CHEMICAL PROPERTIES, BIOAVAILABILITY OF YELLOWFIN TUNA BONE AND ITS POTENTIAL APPLICATION AS CALCIUM SOURCE IN BAKERY PRODUCTS

MAHNAZ NEMATI

UNIVERSITI SAINS MALAYSIA
2019

CHEMICAL PROPERTIES, BIOAVAILABILITY OF YELLOWFIN TUNA BONE AND ITS POTENTIAL APPLICATION AS CALCIUM SOURCE IN BAKERY PRODUCTS

by

MAHNAZ NEMATI

Thesis submitted in fulfillment of the requirements
for the degree of
Doctor of Philosophy

March 2019

This thesis is dedicated to my love

MOHAMMAD

For his endless love, support and encouragement

This work is also dedicated to

My dear parents

Who have always loved me unconditionally

I am truly thankful for having you in my life.

ACKNOWLEDGEMENT

And finally, ***IT IS ACCOMPLISHED***! Praise be to GOD for seeing me through it all.

I am very grateful for getting an opportunity to carry out my Ph. D. work in Department of Food Science, School of Industrial Technology, Universiti Sains Malaysia. All the experiences have made the past years an ever good memory of my life. I have enjoyed to become absorbed by a topic that interests me and to be in a continuous process of learning. I wish to thank the many people who in one way or the other made this thesis possible.

Foremost, I wish to convey my utmost gratitude to my guide, Assoc. Prof. Dr. Fazilah Ariffin, whose unique professional supervision, meticulous comments, thought provoking ideas and support at all levels have been very valuable throughout this work. I am obliged to say that without her patient guidance and encouragement this work could have not been a reality.

I wish to express my heartfelt thanks to my co-supervisor, Assoc. Prof. Dr. Nurul Huda, for his guidance, motivations and optimistic outlook in the course of my research. I also wish to express my sincere appreciation and thank to academic and nonacademic staff of the School of Industrial Technology, who helped me and extend cooperation, one way or the other in the completion of my research work.

An honorable mentions and thank goes to my dear husband, for his patience and motivations during my difficult times and understanding me while finishing my thesis. Without his, I may have the spirit to end up my thesis.

Finally, I would like to express my most sincere and warmest gratitude to my family, my relatives and my friends in Malaysia and in Iran for their prayers, assistance and encouragement throughout my study. I think words can never express enough how grateful I am to my parents. I can only say a word of thanks to my mother and father for her prayers, patience and untiring support in every way during my long absence from the family. My gratitude is also extended to my sisters for their motivation and confidence in me.

MAHNAZ NEMATI

January 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

WHO	World Health Organization
FAO	Food and Agriculture Organization of the United Nations
RDA	Recommended Dietary Allowance
SEM	Scanning Electron Micrograph
n-6	Omega-6 fatty acid
n-3	Omega-3 fatty acid
Ca	Calcium
TF	Tuna Frame
TBP	Tuna Bone Powder
NaOH	Sodium Hydroxide
HCl	Hydrogen chloride
N	Nitrogen
AOAC	Association Of Analytical Chemists
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
UV-VIS	Ultraviolet Visible Spectroscopy
AABA	L-α-amino-n-butyric acid
HPLC	High Performance Liquid Chromatography
FAME	Fatty Acid Methyl Esters
GC	Gas Chromatography
TCP	Tricalcium Phosphate
CC1	Calcium Carbonate
CL	Calcium Lactate
CC2	Calcium Citrate

AAS	Atomic Absorption Spectrometry
$NaHCO3$	Sodium Bicarbonate
SD	Sprague Dawley
AIN	American Institute of Nutrition standard
CO2	Carbon dioxide
K	Potassium
Si	Silicon
Al	Aluminum
Ba	Barium
Na	Sodium
As	Arsenic
Cr	Chromium
Cu	Cupper
Fe	Iron
Mg	Magnesium
Zn	Zinc
P	Phosphorus
Cd	Cadmium
Pb	Lead
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
MUFA	Monounsaturated Fatty Acids
SFA	Saturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
MP	Milk Powder

CaCO3	Calcium Carbonate
TEB	Total Bone Extract from Bovine Femur

CIRI KIMIA, KETERSEDIAAN BIO TUNA YELLOWFIN DAN POTENSI PENGGUNAAN SEBAGAI SUMBER KALSIUM DALAM PRODUK BAKERI

ABSTRAK

Tuna sirip kuning (*Thunnus albacares,* Bonnaterre, 1788) adalah tergolong dalam keluarga *scombridae* dan merupakan antara spesis ikan terbesar digunakan dalam proses pengetinan di Malaysia. Dalam kajian ini, sisa tuna sirip kuning yang merupakan bahagian terbesar di dalam kilang pengetinan tuna telah diambil sebagai bahan mentah dan telah diproses untuk mendapatkan rangka tuna (TF) dan telah didedahkan kepada rawatan alkali untuk mengekstrak serbuk tulang tuna (TBP) sebagai potensi sumber kalsium. Jumlah TF yang berjaya dipulihkan daripada sisa tuna ialah 53.75% dan jumlah ekstrak TBP daripada TF ialah 59.79%. Penampilan umum TBP ialah di dalam bentuk serbuk bersaiz halus, berwarna putih dan tanpa bau hanyir ikan yang tidak di ingini, yang menjadikan ia sumber kalsium yang sesuai untuk tujuan pembangunan suplemen kalsium atau pengayaan produk makanan. Dalam fasa 1, keputusan komposisi proksimat untuk TF dan TBP menunjukkan bahana bahagian terbesar di dalam komposisi proksimat ialah kandungan abu, masing-masing iaitu 53.43% dan 77.97%. Hasil untuk komposisi mineral yang mewakili kalsium dengan 24.56% dan 38.16% adalah elemen terbanyak dalam TF dan TBP. Elemen kedua yang signifikan di dalam TF dan TBP ialah fosforus dengan masing-masing menunjukkan hasil 14.58% dan 23.31%. Ukuran nisbah untuk K:F adalah penting dalam TBP sebagai sumber kalsium di dalam kajian ini, jumlah nisbah ia adalah 1.64. Hasil keputusan kajian terkini menunjukkan, serbuk tulang yang perolehi daripada rangka tuna adalah tinggi dengan kalsium dan mengandungi

kandungan mineral penting yang rendah. Dalam fasa 2, kandungan bioavailabiliti kalsium dalam serbuk susu, TBP dan garam kalsium komersial termasukl trikalsium fosfat, kalsium karbonat, kalsium lactate dan kalsium sitrat diukur dengan menggunakan kaedah dialisis keseimbangan *in vitro*. Kandungan kalsium bioavailabiliti TBP adalah tinggi secara signifikan berbanding serbuk susu dan garam kalsium kecuali trikalsium fosfat; peratusan bioavailabiliti kalsium untuk TBP dan trikalsium fosfat adalah 53.93% dan 57.66%. Dalam fasa 3, TBP dan trikalsium fosfat ditambah sebagai sumber kalsium di dalam bahan-bahan produk bakeri. Keputusan yang diperolehi daripada kajian *in vitro* menunjukkan tiada perbezaan yang signifikan di antara roti putih yang diperkaya dengan trikalsium fosfat dan TBP. Hasil yang sama dapat diperhatikan dalam biskut butter yang diperkaya dengan fosfat trikalsium dan TBP juga. Seperti yang dijangka, kandungan kalsium bioavailability di dalam roti putih dan biskut butter yang diperkaya dengan trikalsium fosfat (roti: 37.43%, biskut butter: 39.48%) adalah lebih sedikit daripada TBP (roti: 36.98%, biskut butter: 38.97%). Kandungan kalsium bioavailabiliti roti mil penuh dan biskut *oatmeal* yang diperkayakan dengan trikalsium fosfat dan TBP menunjukkan tiada perbezaan signifikan di antara roti mil penuh yang diperkaya dengan trikalsium fosfat (21.94%) dan TBP (20.88%). Keputusan yang diperolehi daripada kajian *in vitro* menunjukkan kandungan bioavailabiliti kalsium di dalam roti mil penuh dan biskut *oatmeal* adalah rendah jika dibandingkan dengan roti putih dan biskut butter. Keputusan yang diperolehi daripada analisis sensori menunjukkan produk bakeri yang diperkaya dengan trikalsium fosfat dan TBP mempunyai skor yang sama yang berkaitan dengan rasa, bau, warna, tekstur, penampilan dan penerimaan keseluruhan. Malah, tiada perubahan signifikan dalam sensori atribut di antara produk yang diperkaya atau tidak diperkaya. Dalam fasa 4, kajian *in vivo* tikus

