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Research Article

Development of calcium supplement from the bone of Nile Tilapia (*Tilapia nilotica*)

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Abstract

Fishbone of Nile Tilapia (*Tilapia nilotica*) waste from a fish leather factory, is one potential calcium source. In order to reduce the factory waste and increase the fishbone value, this study aimed to develop a calcium supplement from the bone of Nile Tilapia. The fishbone was pretreated using various methods including boiling at 95°C for 10 minutes, soaking in 10 ppm chlorine for 90 minutes and soaking in 0.8% sodium hydroxide for 90 minutes. Due to ability to remove fish tissue from the fishbone, sodium hydroxide was selected as the chemical solution for pretreatment. The pretreated fishbone was heated under controlled pressure and dried using hot air drying. Increased heating time at 121°C from 30 to 90 minutes under high pressure (15 $lb.in^{-2}$) significantly increased lightness of fishbone ($p \leq 0.05$). In addition, from scanning electron micrograph (SEM) of dried fishbone, heating for 90 minutes yielded more porous structure than heating for 30 and 60 minutes. For drying process, increasing drying temperature from 50°C to 90°C showed a significantly increased kinetic rate constant. The optimal process to develop a calcium supplement from Nile Tilapia was soaking in 0.8% sodium hydroxide for 90 minutes, heating at 121°C for 90 minutes and drying at 90°C for 60 minutes. The obtained calcium supplement (In no.0 capsule) contained 25.01% calcium and its quality met the requirements for calcium supplement standards.

Keywords: fish leather waste, calcium, fishbone, drying, Thailand.

Introduction

Calcium is one of the important minerals for human growth and prevention of osteoporosis in aged persons. According to Thai RDI, Thai people should have an intake of 800 mg of calcium per day. Calcium is generally obtained from dairy products, broccoli, tofu, oysters, sardines and similar soft sources of bone [1]. Edible fishbone contains a high amount of calcium [2]. Fishbone ash consists of 34-36% calcium, particularly calcium phosphate [3]. Fishbone of Nile Tilapia *(Tilapia nilotica)*, available as waste from a fish leather factory, should be a potentially good source of calcium. On the other hand, the fishbone is too hard and too sharp to eat directly. Fishbone may be softened, when the connective tissue is denatured [4]. Previous research [5] has found that using sodium hydroxide, together with heating under high pressure, can eliminate protein, connective tissue and fat. To remove the fish tissue, some chemical solutions have been studied. Alkali solution caused polar side of some fat and thereby increased solubility in water [6]. Acetic acid solution with heating could decrease hardness of fishbone rapidly, as well as the solubility of ash in fishbone [7]. However, hydrochloric solution damages the fishbone shape and increases the loss of calcium content [5]. Chlorine solution was found to be good at bleaching by oxidizing pigment [8]. Codex recommended 10 ppm chlorine in washing water for fishery raw materials [9]. In addition, to remove moisture and extend shelf-life, drying could be applied, after the fish tissue was removed from the fishbone. Therefore, this study aimed to develop a calcium supplement from the bone of Nile Tilapia. Effect of pretreatment, heating and drying processes on the quality of calcium supplement was studied.

Materials and Methods

Sample preparation

Fishbone of Nile Tilapia waste from Jerada Leather and Product Co., Ltd. was prepared by removing the head and tail. The fish tissue was also partially removed from the bone by cutting before storage at -18°C.

Effect of pretreatment on fishbone characteristics

Fishbone was pretreated by various methods: boiling at 95°C for 10 minutes, soaking with 10 ppm chlorine for 90 minutes and soaking with 0.8% sodium hydroxide for 90 minutes. Ratio of fishbone and solutions was 1:5. To select the suitable pretreatment for Nile Tilapia fishbone, physical characteristics including colour and porosity were determined. After pretreatment with chemical solutions, the pretreated fishbone was rinsed with water 5 times (ratio of sample and water = 1:5).

