feed, which is not economical (Kim and Mendis, 2006). Gaining more product value from the tilapia bone would be one strategy to more effectively utilize the fish resource and contribute to sustainability. The preparation of tilapia bone powder has been studied (Hemung, 2013) with the ash content from the bone powder representing the major mineral component, accounting for 75%. The potential of fish bone as a source of mineral supplement was reported by Phiraphinyo *et al.* (2006) and tilapia bone powder has been developed into a supplementary form as a calcium capsule (Techochatchawal *et al*., 2009). Calcium is considered as the rich mineral in the bone (Piccirillo *et al.,* 2013). However, the direct application of fish bone powder to generate the functionality is limited by its low solubility because calcium in the bone is mainly found in the hydroxylappatite form (HA) as calcium phosphate, which is hard to solubilize (Techochatchawal *et al.,* 2009). Moreover, the low solubility of calcium limits its bioavailability. Thus, extraction of calcium from fish bone powder is necessary for generating functionality as well as fulfilling the nutritional value of food products.

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Application of fast, high-intensity, pulsed-electric-field-assisted extraction was reported as an effective method to solubilize calcium from the bone (Yongguang and He, 2008). However, this technique has not been applicable commercially, whereas acid extraction seems to be more feasible and acetic acid has been applied for extraction of calcium from wollastonite (Ptacek *et al*., 2010). Acetic acid is generally accepted as a food additive and as one of the choices for food application. The calcium content in bone soup was affected by vinegar, which consisted of acetic acid (Li and Fang, 2002). The use of acetic acid in softening fish bone has been reported (Ishikawa *et al*., 1988). Thus, calcium extraction by acetic acid would be possible and the calcium extract could be applied directly to the food products as calcium acetate.

Application of calcium in food products to increase their nutritional value is an interesting topic. Fortification of calcium into texturized vegetable protein and its bioavailability were performed in order to evaluate the nutritional quality (Cuptapun *et al*., 2013). Moreover, the direct application of calcium was also carried out in bakery products and the digestibility of calcium-fortified bread was evaluated in *vivo* using young rat as the model (Shanil Juma *et al.,* 1999). Beside the nutritional perspective, the addition of fish bone as a calcium source to improve the functionality of a food product has also been widely considered. Yin and Park (2014) applied fish bone as nano-scale particles into Alaska pollock surimi, concentrated myofibrillar protein, to improve the textural properties of the surimi gel basing their work on Lee and Park (1998), who reported that the addition of calcium compounds could increase the gelling properties of surimi gel. However, the addition of calcium extracted from fish bone powder into fish sausage has not been reported. Thus, the extraction of calcium from fish bone powder and its application as a food additive in fish emulsion sausage should be investigated. This would lead to valuable information regarding the functionality of calcium on the emulsion gel of fish proteins. Therefore, this study aimed to extract calcium from tilapia bone powder using acetic acid and to evaluate the effect of calcium extract on the quality of fish emulsion sausage.

MATERIALS AND METHODS

Fish bone powder preparation

Fish bone powder was prepared according to the method described by Hemung (2013) with slight modifications. The fresh tissue was removed from the bone (main frame) of tilapia (*Oreochromis niloticus*) by soaking in hot alkaline solution (0.8% NaOH) at a ratio of bone to solution of 1:2 (weight by weight) for 1 hr. Subsequently, bone samples were rinsed with distilled water and autoclaved at 121°C at 350 $g.cm^{-2}$ for 1 hr. The obtained samples were dried out overnight in a hot air oven at 105°C. The dried samples were ground into powder and sieved through a 38 μm mesh before keeping the sample for later use as fish bone powder.

Calcium extraction and determination

The fish bone was mixed with acetic acid (0.25 M) at a ratio of bone powder to acid solution of 1:50 (weight by volume) before shaking (200 rpm; SHO-2D; Daihan Scientific; Seoul, Korea) at room temperature for 48 hr. The solution was filtered through No.1, Whatman filter paper and the filtrate was adjusted to pH 7.0 using 3 N NaOH (about 0.5 mL) for consideration as the calcium extract. Wet ashing was performed to determine the calcium content in the extract. The sample was digested with nitric acid (0.1 M) at 95°C for 2 hr and then analyzed for its calcium content using inductively coupled, plasma-optical emission spectrometry (ICP-OES; Optima 4300 DV; Perkin Elmer Instruments; Norwalk, CT, USA). The emission wavelength for calcium was recorded at 317.933 nm and the calcium content in the extract was reported to be 2,376 $mg.L^{-1}$.