Sprague Dawley (SD) diberi makan dengan diet yang diperkaya dengan trikalsium fosfat dan TBP. Berat badan purata tikus yang menerima diet yang diperkaya dengan trikalsium fosfat dan TBP meningkat dalam masa 40 hari. Keputusan tidak menunjukkan perbezaan yang signifikan untuk berat badan di antara dua diet sepanjang kajian dijalankan. Jumlah berat badan yang bertambah secara general dan berat badan yang bertambah mengikut hari untuk tikus adalah tiada perbezaan signifikan juga. Tiada perbezaan signifikan secara statistik dapat dilihat dalam jumlah pengambilan makanan dan kecekapan makanan dianatara diet trikalsium fosfat dan TBP. Tidak hanya jumlah kalsium yang dikumuhkan dalam najis dan air kencing tiada perbezaan tetapi jumlah kalsium di dalam serum darah juga sama. Tiada perbezaan yang signifikan didapati untuk jumlah pengambilan kalsium di antara kumpulan eksperimen. Keputusan menunjukkan trikalsium fosfat dan TBP sebgai sumber kalsium yang berbeza tidam member kesan kepada penyerapan kalsium dan perkumuhan kalsium di dalam tikus. Tiada perbezaan yag signifikan untuk berat kering, berat abu dan kandungan kalsium dalam tulang paha antara dua kumpulan tikus yang diberi makan diet yang diperkaya dengan trikalsium fosfat dan TBP. Keputusan yang diperolehi daripada imbasan mikroskop elektron metunjukkan penggunaan TBP sebgai ganti kepada trikalsium fosfat di dalam diet tidak akan menyebabkan meningkat kan saiz poros dan jumlah poros. Keputusan kajian menunjukkan TBP boleh dianggap sebagai suplemen kalsium yang dipercayai memperbaiki parameter tulang yang berkesan sebanyak trikalsium fosfat. Tambahan pula, kebaikan TBP melebihi trikalsium fosfat berkaitan dengan kos pengeluaran yang rendah, diperolehi daripada sumber semulajadi dan akhir sekali ia adalah mesra alam sekitar.

CHEMICAL PROPERTIES, BIOAVAILABILITY OF YELLOWFIN TUNA BONE AND ITS POTENTIAL APPLICATION AS CALCIUM SOURCE IN BAKERY PRODUCTS

ABSTRACT

Yellowfin tuna (*Thunnus albacores*, Bonnaterre, 1788) belongs to the family scombridae and is the largest species of fish that is significantly used in canning process in Malaysia. In this study, yellowfin tuna wastes as a considerable part of tuna canning factories were taken as raw materials and were processed to recover tuna frame (TF) and were then exposed to the alkaline treatment to extract of tuna bone powder (TBP) as a potential calcium source. The amount of the TF recovered from tuna wastes was 53.75% and the amount of TBP extracted from TF was 59.79%. The general appearance of TBP was in a form of fine particle size powder, white color and without any undesirable fishy odor, which makes it into the appropriate source of calcium for the purposes of development of calcium supplement or enrichment of food products. At phase 1, the results of the proximate composition of TF and TBP showed that the major part of the proximate composition in TF and TBP was ash content, which was 53.43% and 77.97%, respectively. The results of mineral composition represented that calcium with 24.56% and 38.16% was the most abundant element in TF and TBP, respectively. The second significant element in TF and TBP was phosphorus with 14.58% and 23.31%, respectively. The measurement of the ratio of Ca:P is important in TBP as calcium source and in this study, the amount of the ratio was 1.64. Results of present study proposed that, bone powder recovered from tuna frame is rich in calcium and contains a small amount of other essential minerals. At phase 2, the amount of the bioavailability of calcium in

milk powder, TBP and commercial calcium salts including tricalcium phosphate, calcium carbonate, calcium lactate and calcium citrate was measured by using the *in vitro* equilibrium dialysis method. The amount of the calcium bioavailability of TBP was significantly higher than milk powder and calcium salts except tricalcium phosphate; the percentage of calcium bioavailability for TBP and tricalcium phosphate was 53.93% and 57.66%, respectively. At phase 3, TBP and tricalcium phosphate were added as a calcium source to the ingredients of the bakery products. The results obtained from *in vitro* study showed that there was no significant difference between white breads fortified with tricalcium phosphate (37.43%) and TBP (36.98%). The same results were observed in butter cookies fortified with tricalcium phosphate (39.48%) and TBP (38.97%) as well. The amount of the calcium bioavailability in whole meal bread and oatmeal cookies fortified with tricalcium phosphate and TBP showed that there was no significant difference between whole meal breads fortified with tricalcium phosphate (21.94%) and TBP (21.36%). Moreover, no significant difference was observed between oatmeal cookies fortified with tricalcium phosphate (21.31%) and TBP (20.88%). The results obtained from *in vitro* study showed that the amount of the bioavailability of calcium in whole meal bread and oatmeal cookies was lower in comparison with the white bread and butter cookies. The results obtained from sensory analysis showed that the bakery products fortified with tricalcium phosphate and TBP were similar in scores related to the taste, odor, color, texture, general appearance and overall acceptability. In fact, there were no significant changes in sensory attributes between fortified and non-fortified products. At phase 4, in *in vivo* study, Sprague Dawley (SD) rats were fed with diets fortified with TBP and tricalcium phosphate. The average body weight of rats receiving the diet fortified with tricalcium phosphate and TBP increased

during the 40 days. The results didn’t show any significant difference in the body weight between two diets during experiment period. The amount of the body weight gain in general and the body weight gain per day in rats were not significantly different, as well. No statistically significant difference was observed in the amount of the food intake and food efficiency between diet with tricalcium phosphate and TBP. Not only the amount of the calcium excreted in faeces and urine was not different but also the amount of the calcium in blood serum was similar. No significant differences were found in the amount of the calcium intake between two experimental groups. The results showed that tricalcium phosphate and TBP as different dietary calcium sources did not affect the amount of the calcium absorption and calcium excretion in rats. There were no significant differences in the dry weight, ash weight and calcium content of the femur between two groups of rats fed with diets fortified with tricalcium phosphate and TBP. The results obtained from the scanning electron microscope indicated that utilization of TBP instead of tricalcium phosphate in diet was not caused an increase in porous size and in the number of porous. The results of this study indicated that TBP could be considered as reliable supplement of calcium to maintenance of bone parameters effectively as much as tricalcium phosphate. Moreover the advantage of TBP over tricalcium phosphate is related to the low cost of its production, derived from natural sources and finally is environmental friendly.

