UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF CALCIUM CONTENT IN SELECTED CALCIUM FORTIFIED MILK

NUR FARHANAH BINTI OTHMAN

This project entitled "Determination of Calcium Content in Selected Calcium Fortified Milk" was prepared by Nur Farhanah Binti Othman and submitted to the Faculty of Medicine and Health Sciences as a partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Received and examined by:

____

(Dr. Mohd Redzwan Sabran)

Date: _________________

ACKNOWLEDGEMENTS

It is a pleasure to thank the many parties who have been involve in the development and completion of my final year project. First and foremost, I would like to thank God Almighty for giving me the strength, knowledge, ability and opportunity to undertake this research study and to persevere and complete it satisfactorily. It is difficult to overstate my gratitude to my beloved supervisor, Dr. Mohd Redzwan Sabran for his continuous support of my study and research, for his patience, motivation, enthusiasm and guidance which made the journey so much fun and easier. He has given all the freedom to pursue my research, while silently and non-obtrusively ensuring that I stay on course and do not deviate from the core of my research. I could not have imagined having a better supervisor for my final year project.

Besides my supervisor, I would like to extend my heartfelt appreciation to the course coordinator, Dr Siti Raihanah Shafie for constantly keeping track on our progress throughout the semester. I would like to thank the laboratory officers especially En. Hasbullah and assistants from the Department of Nutrition and Dietetics of Faculty of Medicine and Health Sciences, UPM for their constant assistances and guidance. It would be inappropriate if I omit to mention the names of my fellow friends Nurain Farhana, Wong Yi Theng, Feadlind Damia and those who have, in their own ways, kept me going on my path to success and assisting me as per their abilities and stimulating all the fun we had for the last two semesters in UPM.

Last but not the least, I wish to convey my highest gratitude to my parents, siblings and entire extended family for continuously praying for me, providing a

loving environment for me always, supporting me spiritually and physically by giving moral support. I sincerely feel grateful to all the parties involved directly and indirectly in my journey of completing this project and I dedicate this work to all of them. Without them, I may not be able to finish what I have started in the last 4 years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

DETERMINATION OF CALCIUM CONTENT IN SELECTED CALCIUM FORTIFIED MILK

Nur Farhanah Binti Othman

Milk is an important source of calcium and calcium deficiency may lead to other chronic diseases. In order to achieve daily calcium requirement, consumers may choose to consume calcium-fortified milk. Calcium has low solubility in water and must be in the form of salt (i.e phosphate and carbonate) in order to be fortified. The objective of this study was to determine the calcium content in selected calcium-fortified milk. Based on products' list of ingredients, twenty samples with added calcium (i.e tricalcium phosphate, calcium carbonate), were purposively purchased at a hypermarket in Bangi, Selangor, comprising of seven animal- and thirteen plant-based milk. An aliquot of each sample was digested in microwave and analyzed for calcium content with Atomic Absorption Spectrometry. Subsequently, the milk product was shaken using a belly dancer shaker and another aliquot was taken, digested and analyzed for the calcium content. The calcium content was not significantly different ($p>0.05$) between calcium-fortified plant- and animal-based milk. Although the calcium content was higher after the milk products were shaken, the difference was not significant ($p>0.005$) if compared to the calcium content before they were shaken, for most of the samples. Nevertheless, the calcium content in Brand D (full cream milk) and Brand T (almond milk) was significantly higher ($p<0.005$), after they were shaken and the differences were 10.4% and 93.9%, respectively. A lower calcium content in unshaken milk may be due to the sedimentation of added calcium in calcium-fortified milk. Without shaking the product prior use, the added calcium will remain as precipitate at the bottom of the packaging. Besides, low calcium absorption may also be due to plant-based milk contains inhibitory substances such as phytate and oxalate which can bind to calcium and form insoluble complexes. Hence, it is crucial for consumer to shake the products prior use in order to get the maximum amount of calcium stated on the food label.