Effect of heating time under high pressure and drying temperature on quality of dried fishbone

The pretreated fishbone was heated at 121°C with controlled pressure at 15 $lb \cdot in^{-2}$ for various time durations (30, 60 and 90 minutes). Then the heated fishbone was dried using hot air drying at 50, 70 and 90°C. The physical and chemical qualities of the processed fishbone were determined. In addition, drying kinetic rate constants (k) of different drying temperatures were calculated using the following equations.

$$MR = \frac{(M_t - M_e)}{(M_0 - M_e)} \quad (1)$$

$$MR = \exp(-kt) \quad (2)$$

where MR is the moisture ratio, M_t is the moisture content at a specific time (g water · g dry solid^{-1}), M_o is the initial moisture content (g water · g dry solid^{-1}), M_e is the equilibrium moisture content (g water · g dry solid^{-1}). The equilibrium moisture content (M_e) was assumed to be zero [10].

Quality measurement of dried fishbone was undertaken as follows:

- Colour was determined using a spectrophotometer (Minolta, CM-3500d, Japan).The colour was presented in CIE Lab system (L*, a*, b*, C* and h). The fishbone was ground using a blender before measurement.
- Porosity was determined using Scanning electron microscope (JEOL, JSM 5600LV).
- Moisture content by oven method [11].
- Total calcium content [12].

Quality evaluation of calcium supplement from fishbone of Nile Tilapia

Quality of the obtained calcium supplement (In no.0 capsule) from the fishbone of Nile Tilapia was evaluated and followed the Notification of Ministry of Public Health o.293 B.E. 2548 (2005) Food Supplement [13] as follows:

- Total Arsenic content [12].
- Lead [12].
- Total Calcium content [12].
- *Clostridium* spp. [14].
- *Escherichia coli* [14].
- *Salmonella* spp. [14].
- *Staphylococcus aureus* [14].

Experimental design

Experiments were designed by using 3^2 Factorial in Completely Randomized Design (CRD) with two replications. Independent parameters included heating time at 30, 60 and 90 minutes and drying temperatures at 50, 70 and 90°C.

Statistical analysis

The data were analyzed using ANOVA (SPSS® version 12.0). Significant differences between means were estimated by Duncan's Multiple Range test at 0.05 significance level.

Results and Discussion

Effect of pretreatment on fishbone characteristics

From Table 1 it can be seen that soaking in sodium hydroxide solution yielded the highest lightness (L* value) of fishbone. This was possibly due to the ability of sodium hydroxide to make collagen absorb water and swell [5], as well as to destroy connective tissue of the fishbone [4, 15] as shown in Figure 1. In this way fish tissue was easily removed and the resulting fishbone became clean. Boiling yielded the highest redness (a*-value) because of the residual blood in the fishbone. For yellowness (b*-value), pretreatment by either boiling or soaking in sodium hydroxide yielded fishbone with higher yellowness than the treatment from soaking in chlorine. Therefore, soaking in sodium hydroxide was selected as the preferred pretreatment for the fishbone.

Table 1. Colour of the pretreated of fishbone.

Sample	L*	a*	b*
After boiling	$62.18^{b} \pm 8.40$	$1.88^{a} \pm 0.84$	$17.88^{a} \pm 0.49$
After soaking Chlorine	$68.40^{ab} \pm 7.09$	$0.87^{b} \pm 0.38$	$16.73^{b} \pm 0.48$
After soaking NaOH	$73.57^{a} \pm 2.32$	$0.88^{b} \pm 0.38$	$18.44^{a} \pm 1.59$

a-c means significant difference within the same column ($p \leq 0.05$).