CHAPTER 1: INTRODUCTION

Annually, around 32 million tons of fish byproducts are discarded to the environment which is considerable large and leading to an undesirable effect on the environment pollution (Arvanitoyannis and Kassaveti, 2008; Flammini *et al.,* 2016). The recovery and reutilization of the fish bones as animal feed might be effective to reduce the negative impact of fishing activity on the environment (Boutinguiza *et al.,* 2012). Fish bone as a natural source of calcium can be used as food ingredient and calcium supplement. It might be the appropriate strategy to maximally consume of fish resource and effectively decrease in the waste products from fishery industry (Hemung, 2013).

Recently, because of the enhancement in people awareness on the importance of adequate intake of calcium to decrease the risk of osteoporosis, the demand for calcium supplements and calcium fortified food products are growing (Malde *et al.,* 2010a). Calcium is not made in the body and is usually obtained from food, therefore consumption of the food and food product which is rich in calcium would be inevitable. However, the amount of the calcium in most of the ordinary diets is very low; therefore utilization of the calcium supplement along with the foods seems to be necessary. Generally, the main source of the calcium is milk and milk products however a large proportion of the people, especially Asian, are not able to use of the milk and dairy products because of the lactose intolerance and lactose indigestion. Approximately 65 percent of the human population has a reduced ability to digest lactose after infancy. Lactose intolerance in adulthood is most prevalent in people of East Asian descent, affecting more than 90 percent of adults in some of these communities (Techochatchawal *et al.,* 2009).

Calcium supplements and calcium-fortified food products are the most important source of calcium for a large group of people who are suffering from lactose intolerance. These people prefer to use the calcium supplements and calcium fortified products to compensate for the lack of the calcium (Jung *et al.,* 2007). However, the majority of these products are expensive which make them difficult to buy for all group of society. The common calcium fortificant which are commercially used for fortification of food products are including calcium carbonate, tricalcium phosphate, calcium citrate, calcium gluconate and calcium lactate. In fact, complex techniques and paucity of raw materials in fortification process are the main reasons for keeping higher price in spite of commercial motive. Therefore, investigations related to the calcium bioavailability from other resources of calcium that are somewhat cheaper and more available are on the rise (Kim *et al.,* 2003; Logesh *et al.,* 2012; Hemung *et al.,*2013; Framroze *et al.,* 2015). In some countries of Asian, among the population with lactose intolerance, an alternative source of calcium consumed in their diets is consumption of whole small fish with bones (Flammini *et al.,* 2016). The amount of the calcium absorption form such fish (whole small fish with bones) is comparable with the results of the calcium absorption from milk and dairy products in rats and human (Hansen *et al.,* 1998; Larsen *et al.,* 2000). However, fish bones and whole small fish with bones with a significant amount of calcium and phosphorus are regarded as waste products and thrown away in a huge-scale every year (Toppe *et al.,* 2007).

Calcium performs a number of basic functions in your body. Your body uses 99 percent of its calcium to keep your bones and teeth strong, thereby supporting skeletal structure and function. The rest of the calcium in your body plays key roles in cell signaling, blood clotting, muscle contraction and nerve function (Miller *et al.,* 2013). If people aren't getting enough calcium in their diet, the body takes calcium from the bones to ensure normal cell function, which can lead to weakened bones (Theobald *et al.,* 2005).

Calcium is recognized to be a critical element for numerous structural and functional purposes in human body including muscle contractions, nerve functions and enzymatic reactions as a cofactor and though the most important role of the calcium is recognized for strengthening of teeth and bones in our body (Jung *et al.,* 2007). Calcium element plays a fundamental role in bone development and sufficient intake of calcium along with the vitamin D has long been identified to be the most important nutritional factor to achieve the optimal peak bone mass and to preserve of the bone in human body (Anderson *et al.,* 1993; Ross *et al.,* 2011; Flammini *et al.*, 2016). Calcium is effective for bone health in lifetime and calcium requirement is particularly increased during growth, when development of the bones and muscles happens, and finally during old age (Mesias *et al.*, 2011; Schulman *et al.*, 2011; Flammini *et al.*, 2016). Differences in the endocrine system between young and elderly are caused by a reduction in estrogen hormone in postmenopausal women which is significantly affect on the bone health and in particular on the process of bone remodeling, causing to the enhancement of resorption/formation ratio which named osteoporosis (Chapman, 2007; Schulman *et al.*, 2011). Age-related osteoporosis is now considered to be a consequence of the progressive impairment of the hypothalamic –pituitary–gonadal axis in men (Riggs *et al.*, 1998) and, even though a corresponding dramatic fall of sex hormones as happens in menopause does not occur in men, the continuous slow reduction in circulating androgens, as well as changes in total and bioavailable serum testosterone in men, are strongly related to bone loss (Bilezikian *et al.*, 1999; Boonen and Vanderschueren, 2002).

One of the most disorders reported with aging is osteoporosis which is determined by reduction in bone mass and deterioration of the bone structure with significant increase in the percentage of bone fragility and skeletal fracture. Osteoporosis that is related to ovarian hormone deficiency subsequent of menopause is the significant cause of increase in the bone loss which is potentiated by reduction in ovarian estrogen commonly happens during the first decade following menopause onset (Framroze *et al.*, 2015). Current treatment for osteoporosis in postmenopausal women still rely on consumption of medicine such as estrogen hormone for a long term which is associated with side effects (McHorney *et al.*, 2007). We believe that finding a naturally substance that helps to enhancement of the bone formation in body will be effective to decrease the risk of the osteoporosis in elderly. Undoubtedly, the main factors which are useful for decrease in the frequency of osteoporosis are adequate intake of calcium, vitamin D and protein intake (Peters and Martini, 2010; Plawecki and Chapman-Novakofski, 2010).

In recent decades, a few studies have presented that natural supplements may be helpful in preserving bone mass for osteoporosis (Framroze *et al.*, 2015). In particular, studies using fish bone supplements to development of the bone density and growth are rare (Kim *et al.*, 2003; Luu and Nguyen, 2009; Hue *et al.*, 2010; Logesh *et al.*, 2012). Therefore, the beneficial effects of using natural compound have led us to hypothesize that extraction of calcium from fish bone powder would be a considerable source of calcium to use in fortification of food products instead of the commercial calcium salts. With these premises, this study attempts to find a solution for the improvement of the following problem statement;

1.1 Problem statements

The problem statements of present study were:

(i) Large percentage of tuna bone waste in processing factories is not only valueless, but also is a good opportunity to recovery of them to the value added products such as alternative calcium source.

(ii) Determination of calcium bioavailability in fish bone powder and fortified-products by in-vitro and in-vivo experiment could be effective to increase of utilization of them instead of commercial calcium salts.

From the problem statement, few things can be hypothesis as follow;

1.2 Hypothesis

(i) It is expected that, the calcium level in tuna bone powder is considerable.

(ii) The bioavailability of calcium in bakery products fortified with tuna bone powder is similar to the products fortified with calcium salts.

(iii) The porous structure in rats bone fed with diet fortified with tuna bone powder is in agreement with the results from diet fortified with calcium salts.