ABSTRAK

PENENTUAN KANDUNGAN KALSIUM DALAM SUSU DIPERKAYA KALSIUM

Nur Farhanah Binti Othman

Susu adalah sumber penting kalsium dan kekurangan kalsium boleh menyebabkan penyakit kronik yang lain. Untuk memenuhi keperluan kalsium harian, pengguna boleh memilih untuk menggunakan susu yang diperkaya kalsium. Kalsium mempunyai kelarutan yang rendah dalam air dan harus dalam bentuk garam (iaitu fosfat dan karbonat) agar dapat diperkaya. Objektif kajian ini adalah untuk menentukan kandungan kalsium dalam susu yang diperkaya kalsium terpilih. Berdasarkan senarai ramuan produk, dua puluh sampel dengan kalsium tambahan (seperti tricalcium fosfat, kalsium karbonat), sengaja dibeli di pasar raya besar di Bangi, Selangor, yang terdiri daripada tujuh susu daripada haiwan dan tiga belas tumbuhan. Sebilangan kecil sampel dicerna dalam gelombang mikro dan dianalisis kandungan kalsium dengan Spektrometri Penyerapan Atom. Selepas itu, produk susu digegarkan menggunakan alat penari perut dan lain-lain diambil, dicerna dan dianalisis kandungan kalsiumnya. Kandungan kalsium tidak jauh berbeza ($p > 0,05$) antara susu berasaskan kalsium dan susu berasaskan haiwan. Walaupun kandungan kalsium lebih tinggi setelah produk susu digoncang, perbezaannya tidak ketara ($p > 0,005$) jika dibandingkan dengan kandungan kalsium sebelum dikocok, untuk kebanyakan sampel. Walaupun begitu, kandungan kalsium dalam Jenama D (susu penuh krim) dan Jenama T (susu badam) jauh lebih tinggi ($p < 0.005$), setelah mereka digoncang dan perbezaannya masing-masing 10.4% dan 93.9%. Kandungan kalsium yang lebih rendah dalam susu yang tidak digoncang mungkin disebabkan oleh pemendapan kalsium tambahan dalam susu yang diperkaya kalsium. Tanpa menggegarkan produk sebelum digunakan, kalsium tambahan akan tetap seperti endapan di bahagian bawah bungkusan. Selain itu, penyerapan kalsium yang rendah juga disebabkan susu berasaskan tumbuhan mengandungi bahan penghambat seperti fitat dan oksalat yang dapat mengikat kalsium dan membentuk kompleks yang tidak larut. Oleh itu, sangat penting bagi pengguna untuk menggoncang produk sebelum digunakan untuk mendapatkan jumlah maksimum kalsium yang tertera pada label makanan.

CHAPTER 1

INTRODUCTION

1.1 Background

Calcium is the fifth most abundant element in the human body, with approximately 1000 g present in adults (RNI, 2017). Nearly all (99%) of calcium is in the skeleton. The remaining 1% is equally distributed between the teeth and soft tissues, with only 0.1% in the extracellular fluid (Food and Agriculture Organization of the United Nations, World Health Organization, FAO/WHO, 2002). Calcium plays a vital role in skeletal mineralization, as well as a wide range of biological function including vascular contraction and vasodilation, coagulation of blood, transmission of nerve impulses, activation of enzyme reactions and stimulation of hormone secretions and intracellular signaling (Recommended Nutrient Intakes for Malaysia, 2017; National Institutes of Health, 2016a).

Calcium levels in cells and blood are precisely controlled by the body, which the body moves calcium out of bones into blood as needed to maintain a steady level of calcium in the blood. Insufficient calcium intake will cause excessive mobilized calcium from the bones which can weaken the bone and osteoporosis can result. Thus, adequate calcium intake is particularly important to maintain a normal level of calcium in blood without weakening the bones especially for bone development in childhood and adolescence as it is a critical period for skeletal mineralization (Bonjour, Chevalley, Ferrari & Rizzoli, 2009). Calcium intake is also very crucial for post-menopausal and elderly women because lack of calcium is associated with reduced bone density and it can lead to osteoporosis (Lau, Suriwongpaisal, Lee et al., 2001). This is because, adequate calcium intake before the age of peak bone mass can reduce the effect of bone mineral density declines and fracture risk, demonstrating the importance of supporting the build-up of bone mass during the growth stage (National Institutes of Health, 2016a).

There are many sources of calcium-rich foods in the Malaysian diet, for example, fish with edible bones such as canned sardines and anchovies, beans and bean products including yellow dhal, tofu and *tempeh* (fermented soybeans), locally processed food such as shrimp paste, *cincalok* and *budu*, as well as vegetables like spinach, watercress, mustard leaves, *cekur manis*, tapioca leaves, *kai-lan* and broccoli (Tee et al., 1997). However, according to Léon, MsScAgr & Alain (2013), most of the dietary calcium comes from milk and dairy products, while only a few green vegetables and dried fruits are good sources of calcium. Milk and dairy products have high calcium contents of high bioavailability and can be obtained at a low cost in relation to their nutritional value. Moreover, these products also provide other essential nutrients and their intake improves the overall nutritional quality of the diet of children and adolescent (Nicklas, 2003). This is the main reason most people choose milk and dairy products as their source of calcium.