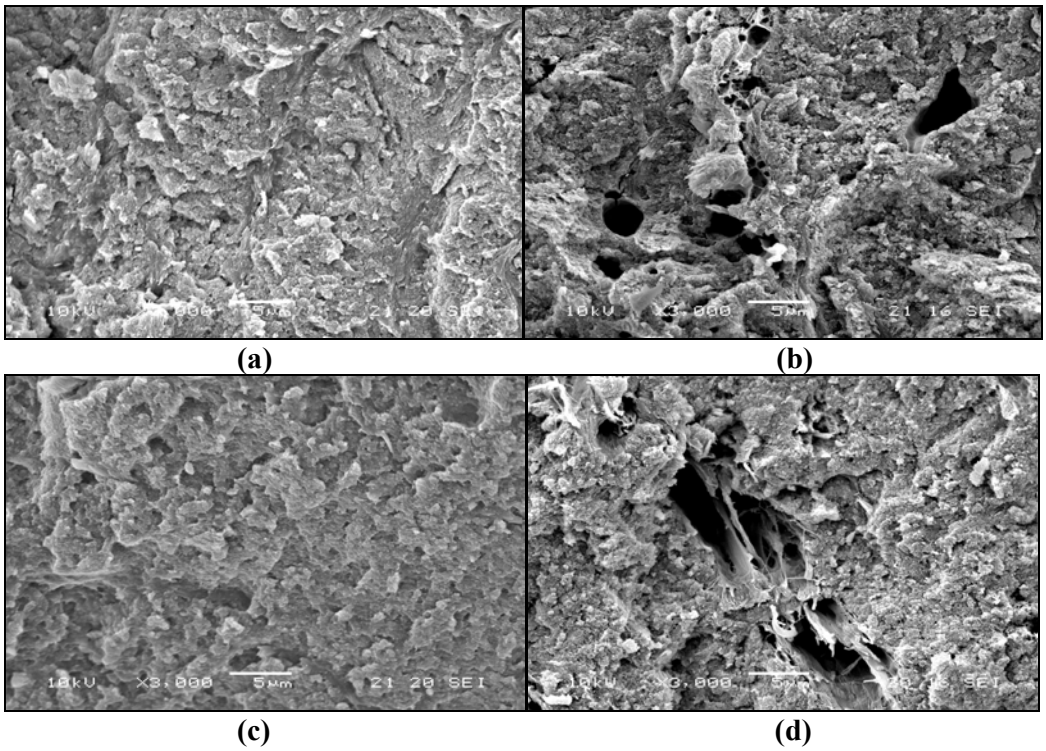

Figure 1. Scanning Electron Microscope (3000x) of fishbone.
(a) Raw material, (b) after boiling for 10 min, (c) after soaking in 10 ppm chlorine for 90 min, (d) after soaking in 0.8% NaOH for 90 min.

Effect of heating time under high pressure and drying temperature on quality of dried fishbone

From the scanning electron microscope (Figure 2), it could be seen that increased heating time under high pressure caused an increase in porous structure. This was because of the increased time for denaturation of protein, connective tissue and fat in fishbone. Therefore, fishbone could be whitened [15]. As shown in Table 2, 90 minute heating time under high pressure yielded light-yellow fishbone (from b*, C and h values) and higher lightness than 30 and 60 minute-heating times. However, increasing heating time from 30 to 90 minutes did not show significant effect on moisture removal during drying (Table 3).

In contrast, drying temperature significantly affected the kinetic rate constant (k) ($p \leq 0.05$). Increasing drying temperature from 50 to 90°C could shorten the drying time, as the kinetic rate constant was increased (Table 3). However, drying temperature affected colour change of fishbone significantly ($p \leq 0.05$). As shown in Table 2, increased drying temperature decreased

lightness and increased redness of fishbone. The same observation was also reported in the previous studies [5, 15].

Finally the processed fishbone contained 24.09 to 25.18 % calcium. From statistical analysis, there was no interaction between heating time under high pressure and drying temperature on L*-value, drying kinetic rate constant and calcium content. Due to high porosity and drying kinetic rate constant, heating condition at 121°C for 90 minutes and drying at 90°C was selected as the best process condition to produce the calcium supplement.

Table 2. L*, a*, b*, C and h from fishbone after heating under high pressure at 121°C for 30, 60 and 90 minutes then drying at 50, 70 and 90°C.