Considering the above problem statement and hypothesis thus this study goes to tackle the following objectives;

1.3 Objectives

The overall objective of the present study is study on the availability of calcium in the bones collected from tuna processing factories as a potential calcium source and fortification of food products, in order to compensate inadequate consumption of dairy products. Therefore, the objectives of the present study were:

(i) To study the chemical characteristics of tuna bone powder

(ii) To compare the bioavailability of calcium in tuna bone powder with commercial calcium salts

(iii) To evaluate of the bioavailability of calcium in the bakery products enriched with tuna bone powder and calcium salts

(iv) To assess the calcium absorption and bone structure in rats fed with diets fortified with bone powder and calcium salts

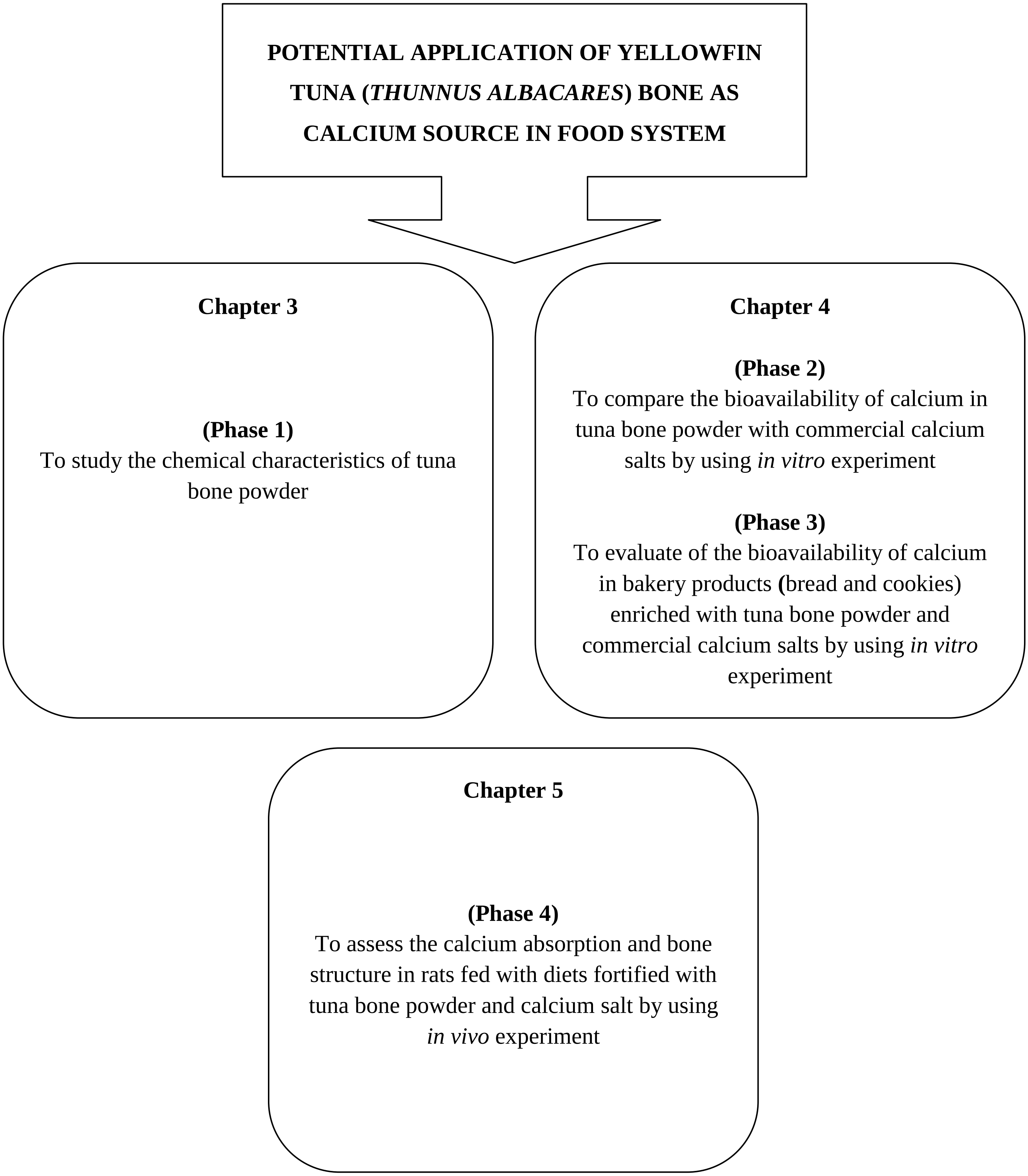

Figure 1.1: Overview of the thesis research design

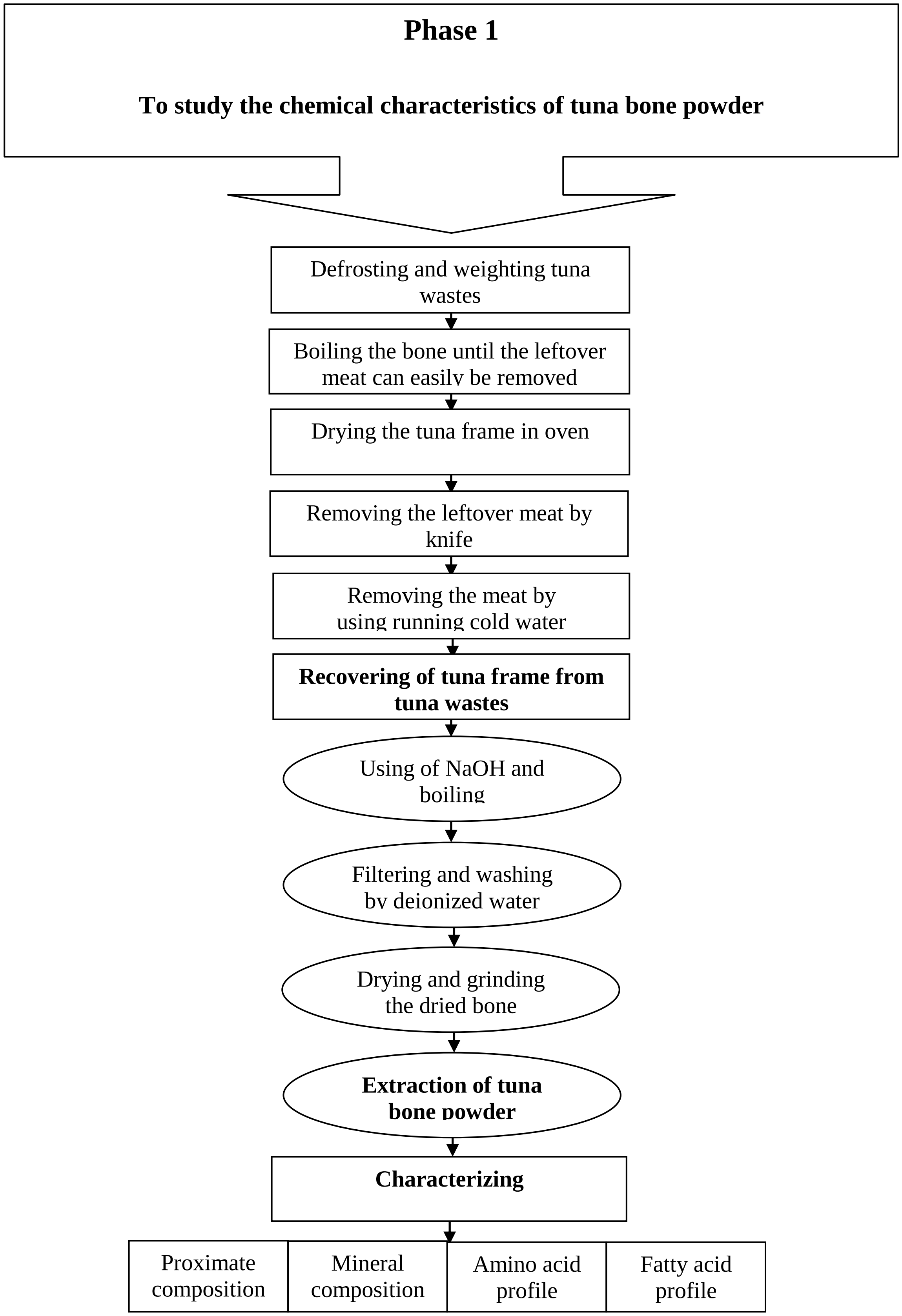

Figure 1.2: Research design in phase 1

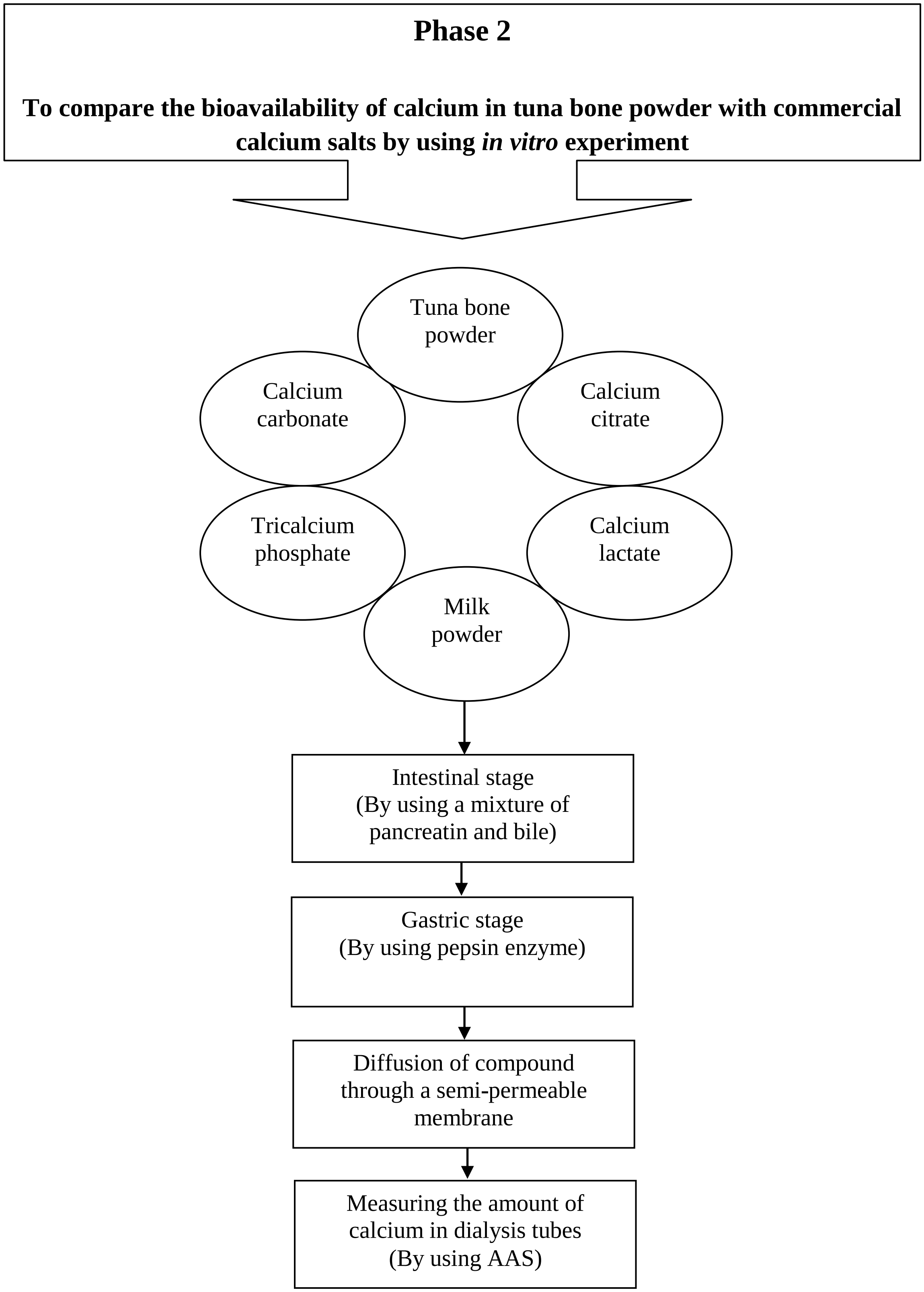

Figure 1.3: Research design in phase 2

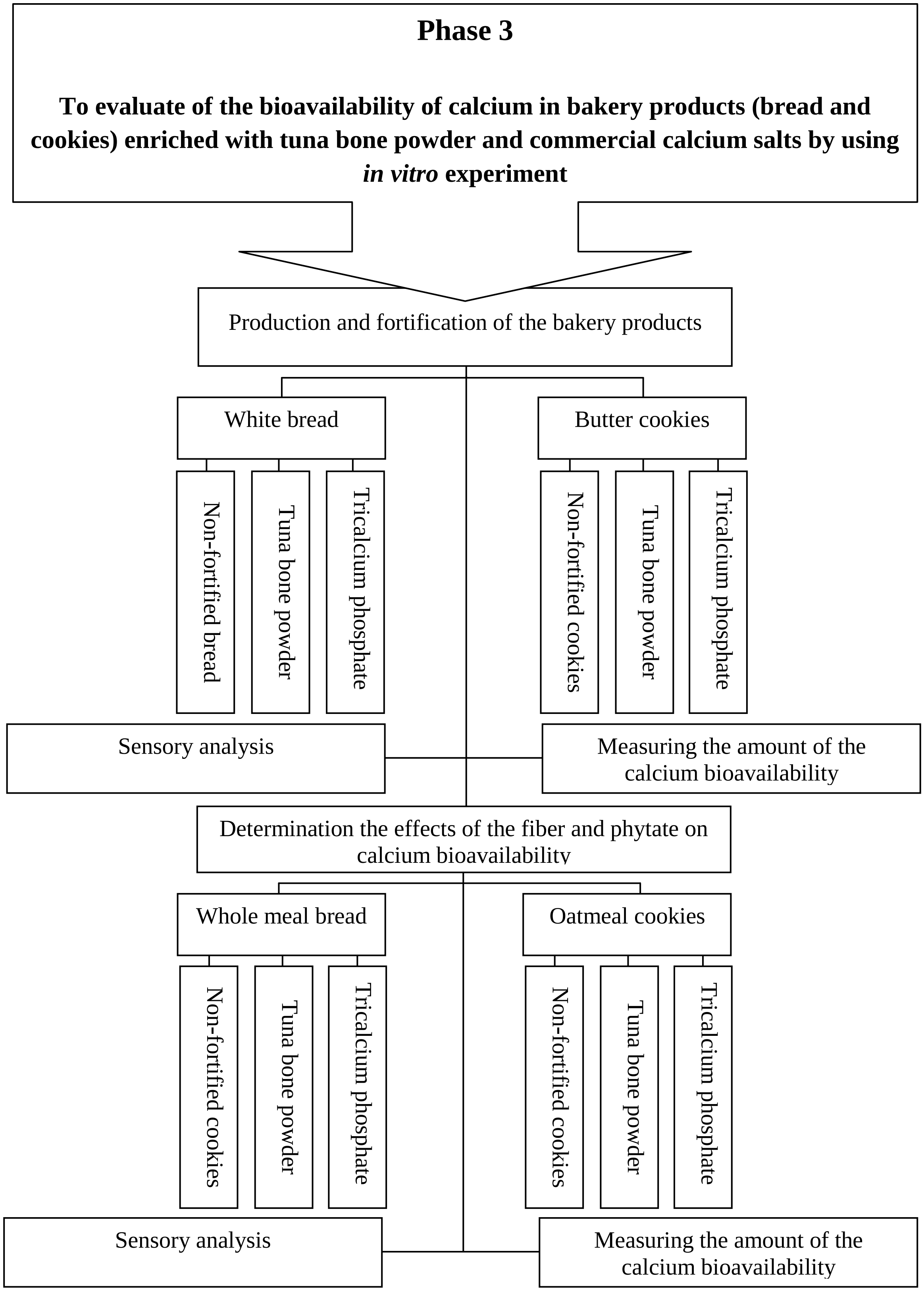

Figure 1.4: Research design in phase 3

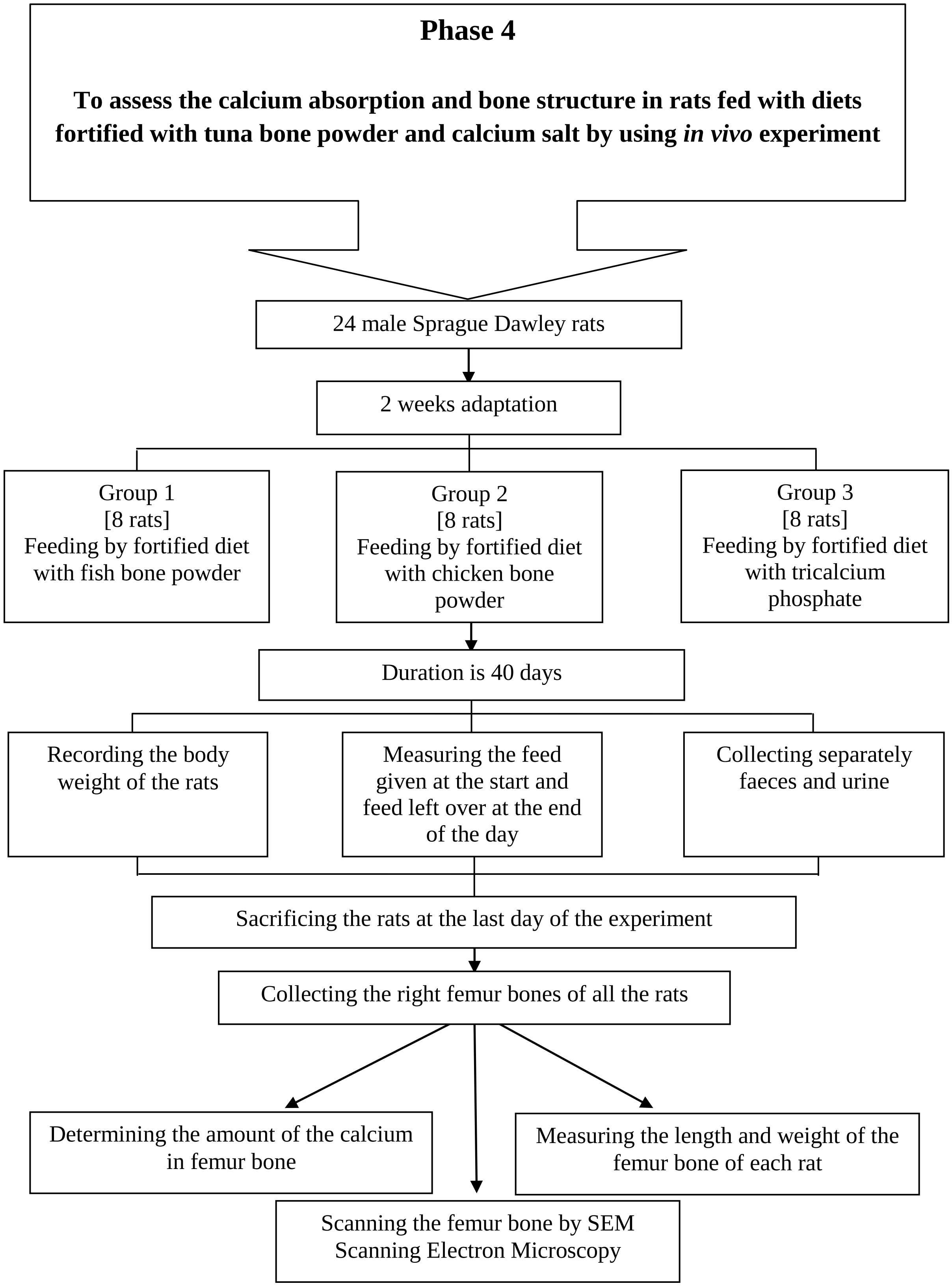

Figure 1.5: Research design in phase 4

CHAPTER 2:   LITERATURE REVIEW

2.1 Calcium