Nonetheless, calcium deficiency is one of the major nutritional problems. According to the International Osteoporosis Foundation (IOF), calcium intake among Malaysian remains low, with several surveys showing daily calcium intakes of below 500 mg daily especially in premenopausal and postmenopausal women (Pon et al., 2006; Chong et al., 2007; Chee et al., 2002). The level of calcium in the body, however, depends not only on the amount of calcium in the food consumed but also depends on how well the calcium is absorbed into the body. Poor absorption of calcium in the body is usually due to plant-based products that contain inhibitory substances such as oxalates and phytates that will bind to calcium and form insoluble salt complexes, which will reduce calcium absorption, hence lead to calcium deficiency. Due to that, food industry has developed calcium fortified foods in securing adequate calcium intake in plant-based products (Nicklas, 2003; Fuente, Belloque & Juárez, 2004).

With fortification, sedimentation of added calcium is another problem that will cause lower intake of calcium. In fact, a study has shown that unshaken calcium fortified soy beverages amounted to average only 31% of their label claim and only averaged 59% when shaken, with both cases making up the remaining stated calcium content in the settled residue (Heaney & Rafferty, 2006). The authors of the study also noted that these percentages were for "moderately vigorous shaking", and that none of the samples which are soy beverages that were fortified with calcium met the label claim for typical use. This is also related to the awareness of the consumer on reading label before consume a certain product and in this case, is calcium fortified milk in order to get enough nutrient that is claim by the products. Thus, this study aims to determine calcium content in calcium fortified milk as well as to compare calcium content in calcium fortified milk before and after being shake.

1.2 Problem Statement

According to the International Osteoporosis Foundation (IOF), calcium intake among Malaysian remains low, with several surveys showing daily calcium intakes of below 500 mg daily especially in premenopausal and postmenopausal women (Pon et al., 2006; Chong et al., 2007; Chee et al., 2002). In addition, a study by Hawa, Sakinah and Hermizi (2013) also stated that there was a high level of calcium deficiency among pre and postmenopausal women in Kelantan. As for children, a survey by Southeast Asia Nutrition Survey (SEANUTS), stated that 50% of the children are not consuming the right amount of calcium which mean that they either consume more or less calcium in their diet. However, there is limited national data regarding calcium deficiency among children in Malaysia.

An adequate intake of calcium has been demonstrated to reduce the risk of chronic diseases such as osteoporosis, hypertension, colon cancer, breast cancer, kidney stones, polycystic ovary syndrome, ovarian cancer and a number of other disorders (Miller, Jarvis & McBean, 2001; Nicklas, 2003). Milk and dairy products are the best natural sources of calcium as study from Cáceres, García, Toro and Selgas (2003) showed that, 75% to 89% of all ingested calcium comes from these sources. Milk products have high calcium contents of high bioavailability and can be obtained at a low cost in relation to their nutritional value (Sara, Reyes, María & Rosaura, 2006). The Malaysian Dietary Guidelines also recommend consumer especially women to obtain adequate calcium from low-fat milk and its products and other calcium-rich food sources to prevent calcium deficiency, which can lead to osteoporosis among pre- and postmenopausal women (Malaysian Dietary Guidelines, 2010). Predicted upon that, there is an increasing trend of calcium-fortified foods especially in milk and milk products in Malaysia.

The deficiency in calcium can also be due to increasing trend of vegetarianism and rising demand for vegetarian products particularly among practicing Chinese and Indian vegetarian communities in Malaysia (Gan, Boo, Seik et al, 2018). It has been reported that vegetarians may have lower intakes in micronutrients especially in calcium as they have higher consumption of plant-based products and poorly planned vegetarian diet. A study by Sousa and Bolanz (2017), found that non-dairy plant-based beverages have much less calcium than cow's milk. The authors also stated that not all calcium that is present in food is absorbed by the body. The bioavailability of calcium depends on the types of food. For example, 30% to 32% of the calcium naturally present in dairy products is absorbed, compared to 20% to 30% of the calcium from plant sources such as almonds and beans (Wardlaw & Byrd-Brednner, 2013). Hence, plant-based products need to be fortified with calcium in order to meet the requirement intake of calcium for people who practice vegetarian or vegan diet. This is also the reason of the increasing trend of calcium fortified products, in this case, in plant-based milk.

Besides that, the deficiency of calcium is also due to misconception about calcium content in plant-based and animal-based milk. This is because a study conducted by Haas et al. (2019) stated that, in the product image analysis, both consumer groups rated cow and soy milk as rich in minerals. However, soy milk or other plant-based products are only rich in calcium if the products are additionally fortified. Besides, it shows the lack of awareness among consumer on the importance of reading food label before purchasing or consuming any products. For example, consumers are not aware on the instructions on the packaging which require them to shake before consuming it. This is an important step and if the consumers do not follow the instructions, the added nutrients will remain as residue at the bottom of the box, hence, they will not receive enough nutrient that they are supposed to get.

