Development of Calcium Supplement from the Bones of Yellow Fin Tuna (*Thunnus albacares*)

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Introduction

The mineral calcium is mainly important in building of bones and teeth and regulating certain metabolic processes in human body. According to the sources of Health ministry, daily dietary requirement of calcium for adults in Sri Lanka is 750-800 mg. Insufficient intakes of dietary calcium produce hypocalcaemia and osteoporosis (Piratheeban, 2013). Therefore, it is important to use food supplement to enhance the calcium content in various kinds of foods. Fish bones are well known to contain a high amount of calcium and fishbone ash normally contains 34 -36% calcium, particularly calcium phosphate (Hamada *et.al*., 1995). Yellow fin Tuna (*Thunnus albacares*) is one of the commercially valued fish species in fish processing industries in Sri Lanka and its' bones are discarded as waste by processing industries causing environmental pollution. The present study therefore, focuses on developing a method for producing a calcium supplement suitable for human consumption using the bones of Yellow fin Tuna (*Thunnus albacares)* and reduces accumulation of fish bone as waste causing environmental pollution. Development of a calcium supplement using fish bone will be a new implication in value addition sector of aquatic products in Sri Lanka.

Methodology

Bones of Yellow Fin Tuna were collected from Global Sea Foods (Pvt) Ltd., Badalgama. Collected samples were manually rinsed to remove adhered muscle residue after transporting to Animal Science laboratory of Uva Wellassa University. The processing steps include pretreatment using NaOH, neutralization, drying, grinding and sifting. Two experimental trials with different treatments were conducted including three replications for each treatment. Trial I was carried out to find out the best NaOH concentration and boiling time combination based on the softness of fish bones (T_1- 9%, 80 minutes, T_2- 9%, 90 minutes, T_3-9%, 100 minutes, T_4-10%, 80 minutes,T_5-10%, 90 minutes,T_6-10%, 100minutes,T_7-11%, 80 minutes,T_8-11%, 90 minutes,T_9- 11%, 100 minutes).The best treatment was selected by assessing the easiness of grinding 10 g of pretreated fish bones by mortar and pestle for 1 -2 minutes. After neutralization (rinsing by water 7 times), selected best pretreated sample was subjected to Trial II. Trial II was conducted to find out the best time and temperature combination (S_1- 80 ºC, 70 minutes; S_2-80 ºC, 80 minutes; S_3-80 ºC, 90 minutes; S_4-90 ºC, 70 minutes; S_5- 90 ºC, 80 minutes;, S_6- 90 ºC, 90 minutes; S_7-100 ºC,70 minutes; S_8-100 ºC, 80 minutes and S_9- 100 ºC, 90 minutes) for powder form final products. All final products were packed using polyethylene cover and stored at room temperature.

Then all final products were analyzed for different parameters. Moisture contents and Drying Kinetic Rate Constants ($MR = (M_t - M_e) / (M_o - M_e)$, $MR = \exp^{(-kt)}$) (MR- Moisture ratio, M_t- Moisture content at a specific time, M_o- Initial moisture content, M_e- Equilibrium moisture content, k- Drying kinetic rate constants, t-Time) (Techochatchawal *et al*., 2009) were evaluated for all final samples of Trial II. Calcium content (AOAC Standard Method 927.02) was determined for final treatments of Trial II and most suitable treatment was selected using above parameters.

Proximate composition of selected final product was determined for Pb content (AAS, Method 7082), crude fat, ash and crude protein level (AOAC, 1995). Microbiological analysis was conducted using Total Plate Count (TPC) for selected treatment at 37 ^{0}C. Finally, best treatment was compared with Standards for Supplementary food (Notification of the Ministry of Public Health o.293 B.E. 2548, 2005). Results were analyzed using Kruskal-Wallis test and Analysis of Variance (ANOVA) incorporated in MINITAB 14 soft ware.