Calcium is one of the most essential mineral involved in human health and nutrition in all age groups. Calcium is most important constituent to maintain a best bone development during childhood and growing ages, and to prevent of osteoporosis in old ages (Martínez-Valverde *et al.*, 2000; Techochatchawal *et al.*, 2009). The lack of calcium in body may lead to deformity and poor quality of bones and teeth named rickets, and calcium deficiency for a long time will result in demineralization of bones and osteoporosis (Logesh *et al.*, 2012). Nowadays, scientists have proven that calcium is considered as an integral part of the diet, and adequate intake of calcium during growth is effective way to achieve of the maximum bone mass and prevent of the mentioned disease (Guéguen and Pointillart, 2000; Sittikulwitit *et al.*, 2004).

Moreover, calcium is not only necessary for strengthening of bones and teeth, but also is required for numerous physiological functions including; nerve transmission, muscle contraction, enzymes reactions, blood clotting, hormones secretion, and regulation of the permeability of sodium ion across cell membranes, and finally for reproductive functions including sperm motility and ovum fertilization (Techochatchawal *et al.*, 2009; Chaimongkol, 2012; Logesh *et al.*, 2012).

The amount of the calcium is a main factor in monitoring of the blood pressure. Enhancement in calcium intake will lead to decrease in blood pressure and avoid of the risk of hypertension, colon and colorectal cancer (Kettawan *et al.*, 2002). In fact, excessive consumption of calcium affects the absorption of the other minerals. High calcium intake reduces the intestinal absorption of magnesium which may lead to various diseases such as hypertension, kidney calcification, cancer and coronary heart disease (Sittikulwitit *et al.,* 2004).

In the human body, calcium considered as fifth element after oxygen, carbon, hydrogen, and nitrogen and it accounts for almost 1.5% of the body weight. The amount of the calcium is around 0.l - 0.2% of early fetal fat-free weight and it reaches to around 2% of adult fat-free weight (Nordin, 1997). Calcium is distributed in three main parts of the body, first, in the skeletal system, second, in the extracellular fluid, and third, in the cellular compartment. Approximately 99% of calcium is stored in bones and teeth, and only 1% of calcium is remained in blood (Phiraphinyo *et al.,* 2006). The amount of the calcium in the blood plasma is approximately constant and differs slightly over time. Indeed, when the amount of calcium in blood drops, it will be compensate with calcium from the bones and teeth to keep it stable (Kim *et al.,* 2003).