There is very limited study about the calcium content in milk especially calcium fortified milk in Malaysia. Even though, there have been many studies on the potential health benefits of components in plant-based milk, there are no studies have compared plant-based milk with cow's milk from the perspective of whether or not calcium content in plant-based milk has similar amount to the calcium content in cow's milk. Moreover, there is also limited study or research about the calcium content in milk especially calcium fortified milk before and after being shaken, even though, certain milk products have stated in the instruction label to shake prior drink but vigorous shaking may not be enough to resuspend the calcium salt added in the calcium fortified milk. Hence, this study aims to determine the calcium content in calcium fortified milk in both plant-based and animal-based milk and to compare calcium content in calcium fortified milk before and after being shake.

1.3 Significance of the study

There are limited studies conducted to measure the amount of calcium that is being fortified in the calcium fortified milk, as well as to compare the calcium fortified milk between plant-based milk and animal-based milk in Malaysia. Therefore, in this study, several plant-based milk and animal-based milk that are fortified with calcium is screened for calcium content and a comparison will be made between these two sources of calcium fortified milk in order to determine which one contains the actual or highest amount of calcium that has been claimed on the packaging. Such information can urge the food manufacturer to emphasize the instruction on the product packaging. Moreover, information from this study can also correct the misconception regarding the calcium content on the animal-based and plant-based milk.

Besides that, this study can also help in raising awareness among consumers on reading food label before purchasing or consuming any food products. Nowadays, consumers have tended toward a plant-based diet because of varied reasons such as an aversion to animal cruelty, a desire for a healthy lifestyle and environmental awareness (Janssen, Busch, Rodiger & Hamm, 2016); Sebastiani et al., 2019). However, they do not aware of the instruction on the packaging on how to consume it thus, they actually do not get the actual amount of nutrient that they have paid for. Therefore, this study is conducted to see whether or not the amount of calcium in the calcium fortified milk have a difference between before and after the milk being shake. Hence, the information that will be gather in this study can help consumer to be more alert and aware in choosing and consuming their food products in order to get adequate amount of nutrient that is required for them.

Lastly, information regarding the comparison between plant-based milk that is being fortified with calcium and the fresh cow's milk can also be significant to consumer especially pre and postmenopausal women who are in need to consume adequate amount of calcium in their diet due to prevent certain chronic diseases such as osteoporosis. This is because according to Miller, Jarvis and McBean (2001), adequate intake of calcium has been demonstrated to reduce the risk of chronic diseases such as osteoporosis, hypertension, colon cancer, breast cancer, kidney stones, polycystic ovary syndrome, ovarian cancer and a number of other disorders. Hence, this information can also be beneficial to help more future investigations to persuade baseline data regarding calcium content in other calcium fortified products.

1.4 Objectives

1.4.1 General Objective

To study the calcium content in selected calcium fortified milk.

1.4.2 Specific Objectives

1. To determine the calcium content in calcium fortified milk in plant-based and animal-based milk.
2. To compare the calcium content in calcium fortified milk in plant-based and animal-based milk.
3. To compare the calcium content in calcium fortified milk in plant-based and animal-based milk before and after shake.

1.5 Hypothesis

1. There is a significant difference in the calcium content between calcium fortified milk in plant-based and animal-based milk.
2. There is a significant difference in the calcium content between calcium fortified milk in plant-based and animal-based milk before and after being shake.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk

Milk is a nutrient-rich, opaque white fluid secreted by the mammary glands of mammals. It is the primary source of nutrition for infant mammals (including humans who are breastfed) before they are able to digest or consume other types of food. Based on Online Etymology Dictionary, the term "milk" comes from "Old English *meoluc* (West Saxon), *milc* (Anglian), from Proto-Germanic **meluk* "milk" (source also of Old Norse *mjolk*, Old Frisian *melok*, Old Saxon *milk*, Dutch *melk*, Old High German *miluh*, German *Milch*, Gothic *miluks)* (Douglas Harper, 2001). According to the General Standard for the Use of Dairy Terms (1961), the term *milk* has been defined under Codex Alimentarius standards as "the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing. Other than that, as an agricultural product, the term *dairy* relates to animal milk and animal milk production.

According to Godden (2008), dairy milk is produced by the mammary glands of healthy mammals and it is the primary source of nutrition to nourish their young after birth and excludes secretions made between 15 days before and 5 days after parturition or until the milk is free of colostrum. Milk has been found to be the only food that has adapted to the nutritional needs of new born containing all the nutrient required to support the growth and development (Fulgoni, Keast, Auestea & Quann, 2011; Hauq, Hostmark & Harstad, 2007; Pereira, 2014). Furthermore, milk contains several properties that can enhance the absorption and bioavailability of nutrients (Wijesinha-Bettoni & Burlingame, 2013). Casein is one of the proteins present in dairy milk as calcium salt and calcium caseinate. Moreover, a mixture of alpha, beta and kappa caseins to form a cluster called micelle, where it can help to coagulate and gel when exposed to the acidic environment of the stomach, subsequently slow the digestion process and provides satiety and more time to efficiently digest the nutrients in milk (Lambers et al., 2013). These micelles are responsible for the white opaque appearance of milk.