Results and Discussion

The results has shown that there is an effect for NaOH - time combination for softness of the samples ($p<0.05$).The lowest softness is shown by T_1, T_2, while T_3 showed a moderate softness. Highest softness is recorded for T_4.There was a tendency to dissolve fish bones in other NaOH treatments (T_5, T_6, T_7, T_8 and T_9). So T_4 was selected as the best treatment. When producing a calcium supplement using fish bones, it is essential to dissolve connective tissue of the fish bones and fat should be eliminated. Research finding of Ishikawa *et al.* (1987) has revealed that NaOH, with heating under high pressure, can eliminate protein, connective tissue and fat. According to research finding of Techochatchawal *et al.* (2009) fish bones can be softened, due to denaturation of connective tissue. So softness of pretreated bones can be used as an indicator of fat elimination while increasing the fat solubility in water (Ockerman, 1988). Since T_4 recorded highest softness for pretreated bone sample, soaking the bones in 10% Sodium hydroxide solution for 80 minutes was considered as the optimum pretreatment method of Trial I.

Moisture content and Drying Kinetic Rate Constant (k) of final samples were significantly different ($p<0.05$) according to drying temperature and drying time period of the final samples (Table 1).

Table 1. Moisture %, Drying Kinetic Rate Constant (k and Calcium % (dry weight basis) for final treatments .

Parameter	Treatment								
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9
Moisture content %	12.63*	12.16*	9.20*	8.80*	8.46*	5.56*	5.20*	4.50*	4.26*
k (min^{-1}) ($x\ 10^{-2}$)	1.60^{a}	1.44^{a}	1.59	2.11^{a}	1.90^{a}	2.15^{a}	2.86^{a}	2.68^{a}	2.45^{a}
Calcium Content %	30.75	30.45	30.69	30.74	30.60	30.29	30.78	30.62	30.71

*^{a} – Significantly different at 0.05 level

There is no significant effect for the recorded final calcium content of the samples according to the treatment (Drying temperature and time) (Table 1). Results clearly indicate that the moisture content of samples is decreasing with increasing drying time - temperature combination. Therefore, it can be concluded that there is an inverse relationship between the moisture content and drying time / temperatures combinations. The highest Drying Kinetic Rate Constant was recorded by S_7. Moisture of the product is eliminated efficiently with increasing Drying Kinetic Rate Constant. There by the production process becomes cost effective, while quality of the final product retains. Hence, it can be recommended S7 (100 ºC, 70 minutes) as the best treatment that records greatest k of Trial II. Proximate composition of selected S_7 sample was recorded as, 13.43 0.25 for Crude fat, 7.30 0.59 for Crude protein, 75.33 2.52 for ash, and 5.20 0.30 for Moisture content. The Total Plate Count was zero for the final product (S_7). It indicates that there is no microbiological contamination of selected treatment.

High levels of Pb can cause toxic effect for consumers as a heavy metal. Results imply that Pb content of developed Ca supplement is in minor quantity (0.01 mg/Kg). According to results of Table 2, developed calcium supplement using Yellow fin Tuna bones satisfies the standards for supplementary food. Therefore this calcium supplement can be used to fulfill calcium requirement in human body.

Table 2. Comparison of the developed calcium supplement with the standards for Supplementary foods.

Characteristics	Standard for Supplementary food (Food and Drug Administration, 2005)	Developed Calcium supplement
Lead	≤ 1 mg/Kg	0.01 mg/Kg
Clostridium spp.	Not detected in 0.1 g	Not detected in 0.1 g
Escherichia coli	< 3 MPN/g	Not detected in 0.1 g
Salmonella spp.	Not detected in 25 g	Not detected in 0.1 g

Conclusions

The most appropriate processing method for processing the calcium supplement using Yellow fin tuna bone is soaking the fish bones in 10 % NaOH at constant temperature (90 ºC) for 80 minutes, neutralization by 07 times (pH - 7.2) and drying the neutralized pretreated fish bones, at 100 ºC for 70 minutes. The calcium composition of the selected final product was 30.6 % and it met the standard requirements for supplementary food. Development of calcium supplement using Yellow fin tuna fish bones could play a significant role in value addition sector of aquatic byproducts in Sri Lanka.

References

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