To meet calcium recommendations, the bioavailability of calcium is an important factor to consider beyond simply the calcium content of foods. Bioavailability is the degree to which a nutrient is absorbed and utilized by the body. The bioavailability of calcium refers to the fraction of dietary calcium that is potentially absorbable and the incorporation of the absorbed calcium into bone (Theobald *et al.,* 2005)

2.2 Calcium intake

Adequate intake of calcium is one of the main factors in prevention of several disorders in related to the bone and teeth in human health, because adequate intake of calcium during growth is a serious way to develop of peak bone mass which is effective to decrease the risk of osteoporosis during old ages (Tongchan *et al.,* 2009). The amount of calcium intake in developing countries is completely below the WHO/FAO recommendation. Based on the (Recommended Dietary Allowance) RDA, the adequate intake of calcium should be around 900 mg/day for adults (800 to 1000 mg, depending on the country), and increasingly to 1200 mg/day for adolescents and the elderly (Guéguen and Pointillart, 2000). This amount of calcium or safety levels of calcium will ensure to provide body with the highest protection against a harmful calcium imbalance and bone loss. In this case, adolescents will be assured that body stores the maximum amount of calcium in bones which is possible to save bones from fractures threshold when they become older. Although statistics show that a high percentage of people consume less than RDA in all over the world. Therefore, the amount of the frequency of osteoporosis in such countries is another indicator to prove the inadequate intake of calcium (Guéguen and Pointillart, 2000).

The National Institute of Health announced the RDA for adequate intake of calcium. The optimal rate of adequate intake of calcium was estimated to be 800 mg/day for childhood below 5 years of age, 800-1000 mg/day for children from age 6-10, 1200-1500 mg/day for adolescence or young adults from age 12-24 and pregnant or lactating women, 1000 mg/day from age 25 to the time of estrogen deprivation or age 65, and 1500 mg/day for elderly people (Kim *et al.,* 2003).

In addition, in developing countries, the amount of consumption of milk and dairy products is very low because of cost and limited availability, and in some area because of the lactose intolerance. In some low income countries, the amount of calcium intake is not enough and in addition, milk and dairy products are only the small part of the daily diet. So, investigation for finding of alternative sources of calcium instead of milk and dairy products for having the better growth in young children is critical (Larsen *et al.*, 2000).

In addition, the amount of RDA is different in various countries. The lower RDA for adults in India may be due to higher circulating levels of parathyroid hormone and calcitriol, promoting the active component of the intestinal calcium absorption and renal tubular calcium reabsorption (Kansal, 2002). The apparently lower calcium requirement in developing countries may be because of lower protein and/or sodium intakes than in the west since both these nutrients increase calcium excretion (Nordin, 1997; Singh *et al.*, 2007).

The amount of adequate calcium intake recommended for prevention of osteoporosis is 1000 to 1500 mg per day to decrease of the incidence of osteoporosis in postmenopausal women. However, a large number of women during the ages of 18 to 70 years consume less than 500 mg per day. So, the extra amount of calcium must come from fortified food products and calcium supplements (Sheikh *et al.*, 1987).

With the highest reported risk of hip fractures in the world, researchers have good reason to consider the benefits and risks of calcium supplements. The challenge is that too little calcium and vitamin D in your diet leads to an increased risk of osteoporosis and broken bones, which taking supplements have been shown to help prevent. However, some studies have also shown that taking supplemental calcium may also increase your risk of heart attack and stroke (Hagen *et al.*, 2016)

Some studies show that getting enough calcium might decrease the risk of heart disease and stroke. Other studies find that high amounts of calcium, particularly from supplements, might increase the risk of heart disease. But when all the studies are considered together, scientists have concluded that as long as intakes are not above the upper limit, calcium from food or supplements will not increase or decrease the risk of having a heart attack or stroke. In adults, too much calcium (from dietary supplements but not food) might increase the risk of kidney stones. Some studies show that people who consume high amounts of calcium might have increased risks of prostate cancer and heart disease, but more research is needed to understand these possible links (Li *et al.*, 2012).

2.3 Osteoporosis

Osteoporosis means "porous bone". Osteoporosis is a medical condition in which the bones become brittle and fragile from loss of tissue, typically as a result of hormonal changes, or deficiency of calcium or vitamin D. Osteoporosis is a major public disease in elderly people as a result of significantly reduction in bone density (Soltan, 2013). The serious problem of osteoporosis is the producing incapability through the development of bone fractures that lead to morbidity and mortality in the older age groups (Pongchaiyakul *et al.*, 2008). Adequate intake of calcium during growth ages and between the ages of 20 to 30 years old can be effective to promote the optimal bone mass in children and prevent of osteoporosis in elders (Chaimongkol, 2012).

Fractures in osteoporosis can happen in any part of the bones, but mostly happen at the hip especially over the age of 50 (Lau *et al.,* 2001). In Asia, osteoporosis has become a crucial degenerative disease, and it has been expected that in the next century, more than 50 percents of all hip fractures in the world will happen in Asia (Wahab, 2010). Researchers reported that the calcium deficiency is one of the most significant risk factors causing osteoporosis followed by physical inactivity, cigarette smoking and coffee consumption (Martin *et al.,* 1987; Wardlaw et al., 1988). Consumption of foods rich in calcium throughout life is effective to prevent or postpone of osteoporosis in both pre- and post-menopausal women and elderly men (Okano *et al.,* 1994).

In the world, every three women over 50 will experience osteoporotic fractures. As will five men. In generally, statistics shows that in Malaysia in every 3 women, 1 women and in every 5 men, 1 men is at risk of osteoporosis. Unfortunately, hip fractures are associated with a high morbidity and mortality rate of up to 20% (Yeap *et al.,* 2013). In Malaysia, it is estimated that over 1 million people are at risk from osteoporosis. This is the serious warning. Because in Malaysia, many people doesn’t like drinking milk because of suffering from the lactose intolerance problem. So, the risk of osteoporosis will increase (Khan *et al.,* 2014)

The prevalence of osteoporosis in women is three times than in men, to some extent due to changes in the feminine hormones that happen during menopause (WHO, 2003). The negative aspects of osteoporosis affects on quality of life in elderly patients.

Statistics shows that 10% in women between 55-64 years old and 22.5% in women aged 65 years old are involved with osteoporosis (Soltan, 2013).

Osteoporosis is related significantly with the deficiency of ovarian hormone (estrogen) following menopause and occurs among the post-menopausal women. Conventional drug therapies for osteoporosis in postmenopausal women have emphasized agents which reduce of bone resorption including estrogen, calcitonin and bisphosphonates (Ranjan *et al.,* 2005). At present, the most traditional methods to decrease the rate of bone loss in postmenopausal women is estrogen substitution therapy as anti-resorptive agents and growth hormone as formation-stimulating agents. However, they may be attended by side effects, so they are recommended only for patients who are severely affected by osteoporosis and or who have no contraindications. Hence, it would be beneficial to found a naturally component that minimizes the necessity for therapy, and reduces the bone losses in postmenopausal women (Arjmandi *et al.,* 1996). At present, estrogen and bisphosphonate with potential side effects (e.g. thrombosis, liver cirrhosis, spider angioma, palmary erytherma, gynecomastia and testicular atrophy) are consumed for treatment of osteoporosis (Framrose *et al.,* 2015). So, the natural compound as supplement which is rich in calcium and can improve bone and skeletal health is necessary to use as an alternative to drugs (Yoon *et al.*, 2005).

2.4 Calcium sources

Calcium is not made in the body and is usually obtained from the diets. However most of the regular diets are severely poor in calcium and it is necessary to obtain calcium as a supplement along with the foods. In general, calcium is mainly received from milk and dairy products and followed by calcium fortified products, supplements or a mixture of mentioned sources as well as broccoli, tofu, oysters, sardines and similar soft sources of bone (Techochatchawal *et al.,* 2009). Around 70% of calcium can easily be achieved from common calcium sources; milk and dairy products, especially cheese in adults and only a few dried fruits and green vegetables as good sources of calcium (about 16%), and also drinking water, such as mineral water (about 6-7%) (Guéguen and Pointillart, 2000). However, consumption of milk and dairy products may be limited in certain population due to the lactose intolerance.