According to The Malaysian Food Regulation (1985), in the Regulation 82, “milk”, “raw milk” or “fresh milk” means the normal, clean, fresh mammary section of healthy cow, buffalo, goat or sheep that is properly fed and kept, excluding that obtained during the four days immediately following calving. It also stated that, milk shall contain not less than 3.25% of milk fat and 8.5% of non-fat milk solids and shall not contain any added water, permitted food additive, other added substances or trace of antibiotics substance. It means that milk can be obtained from many different sources such as goats, cattle, buffalos and camels; yet the cow milk is the most popular. According to Gerosa and Skoet (2012), the buffalo, goat, sheep and camel respectively produced about 11%, 2%, 1.4% and 0.2% of all milk worldwide in 2011. In fact, India is the world’s largest producer of milk and is the leading exporter of skimmed milk powder, yet it exports few other milk products, followed by United States, China, Pakistan and Brazil (IFCN, Dairy Report, 2014). Besides that, New Zealand, United States and European Union are the major exporters of milk products while China and Russia are the world's largest importer of milk.

There are also non-dairy milks that preclude non-animal products which resembles dairy milk in colour and texture, such as almond milk, coconut milk, rice milk and soy milk. In English, the word "milk" has been used to refer to "milk-like plant juices" since 1200 AD (Douglas Harper, 2018). Traditionally, a variety of non-dairy products have been described with the word "milk". Besides that, latex, the complex inedible emulsion that exudes from the stems of certain plants is generally described as milky and is often sold as "rubber milk" because its white appearances. According to Rauf and Monika (2014), the word latex itself is deducted from the Spanish word for milk. However, the definition of milk has come under scrutiny in recent years, as the presence of many different plant-based milk-like beverages on the market have opted to use the term 'milk' to describe the product formulated to imitate and replace bovine milk as an ingredient, beverage and source of nutrition.

2.1.1 Milk consumption

Milk has been one of the most complete of foods, containing almost all the important nutrients for optimal growth. However, research shows that there is low milk consumption among Malaysians, in both adults and children. A few studies have examined Malaysian households' dairy consumption patterns (Bobalian Hendijani & AbKarin, 2010; Norimah et al., 2008; Prescott el al., 2002). According to the Malaysian Adults Nutrition Survey conducted in 2003, milk or dairy products consumption among Malaysians was said to be only 0.14 serving per day which is equivalent to one fifth of a glass of milk compared to the recommendation of one to two glasses per day. The study revealed that adults aged 50-59 were the most frequent consumers of full cream milk and only 15 % of consumers under age 20 consumed milk daily (Norimah et al., 2008). Women were more likely to consume full cream milk daily while men were more likely to prefer and consume less-healthy sweetened condensed milk daily. Norimah et al. (2008) suggest that this difference is likely due to women being more knowledgeable than men about potential health benefits of consuming milk.

The consumption of milk from domesticated animals can be linked all the way back to 7th millennium BC in northwestern Anatolia (Evershed et al., 2008). Since then, the consumption of milk and its use as a food ingredient, especially bovine milk, and proliferation has arisen in every popluated continent, playing a crucial role in the nutrition of humans of all ages (Evershed et al., 2008; Wijesinha-Bettoni & Burlingame, 2013). Today, the dietary role of dairy and dairy-based foods has been well established by the Food and Agriculture Organization (FAO) and the other reputable government and academic groups in countries around the world (Wijesinha-Bettoni & Burlingame, 2013). In the western countries, milk has been widely consumed around the world for hundreds of centuries and is a crucial source of protein. Researchers have also shown that consumption of milk can help human body by providing wide range of host defense protein (Hettinga et al., 2011; van Neerven et al., 2012). The host defense proteins in the milk of different mammalian species are expected to reveal signatures of evolution. For example, proteins are a major contributor to host defense components in milk (German et al., 2002). A study by Hettinga et al. (2011), studied on the difference in the host defense proteome of human and bovine milk and they found out that a number of antimicrobial proteins were abundant in bovine milk compared to human milk. In humans, a positive relation between breastfeeding and health of the babies has been noted from the time of the first recorded use of human-milk substitutes (Newburg, 2001).