Heating time under high pressure	Drying temp.	L*	a*	b*	C	h
30 min	50°C	$89.70^{bc} \pm 1.54$	$0.14^{e} \pm 0.06$	$12.58^{d} \pm 1.69$	$12.58^{d} \pm 1.69$	$89.38^{a} \pm 0.19$
60 min	50°C	$86.86^{de} \pm 1.43$	$0.26^{cde} \pm 0.04$	$14.27^{cd} \pm 0.74$	$14.27^{cd} \pm 0.74$	$88.94^{ab} \pm 0.14$
90 min	50°C	$92.27^{a} \pm 0.87$	$0.25^{de} \pm 0.03$	$10.51^{e} \pm 2.34$	$10.52^{e} \pm 2.34$	$88.63^{abc} \pm 0.15$
30 min	70°C	$87.80^{cde} \pm 0.88$	$0.52^{bc} \pm 0.14$	$16.41^{b} \pm 0.07$	$16.42^{b} \pm 0.06$	$88.19^{bcd} \pm 0.50$
60 min	70°C	$85.92^{ef} \pm 0.13$	$0.46^{bcd} \pm 0.12$	$16.87^{b} \pm 0.23$	$16.88^{b} \pm 0.24$	$88.44^{bc} \pm 1.41$
90 min	70°C	$90.65^{ab} \pm 0.51$	$0.48^{bcd} \pm 0.23$	$14.54^{c} \pm 0.21$	$14.55^{c} \pm 0.21$	$88.13^{cd} \pm 0.89$
30 min	90°C	$84.52^{fg} \pm 2.18$	$1.11^{a} \pm 0.25$	$21.17^{a} \pm 2.16$	$21.19^{a} \pm 2.17$	$87.03^{e} \pm 0.39$
60 min	90°C	$83.37^{g} \pm 2.97$	$0.87^{a} \pm 0.14$	$19.79^{a} \pm 1.12$	$19.80^{a} \pm 1.12$	$87.49^{de} \pm 0.27$
90 min	90°C	$88.49^{bcd} \pm 3.31$	$0.54^{b} \pm 0.17$	$16.49^{b} \pm 2.49$	$16.49^{b} \pm 2.49$	$88.16^{bcd} \pm 0.29$

a-g means significant difference within the same column ($p \leq 0.05$).

Table 3. Drying kinetic rate constant (k) and calcium content from fishbone after heating under high pressure at 121°C for 30, 60 and 90 minutes then drying at 50, 70 and 90°C.

Heating time under high pressure	Drying temp.	k (min^{-1})	Calcium content (%db)
30 min	50°C	0.0133^{c}	$25.18^{a} \pm 0.39$
60 min	50°C	0.0125^{c}	$24.92^{abc} \pm 0.26$
90 min	50°C	0.0139^{c}	$24.60^{abc} \pm 0.31$
30 min	70°C	0.0164^{b}	$24.20^{bc} \pm 0.27$
60 min	70°C	0.0159^{b}	$24.73^{abc} \pm 0.79$
90 min	70°C	0.0163^{b}	$24.72^{abc} \pm 0.07$
30 min	90°C	0.0206^{a}	$24.09^{c} \pm 0.26$
60 min	90°C	0.0200^{a}	$24.66^{abc} \pm 0.31$
90 min	90°C	0.0198^{a}	$25.01^{ab} \pm 0.12$

a-c means significant difference within the same column ($p \leq 0.05$).