Although in the intestine, calcium from milk and dairy products is easily absorbed rather than the calcium from phytate-oxalate rich vegetables and cereals (Kansal, 2002). Absorption of the calcium from plant sources is low compared to animal. Generally, the plants often have a large quantity of substances that seriously inhibit of the absorption of calcium and also other minerals. The main components which inhibit the absorption of the calcium are oxalate, phytate and uronic acid (Kettawan *et al.,* 2002; Hambidge *et al.*, 2005).

Undoubtedly, milk and dairy products are evaluated as complete foods in a daily diet and are the most varied in the natural foods which contain more than 20 different trace elements. Most of them are necessary and very important including calcium, zinc, copper, iron and manganese. These elements are co-factors in enzymatic reactions and play a key role in several physiological functions of the human body and lack of these elements causes disorders and pathological conditions (Enb *et al.*, 2009).

Other sources of calcium are including some vegetables and non-fortified foods are not rich as much as milk and dairy products. For example, the amount of the calcium in soymilk and cow milk is 200 mg/L and 1200 mg/L, respectively, which is incomparable. Therefore, natural soy milk is not a considerable source of calcium and only calcium fortified-soymilk can be replaced as an alternative source of calcium to cow's milk (Zhao *et al.,* 2005).

2.5 Needs for alternative calcium supplements

Utilization of milk and dairy products has reduced gradually over the past few decades due to the high fat levels of milk and its products and their effects on weight gain and obesity (Patwardhan *et al.,* 2001). In addition, in some area the majority of the people have limited because of lactose indigestion and intolerance (Kamchan *et al.,* 2004).

Lactose intolerance is the inability to break down a type of natural sugar called lactose. Lactose is commonly found in dairy products, such as milk and yogurt. A person becomes lactose intolerant when his or her small intestine stops making enough of the enzyme lactase to digest and break down the lactose (Heyman, 2006). Some oriental people cannot drink milk because of lactose intolerance and indigestion which make them sensitive to milk. Usually, they suffer from some unfavorable effects including abdominal distention, abdominal cramps, flatulence, numerous bowel movements and or diarrhea (Kettawan *et al.*, 2002).

That's why, finding alternative sources of calcium would be valuable and beneficial. Moreover, milk and dairy products is well balanced in terms of nutrients specifically calcium but because of having high price, it is beyond the purchasing power of a large group of population (Wahab, 2010). Since milk and dairy products are the main dietary sources of calcium, so avoidance of these products by populations who are lactose intolerant or dieters or low-income people or by those who concerned about cholesterol intake commonly results in submarginal intake of calcium. So, investigation on the finding of the alternative sources of calcium to compensate the inadequate intake of calcium is unavoidable.

2.6 Calcium fortified products

In recent years, supply and demand for calcium supplements and calcium fortified food products are growing fast due to the enhancement in people knowledge of adequate intake of calcium to meet the bone and teeth requirements needed to decrease the risk of osteoporosis (Malde *et al.,* 2010a). Fortification of main foods is generally accepted as an effective way for providing the daily requirements for a range of vitamins and minerals. Fortified-foods such as various juices, yeast breads, breakfast foods, soy products, carbonated beverages and snack foods have been developed to improve calcium intake (Fairweather-Tait and Teucher, 2002; Babarykin *et al.,* 2004). Consumption of these food products can be a good decision for those who do not drink milk and milk products. Selection of the appropriate dietary foods combined with proper calcium source is a significant consideration in the growing of the fortification of food products (Chaimongkol, 2012).

Numerous commercial calcium salts are used for fortification of food products; however different types of calcium salts have different effects on the bioavailability of calcium (Ranjan *et al.*, 2005). Commercial calcium salts commonly used are including calcium carbonate, calcium phosphate, tricalcium phosphate, calcium chloride, calcium citrate malate, calcium gluconate, calcium lactate, calcium lactate gluconate and natural milk calcium (Singh *et al.*, 2007). The most regular calcium supplement is calcium carbonate due to the high concentration (40%) of calcium by weight and lower price. Investigations showed that calcium obtained from calcium carbonate is as good source of calcium, since the bioavailability of calcium in calcium carbonate is equal to milk (Toba *et al.*, 1999).

Calcium supplement from natural sources has been suggested to be more useful for bone health than purified CaCO3 because natural sources of calcium also contain other nutrients such as certain amino acids which might increase calcium metabolism (Suntornsaratoon *et al.*, 2018)

Nowadays, numerous calcium-fortified food products and supplements are quickly mushroomed in the market and also demands for them are growing. However majority of these products are sold at higher price which make them difficult to utilize for all stratum of society. The complex techniques and paucity of raw materials in their production are the main reasons for keeping higher price in spite of commercial motive.

Therefore, investigations related to the calcium bioavailability from other resources of calcium that are cheaper and more available are on the rise (Logesh *et al.*, 2012).

By-products from food processing units including egg shell, fish bone, chicken bone and bovine bone are promising as potential new natural calcium sources with low production cost which can replaced commercial calcium salts (Phiraphinyo *et al.*, 2006; Chuamani, 2010). In present study, an attempt has been made to check the availability of calcium in the bones remained from tuna canning factories. Because the utilization of fish bone powder can be more acceptable by consumers as natural source of calcium instead of utilization of commercial calcium fortificant. Moreover, fish bone powder would be more effective in terms of existanec of calcium phosphate compound in which is similar to the human bone components (Phiraphinyo *et al.*, 2006; Chuamani, 2010).

2.7 Fish bone as potential source of calcium

Fish bone consists of a considerable amount of calcium and phosphorus. This natural source of calcium will be effectively absorbed and it will be a significant dietary contribution in population with inadequate intakes of milk and milk products (Kim *et al.*, 2003; Luu and Nguyen, 2009). Fish bone is determined as a main non-edible part of fish, after separating the fish muscle from the skeletal frame, which could be counted as a valuable source of different health-promoting substances (Logesh *et al.*, 2012).

In some area, mostly in Asia, where milk and dairy products are not significantly included in the daily diet because of the lactose indigestion and lactose intolerance, people find an alternative source to compensate of the lack of calcium is their diet. In fact, a large group of people in mentioned area prefer to use of the whole small fish with bones to achieve the sufficient amount of calcium without undesirable effects of consumption of dairy products. Investigation on the utilization of whole small fish with bones showed that the amount of the calcium absorption from such fish not only is not less than milk and dairy products but also it is comparable with calcium absorption from food products fortified with commercial calcium salts both in rats and humans (Larsen *et al.*, 2000; Flammini *et al.*, 2016).

Fish bone mainly composed of organic and inorganic components. The organic component is made up of collagen that is consisting of 30% of the material (Nagai *et al.*, 2004), which is potentially able to consider as an excellent source of collagen and gelatin (Nagai and Suzuki, 2000). Whereas the inorganic component is mainly made of calcium phosphate and hydroxyapatite that is consisting of 60-70%, which is considered as a potential source of calcium (Logesh *et al.*, 2012). A significant amount of the whole fish catch is threw away as processing leftovers, such as head, bones, fins, skin and viscera. The amount of bone fraction, which is still regarded as a waste product, is approximately 10-15% of the whole body weight. (Jung *et al.,* 2005; Malde *et al.*, 2010a; Malde *et al.*, 2010b; Amiza *et al.*, 2013). While a considerable amount of calcium and phosphorus which is easily available in the huge quantities are discarded (Toppe *et al.*, 2007). Statistical reports showed that the current world's fisheries discards exceeds 20 million tons, or equivalent to 25 percent of the whole production of fisheries capture (FAO, 2003). Fish bones are the major solid by-product remained from frozen fish processing industry (Tongchan *et al.*, 2009).