2.1.2 Plant-based milk consumption among vegetarian and nonvegetarian

Vegetarian diets defined as being devoid of flesh floods such as meat, poultry, wild game, seafood and their products (Leitzmann, 2014). The author also said that vegetarian diets are followed by a growing number of people worldwide because of ethical, health and environmental reasons. There are variations of vegetarian diets which are ovo-lacto vegetarianism, ovo vegetarianism, lacto vegetarianism and vegan diet. Vegetarian diets include a variety of plant-based foods such as grains, legumes, nuts and seeds, fruits and vegetables and for lacto-ovo-vegetarians they may or may not include dairy products and eggs while vegan diets exclude all animal foods. The prevalence of vegetarianism varies widely around the globe. According to Agrawal et al. and Shrindhar et al. (2014), India has the highest proportion of vegetarians, with about 30% of the population following a vegetarian diet. In South Asia, the prevalence of vegetarian was 33% (Jaacks et al., 2016) yet there is no published national data showing the percentage of vegetarians among Malaysians. However, there is an increased trend of vegetarianism and a rising demand of vegetarian food products among practicing Chinese and Indian vegetarian communities in Malaysia (Gan et al., 2018).

Many studies have assessed the nutritional adequacy of vegetarian diets. Overall, these have shown that well-planned vegetarian or vegan diet can supply all the nutrients required for good health (Craig & Mangels, 2009). Vegetarians have been reported to have higher consumption of plant foods; the antioxidant components in vegetables and fruits are the most well know factors that alter the risk of chronic diseases (Szeto et al., 2004). Thus, some concern remains over the possibility of low intakes of some nutrients such as vitamin B12, vitamin D, calcium and omega 3 fatty acids in poorly selected and or unfortified vegetarian or vegan diets (Pawlak, Lester & Babatunde, 2014; Tucker, 2014). Thus, vegetarian or vegan usually consume food fortified with nutrients, in this case is calcium. However, the use of calcium-fortified food does not solve the underlying problem of inadequate dietary patterns of food selection generally low calcium intakes (Miller & Anderson, 2001). Therefore, it is important for individuals who choose to meet their calcium intake needs through calcium-fortified foods to ensure that their needs for other nutrients supplied by foods such as dairy foods, are also met (Miller, 2001).

Besides that, numerous studies have shown that strict-vegetarians who avoid consumption of dairy products in their early age tend to have lower intake of calcium and they are at higher risk of osteoporotic fractures in elderly (Lin, 2008; Larson and Johansson, 2006). According to Léon, MsScAgr & Alain (2013), most of the dietary calcium (70%) comes from milk and dairy products, thus to those who avoid consumption of milk and dairy products tend to have lower intake of calcium. However, it was encouraging to find that the Malaysian strict-vegetarians succeed in meeting their recommended intake especially calcium. This was further proven in local studies done by Lin who conducted a food frequency questionnaire among vegetarians in Klang (Lin, 2008). The result obtained agreed with the statement that Malaysia vegetarians frequently consumed milk and soy products which are rich in calcium. But a local study done by Wong et al. (2013) proven that, only a quarter of the ovolacto-vegetarians and strict-vegetarians achieved Recommended Nutrient Intake (RNI) for

Malaysia for calcium (22.86 and 25.71% respectively). Female ovolacto-vegetarians were also noted to have insufficient intake of calcium (Wong et al., 2013).

Recently, plant-based or non-dairy milk alternative is the fast-growing segment in newer food product development category of functional and specialty beverage across the globe (Swati, Tyaqi & Rahul, 2016). This is due to cow milk allergy, lactose intolerance, calorie concern, prevalence of hypercholesterolemia as well as increase number of vegetarians thus more preference to vegan diets has influenced consumers towards choosing plant-based milk (Swati, Tyaqi & Rahul, 2016). There has been an expansion of milk alternative beverages originating from plant-based sources including soy, oat, hemp, coconut, rice and nut, referred to as milk. Plant-based milk is opted as milk substitute to recover some nutritional deficiency however some plant-based milk has low amount of calcium. When comparing the calcium content of the beverages, a review by Sebastian, Chloe & Benjamin (2018), showed that the nutrient composition of milk and plant-based milk, especially the calcium content of plant-based milk was highly variable; although when fortified, often contained higher total calcium amounts compared to the bovine milk. The review found out that one serving of 2% bovine milk contains 120 mg of calcium, while plant-based milk when fortified had higher levels of calcium than bovine milk. However, they also stated that unfortified milk and milk alternatives had drastically lower level of calcium ranging from 0 to 12 mg per 100 g whereas fortified beverages had calcium contents ranging from 43 to 197 mg per 100 g, with most fortified to levels around 185 mg per 100 mL (Sebastian, Chloe & Benjamin, 2018). Thus, vegetarianism must ensure that they get adequate calcium intake by choosing the right milk and dairy product even though it is non-dairy products.