Sausage preparation

The tilapia samples were obtained from a local market in Nong Khai province, Thailand. The live fish were killed and kept in a foam-box covered with ice and were transported to the Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus within 15 min. Each fish was scarified and eviscerated manually upon arrival. Only fish fillets without skin were ground using a mincer with a screen of 3 mm perforations (TC12-C; Champ; Kent, UK). The emulsion sausage was prepared using a similar recipe to the Bologna style (Chin *et al.,* 1999) with slight modifications. The fat content was reduced using konjac flour and carrageenan as the fat replacer. A sample of 6 g of a mixture of konjac flour and carrageenan (5:1) was blended with 100 g of distilled water as the fat replacer. The amounts of tilapia mince, fat replacer, soy bean oil, salt, corn flour, sugar and mixed spices were controlled at 40, 10, 10, 2, 3, 3, and 2%, respectively. The ice in the recipe (30%) used in the controlled sample was made with water adjusted to pH 7.0. The equivalent amount of ice was replaced by frozen calcium extract yielding added calcium of 710 $mg.kg^{-1}$. To prepare the sausage, fish mince was chopped with other ingredients using a chopper (Champ; Kent, UK) and the temperature of the batter was approximately 12°C. The batter was stuffed into a 25 mm diameter collagen casing (Nippi Corp.; Yokohama, Japan). The raw sausage was incubated at 40°C for 30 min and subsequently cooked in the controlled temperature of a water bath (Memmert, GmbH; Schwabach, Germany) at 90°C for 15 min. Then, samples were cooled down immediately by soaking in iced water and kept in a refrigerator (4 °C) overnight.

Analysis of sausage

pH measurement

The sausage sample (1 g) was mixed with distilled water (9 mL) before homogenization for 1 min. The pH of the homogenate was measured at room temperature using a pH meter (Mettler-Toledo AG; Schwerzenbach, Switzerland). Three replications were used to determine a mean value.

Color measurement

The samples were brought to room temperature and cut into lengths of 1.5 cm. The color of the cross-sectional area was evaluated using a colorimeter (CR-10; Minolta; Tokyo, Japan). The Hunter *L*, *a*, and *b* values (Hemung and Chin, 2014) were reported from three replications.

Proximate analysis

The chemical compositions in the sausage were estimated using proximate analysis according to the methods of analysis of the Association of Official Analytical Chemists, with the protein content based on the nitrogen content using the Kjeldahl method (Tennyson and Winlers, 2000) and a conversion factor of 6.25 was used to convert that content to protein. The moisture content was determined using oven drying at 105°C until a constant weight of each sample was obtained which took about 12 hr. The crude fat content was determined by Soxhlet extraction using petroleum ether as the solvent. Based on the dry ashing technique, thermal combustion at 550°C for 16 hr in a muffle furnace was applied to determine the ash content.

Texture profile analysis

The sausage was cut into rectangular pieces (1×1×1.5 cm) to trim out the collagen casing. The texture profile analysis (TPA) of sample was performed using TA-XT2 equipment (Micro Stable Systems; Godalming, UK). The diameter of the cylindrical probe was 25 mm and the pre- and post-speeds were both $5\ mm.s^{-1}$. The samples were compressed in two consecutive cycles until they reached 75% of the original sample height with a 2 s interval between cycles. The TPA parameters were calculated from seven measurements.

Sensory evaluation

The sensory evaluation was performed by 20 panelists (5 male and 15 female), aged

between 18 and 22 yr in the sensory evaluation room. The sausage samples were cut into 1.5 cm cubes before boiling for 1 min and three pieces for each treatment were served to each panelist. A nine-point scale was used to evaluate the sensory attributes, consisting of appearance, color, flavor, texture and overall acceptance.

Statistical analysis

The difference between means in each treatment was performed using the *t*-test and the significant difference was considered at the level of 95% ($P < 0.05$) using the SPSS 16.0 software package (SPSS Inc.; Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate analysis

Based on the proximate analysis, the fat content in the fish sausage was only approximately 11% (Table 1), which was lower than in commercial emulsion sausages (25–30%), since the carrageenan and konjac flour were used as the fat replacer. In addition, the fat content was not affected by adding the calcium extract. Moreover, the protein content was not significantly different between the control and treated samples. This suggested that neither the protein nor fat contents of fish sausage were affected by the addition of the calcium extract. Hemung (2013) used a similar protocol to prepare tilapia bone powder and found that the protein and fat contents were about 14.8 and 5.8%, respectively. This indicated that protein and fat were removed during calcium extraction yielding mainly mineral in the extract. The protein and fat residues could be removed by filtering and the remaining residues were observed in the filter paper. As expected, the ash content was increased slightly but significantly when the calcium extract was added, suggesting that more mineral was included in the sausage formula. However, the increased mineral content may not have resulted only from calcium ions but may also have been due to other ions such as from NaCl. A reduction in the moisture content was observed in the sausage when the calcium extract was introduced. The frozen calcium extract was used in the treatment instead of ice, resulting in a lower amount of water in the recipe. This would partly explain why the moisture content of the sausage incorporated with calcium extract was reduced.