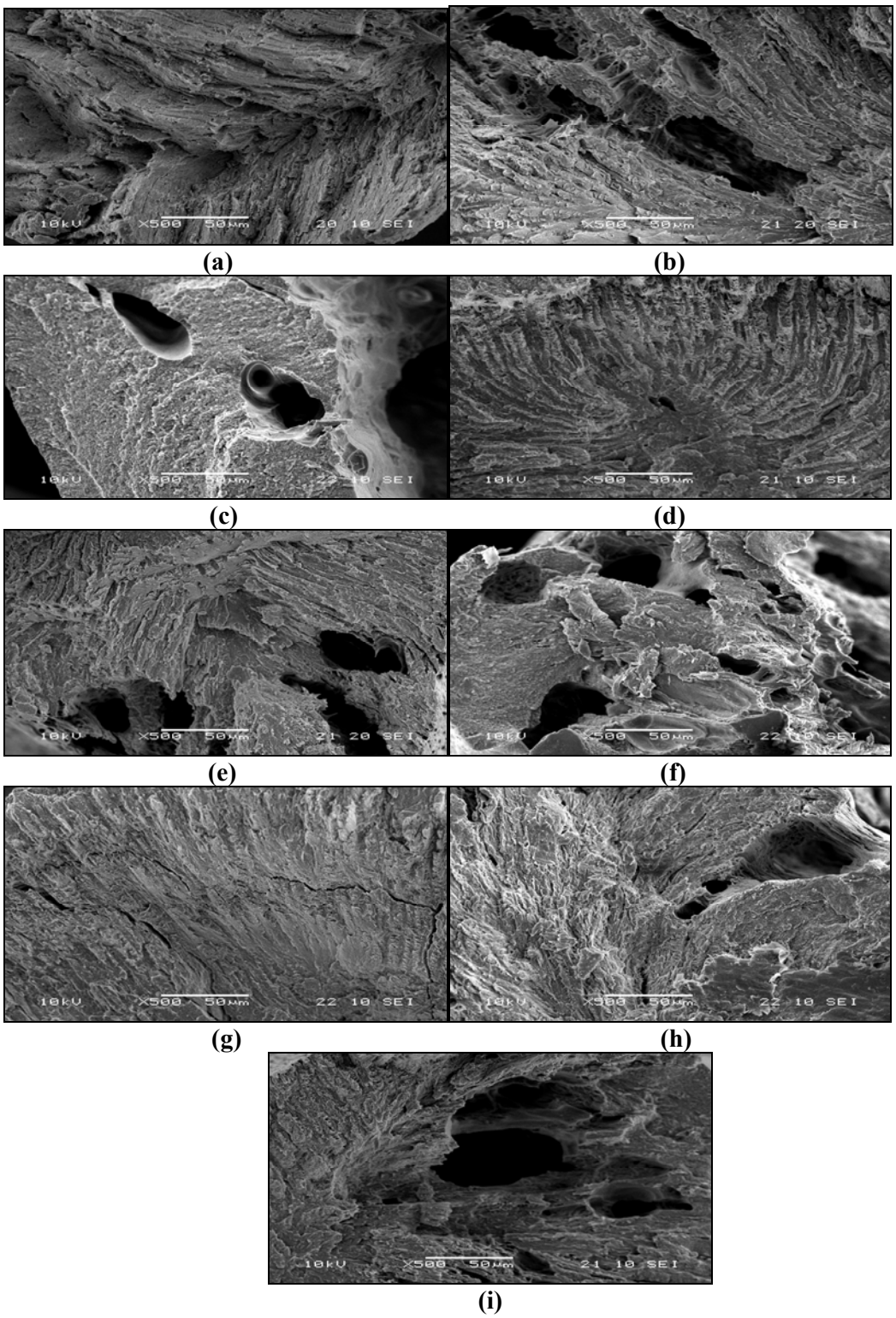

Figure 2. Scanning Electron Microscope (500x) of dried fishbone.
(a-c) after heating at 121°C for 30 60 and 90 min respectively and dried at 50°C
(d-f) after heating at 121°C for 30 60 and 90 min respectively and dried at 70°C
(g-i) after heating at 121°C for 30 60 and 90 min respectively and dried at 90°C.

According to the Notification of the Ministry of Public Health o.293 B.E. 2548 (2005) [13], the calcium supplement from Nile Tilapia fishbone in No. 0 capsule met the requirements of the supplementary products standard (Table 4).

Table 4. Quality of calcium supplement from fishbone of Nile Tilapia.

Characteristics	Standard for supplementary food	Calcium supplement from the bone of Nile Tilapia
Chemical Characteristics		
Total Arsenic	≤ 2 mg/kg	0.36 mg/kg
Lead	≤ 1 mg/kg	0.28 mg/kg
Total Calcium	120mg < Ca ≤ 800 mg/capsule	150 mg/capsule
Microbiological Characteristics		
Clostridium spp.	No detect in 0.1g	No detect in 0.1g
Escherichia coli	< 3 MPN/g	< 3 MPN/g
Salmonella spp.	No detect in 25 g	No detect in 25 g
Staphylococcus aureus	No detect in 0.1g	No detect in 0.1g

Conclusions

To develop a calcium supplement from Nile Tilapia fishbone, various conditions for pretreatment, heating and drying were studied. Pretreatment with NaOH and increased heating time under high pressure caused lighter colour of the processed fishbone. In addition, increased heating time yielded a porous structure of the fishbone, while increased drying temperature could increase drying kinetic rate constant. Therefore, soaking with 0.8% NaOH (ratio of fishbone and NaOH = 1:5) for 90 minutes before heating under high pressure (15 lb/in^2) at 121^0C for 90 minutes and finally hot air drying at 90^0C for 60 minutes were selected as the best process conditions for development of dried fishbone. The dried fishbone was ground to yield 150 μm particles before packing in a capsule (600 mg). One capsule of calcium supplement from Nile Tilapia fishbone contained 25.01% calcium and its quality met the standard requirements established by the Thai government**.**

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