However, for the non-vegetarian or non-vegan, the increased availability and use of calcium-fortified foods raises several concerns, including the potential for calcium toxicity, lower calcium absorbability than expected and inadequate intakes of other essential nutrients. This is due to excess amount of calcium that is consume by individual which is more than the requirement. In 1997, the Institutes of Medicine (IOM) had established a tolerable upper level (UL) of 2500 mg per day for all age groups for calcium. Calcium intakes in excess of this amount can potentially increase risk for milk-alkali syndrome, aggravate kidney stone formation in stone-formers who have a higher levels of calcium absorption from the intestine and inhibit the body's absorption of iron and zinc (Dietary Reference Intakes for Calcium, 2011). Although calcium toxicity is rare, excessive use of calcium-fortified foods, especially by individual who are already meeting their calcium needs, is a possibility. (Dietary Reference Intakes for Calcium, 2011). To avoid calcium toxicity, calcium should be added to fortified foods in small amount. Also, individuals should base their decision on whether or not to use calcium-fortified foods on their current dietary calcium intake, which can be assessed by examining the Nutrition Facts labels on the foods and beverages.

2.2 Calcium

Calcium is a key nutrient in the human body. The primary emphasis on calcium consumption during its initial scientific discovery was focused on early human life primarily during growth periods of infancy and childhood. According to the Recommended Nutrient Intakes for Malaysia (RNI), calcium is the fifth most abundant element in the human body, with approximately 1000 g present in adults. It plays a vital role in skeletal mineralization, as well as a wide range of biological function, which includes a "structural role" providing rigidity such as structure and strength to the skeleton. Besides, calcium also required for a number of basic regulatory functions including contraction and relaxation of muscle, coagulation of blood, transmission of nerve impulses, activation of enzyme reactions, and stimulation of hormone secretions and integrity of intracellular cement substances (Food and Nutrition Board, 1997). Therefore, calcium is vital for bone health.

Milk, yoghurt and cheese are rich natural sources of calcium and are the major food contributors of this nutrient to people in the United States (Dietary Reference Intakes, 2010). According to U.S. Department of Agriculture (USDA) (2019), most grains and plant-based products do not have high amounts of calcium unless they are fortified; however, they contribute calcium to the diet because they contain small amounts of calcium and people consume them frequently. However, currently, food manufacturers in Malaysia have also made available in the market calcium fortified products such as high-calcium milk, yoghurt, breakfast cereals, biscuits and even rice.

Both scientist and the general public are becoming increasingly aware of the importance of dietary calcium. This is largely due to many research studies that have demonstrated links between dietary calcium intake and diseases such as osteoporosis, arterial hypertension and colon cancer (National Osteoporosis Foundation, 2011; Baron & Paganelli, 1999; National Institutes of Health, 2016). Calcium requirements are the best derived from balance studies, which is a careful measurement of calcium absorbed and calcium losses across a range of calcium intakes. The intakes which provides just enough absorbed calcium to meet losses (zero balance) is then derived and set as the mean calcium requirement of an adult while in children, adolescence and pregnancy, the factorial approach is used to estimate calcium requirement because these groups need to be in positive calcium balance (Recommended Nutrient Intake, 2005). According to Recommended Nutrient Intake (RNI) for Malaysia (2005), the average recommended nutrient intakes of calcium is about 850 mg per day (700 to 1000 mg, depending on the age) for children, rising to 1300 mg per day for adolescents, 1100 mg per day (1000 to 1200 mg, depending on the age) for adult, and 1200 mg per day (1000 to 1300 mg, depending on age) for pregnancy and lactation. These RNIs are safety levels designed to provide adults with maximum protection against a negative calcium balance and hence, against bone loss.

According to Dietary Reference Intake for Calcium and Vitamin D (2011), indicators of dietary calcium intakes include calcium balance for example, calcium accretion, retention and loss, which can be measured using stable isotopes techniques. However, bone mineral density can be considered as surrogate marker of fracture risk and calcium status (Dietary Reference for Calcium and Vitamin D, 2011). Low calcium intake, poor calcium absorption and excessive losses contribute to reduced mineralization of bone (Judith, 2015). Calcium deficiency has been associated with increased risk of osteoporosis, hypertension, colon cancer, kidney stones and lead absorption (National Institutes of Health, 2016). The major population groups that are at highest risk of dietary calcium deficiency include women with amenorrhoeic, the female athlete triad and postmenopausal women, individuals with milk allergy or lactose intolerance, vegetarians and at-risk groups for dietary deficiency intake such as adolescents and the elderly (Judith, 2015). At particular risk are female adolescents when bone formation and growth is most crucial. Later in the life cycle, women continue to be at highest risk and this risk is elevated if early baseline bone is not strong during adolescence. Several studies have shown that women who have been diagnosed eating disorders or exhibit physical hyperactivity with female athlete triad syndrome are shown to be at high risk for calcium deficiency, as for the postmenopausal women, hormonal changes may affect bone mineralization processes, which has also been widely studied for calcium deficiency risk (Velde, Brouwers, Geusens, Lem & Bergh, 2014; Bonjour, Kraenzlin, Levasseur, Warren & Whiting, 2013). Individuals with milk allergy or lactose intolerance often exhibit calcium deficiency due to the dietary restriction of calcium-containing foods (Dietary Reference Intakes for Vitamin D and Calcium, 2011).