pH and color values

The pH value of the emulsion sausage made from tilapia was about 6.7 and the addition of the calcium extract did not affect the pH of the sausage. Since the calcium extract and water were adjusted to pH 7.0 before being added to the sausage, comparable hydrogen ions in both systems (the control and the calcium-added sausage) could be found. This explained why the pH of the sausage did not change with the addition of the calcium extract. The observed pH value was higher than that of reduced fat frankfurters, which had a pH of 6.36 (Osburn and Keeton, 2004). The different pH values between the previous and current studies may have resulted from using different meat types.

Table 1 Proximate analysis of tilapia emulsion sausage with and without calcium extract.

Composition (%, wet basis)	Sample	
	Control	Calcium-added sausage
Moisture content	71.67±0.72[a]	69.68±0.28[b]
Crude fat	11.38±0.21[a]	11.52±0.35[a]
Crude protein	3.64±0.36[a]	3.57±0.13[a]
Ash	4.24±0.05[b]	4.30±0.07[a]

Mean values ± SD are calculated based on three replications.

[a, b] = Different lowercase superscript letters in the same row indicate a statistical difference at $P < 0.05$.

The color of the sausage was evaluated on a cross-sectional diameter to eliminate the effect of the collagen casing. The results shown in Table 2 indicate that the addition of the calcium extract resulted in an increase in the lightness of the sausage as evidenced by a significantly higher *L* value than the control. This suggested that the addition of more minerals in the calcium extract would result in an increase in sausage lightness. It can be clearly seen that the *a* and *b* values were not affected ($P > 0.05$) by the addition of the calcium extract. This might have been due to the elimination of browning pigments, proteins and lipids during calcium extraction. This was supported by the similar protein and fat contents between the control and treated sausage samples. Therefore, the addition of the calcium extract in the fish sausage would be feasible since it did not have any major effect on the color attributes of the sausage.

Textural properties

The textural properties of the fish sausage were evaluated by compressing the sample twice to simulate the characteristics of samples during chewing. As shown in Table 3, the chewiness, springiness, cohesiveness and adhesiveness were not affected by the addition of the calcium extract. The hardness and gumminess of the sausage were increased significantly by about 12%. An increase in hardness may have been partly due to the different moisture content (Table 1).

Yin and Park (2014) showed that the addition of nano-particles of fish bone powder at a concentration of 0.1% as the calcium source into Alaska pollock surimi resulted in an increase

Table 2 pH and color values of tilapia emulsion sausage with and without calcium extract.

Parameter	Treatment	
	Control	Calcium-added sausage
pH value	6.72±0.03^{ns}	6.69±0.02^{ns}
Color value		
L	72.69±0.71^{b}	73.76±0.30^{a}
a	1.4 ±0.08^{ns}	1.39±0.2^{ns}
b	9.10±0.11^{ns}	9.68±0.18^{ns}

Mean values ± SD are calculated based on three replications.

$^{a, b}$ = Different lowercase superscript letters in the same row indicate a statistical difference at $P < 0.05$.

^{ns} = non significant difference.

L, a and *b* values describe the color (Hemung and Chin, 2014).

Table 3 Texture profile analysis of tilapia emulsion sausage with and without calcium extract.

Texture profile analysis	Treatment	
	Control	Calcium-added sausage
Hardness (kg)	2.49 ±0.10^{b}	2.79 ±0.28^{a}
Springiness	0.64 ±0.21^{ns}	0.67 ±0.12^{ns}
Gumminess (kg)	1.75 ±0.09^{b}	2.03 ±0.18^{a}
Chewiness (kg)	1.12 ±0.09^{ns}	1.36 ±0.10^{ns}
Cohesiveness	0.70 ±0.02^{ns}	0.73 ±0.10^{ns}
Adhesiveness (kg.s)	0.025±0.008^{ns}	0.029±0.111^{ns}

Mean values ± SD are calculated based on three replications.

$^{a, b}$ = Different lowercase superscript letters in the same row indicate a statistical difference at $P < 0.05$.

^{ns} = nonsignificant difference.

in the breaking force and breaking distance. The addition of calcium compounds also improved the gel functionality of Pacific whiting surimi gel (Lee and Park, 1998). In the current study, the addition of the calcium extract at a concentration of 710 $mg.kg^{-1}$, which was calculated to be approximately 17 mM, could improve the textural properties of fish sausage. This indicated that the functional property of the calcium extract from the current study was apparent because it is in a soluble form. It has been reported that the effective utilization of calcium in fish bone powder was limited by its low solubility (Yin and Park, 2014). Thus, the extraction of calcium using acetic acid provided more soluble calcium to improve the textural properties of the fish sausage.

Incubation of fish protein at a certain temperature for a period of time is generally accepted as the "setting process" (Lanier, 2000). The strength of fish protein gel set under appropriate conditions followed by heating is stronger than that without setting as the hydrophobic interactions and disulfide bond are the prime factors governing the protein aggregation in this process (Gill *et al*., 1992; Chan *et al*., 1995). It has been reported that calcium at a concentration of 10–100 mM could induce changes in the conformation of myosin, the major protein in myofibrillar proteins, facilitating the inter- and intra-molecular interactions in a setting model (40 °C for 30 min) of threadfin bream myosin (Hemung and Yongsawatdigul, 2005). In addition, the destabilization of actomyosin from tilapia by the action of calcium ions was found to promote hydrophobic interactions during incubation at 40°C (Yongsawatdigul and Sinsuwan, 2007). Therefore, the calcium increased the textural properties of the fish protein gel, which was partly explained by inducing the conformational changes of muscle proteins, facilitating the hydrophobic interactions and the disulfide bond.

Beside the interactions mentioned above, calcium is considered to be the activator for several enzymes, including the cross-linking enzyme (endogenous TGase). This enzyme catalyzes the covalent peptide bond between the side chain of the glutamine-bound peptide and the side chain of the lysine-bound peptide. Such interaction results in the so-called "isopeptide bond" and provides the protein cross-linking that produces macromolecules. TGase has been found in several fish species and the purified TGase from those fish enhanced the viscosity of fish mince due to the protein cross-linking (Binsi and Shamasundar, 2012). Threadfin bream TGase participated in gelation of its surimi (Yongsawatdigul *et al*., 2002). Endogenous TGase has been found in tilapia tissue and its cross-linking ability has also been documented (Worratao and Yongsawatdigul, 2005). This enzyme requires calcium as the activator and the optimal calcium concentration is varied, depending on the fish species, as well as on the enzyme purity (Yin and Park, 2014). Worratao and Yongsawatdigul (2005) reported that calcium at only 5 mM was required by the purified TGase from tilapia for full activation. Moreover, the soluble calcium extracted from tilapia bone powder activated the activity of crude tilapia TGase at 162 nM when the synthetic amine (monodansyl cadaverine) was incorporated into dimethylated casein (Hemung, 2013). The endogenous TGase bound tightly with tilapia actomyosin and the remaining residue could catalyze protein cross-linking, when calcium ions were added (Yongsawatdigul and Sinsuwan, 2007). Based on these data, it is possible to speculate that the soluble calcium in the extract could be the activator for endogenous TGase to catalyze the cross-linked proteins, which were accepted to reinforce the emulsion gel matrix.

Sensory evaluation

The hedonic test was used to evaluate the preference of consumers toward the fish sausage and the results are provided in Table 4. The consumer preference for the color and appearance of sausage was not different between the control and the calcium-added sausages. This

Table 4 Sensory attributes of tilapia emulsion sausage with and without calcium extract.

Sensory attributes	Treatment	
	Control	Calcium-added sausage
Appearance	5.70±1.34^{ns}	5.90±1.18^{ns}
Color	5.80±1.44 ^{ns}	5.90±1.13^{ns}
Texture	5.70±1.57^{b}	7.30±1.11^{a}
Flavor	6.40±1.86^{ns}	6.60±1.67^{ns}
Overall acceptance	5.80±1.54^{b}	7.20±1.13^{a}

Mean values ± SD are calculated based on 20 panelists.

$^{a, b}$ = Different lowercase superscript letters in the same row indicate a statistical difference at $P < 0.05$.

^{ns} = nonsignificant difference.

suggested that an increase in lightness (Table 2) upon the addition of the calcium extract did not affect consumer preference. It appears likely that the taste of both treatments was evaluated to be similar, regardless of the addition of calcium extract. This was partly due to the similar pH values of the samples. However, the results clearly showed that the addition of the calcium extract significantly improved the sensory score for the textural attribute of fish sausage. This resulted in an improvement in the overall acceptance. Therefore, the significant difference in the textural properties of the fish sausage determined by TPA (Table 3) was enough to be distinguished in the sensory evaluation.

CONCLUSION

The extraction of calcium from fish bone powder was performed successfully using acetic acid. The calcium extract could be used as an ingredient to improve the textural properties of fish sausage. In addition, the product preference was increased, particularly with regard to the textural attribute. The ash content and lightness were increased slightly on addition of the calcium extract. Based on the complete study, the addition in the fish sausage of calcium extract from fish bone provided benefit by increasing the textural properties and product preference.

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