Chronic calcium deficiency due to low intake or poor intestinal absorption is one of the causes of reduced bone mass and osteoporosis (National Osteoporosis Foundation, 2011). The greater the peak bone mass, the longer one can delay serious bone loss with increasing age thus, everyone should therefore consume adequate amounts of calcium and vitamin D throughout childhood, adolescence and early adulthood (National Institutes of Health (US), 2013). It is known that osteoporosis is most associated with fractures of hip, vertebrae, wrist, pelvis, ribs and other bones (National Institutes of Health, 2000). It is estimated 1.5 million fractures occur each year in United States due to osteoporosis (National Osteoporosis Foundation, 2011). In Malaysia the information on the incidence of hip fractures is limited. However, the data in 2007 showed the incidence of hip fractures in Malaysia ranged from 10/100,000 population from age 50 years old to 510/100,000 populations at age above 75 years old with a rapid increase at age 65 years and above. The report also showed that the Chinese had the highest incidence of hip fractures compared to the Malays and Indians (Lee & Khir, 2007).

2.2.1 Calcium absorption

Efficient dietary intake and absorption of calcium is essential to maintain healthy body stores. The absorption occurs mostly in the duodenum and the jejunum (Gueguen

& Pointillart, 2000). Absorption is the result of two processes; active transport across cells, mainly in the duodenum and the upper jejunum and passive diffusion, which occurs throughout the small intestine, but mainly in the ileum and very little in the large intestine (Bronner, 2009). Typically, at normal calcium intake, 1-25$(OH)^2$D dependent transport accounts for the majority of absorption, whereas as little as 8 to 23% of overall calcium absorption is by passive diffusion (McCormick, 2002). The rate of paracellular calcium uptake is considered non-saturable, while transcellular transport can be upregulated under conditions of low calcium intake (Beto, 2015). Recently evidence by Christakos (2012) suggests that paracellular calcium transport is also regulated, at least in part, by 1-25$(OH)^2$ vitamin D. Since a concentration gradient is not a prerequisite for this process, transcellular transport accounts for most of the absorption of calcium at low and moderate intake levels. The serum calcium level and the 24-h calcium excretion can be measured accurately in clinical practice but, the actual quantity of calcium that is absorbed by the bones is much more difficult to measure. This requires the use of 'bio-markers', absorption of calcium isotopes, or treatment bone densitometry.

Calcium absorption also depends on the salt for fortification as well as the food matrix (Rafferty et al., 2007). A study by Heaney et al. (2000) and Zhao et al. (2005), compare between cow's milk and soy milk fortified with tricalcium phosphate revealed a 75% absorption in soy milk compared to cow's milk, while no differences have been observed when calcium carbonate was used. Ionic calcium precipitates soy proteins especially when subjected to thermal treatments, which may influence the calcium content of the beverage consumed (Pathomrungsiyounggul et al., 2010). Indeed, 82% to 89% of the calcium in soy and rice milks, respectively are separately by centrifugation at 3740 *g*, whereas the value for cow's milk is 11%, which may indicate a decrease in the calcium content of a beverage that is not properly shaken before use (Heaney et al., 2005). Despite these shortcomings, fortified plant milk substitutes may be a valuable source of calcium for individuals with medical conditions that prevent the consumption of dairy products and offering soy milk as an option in elementary schools has been reported to increase the selection of a calcium rich beverage slightly (Reilly et al., 2006).

2.2.2 Fortified milk Act (Malaysian Food Act 1983 and Food Regulations 1985)

According to the Malaysia Food Act 1983 and Food Regulations 1985, milk products shall be any products prepared from milk and includes the food for which a standard is prescribed in regulations 84 to 87 and regulations 89 to 116. In the Regulations 26 (1) stated that the “added nutrient” includes any mineral, vitamin, amino acid, fatty acid, nucleotide or other food components which, when added singly or in combination to food, improves the nutritional value of the food. The added nutrients specified in Figure 1 to the Twelfth Schedule or with prior written approval of the Director shall be the permitted added nutrient within the meaning of and for the purposes of these Regulations. The Regulations 26 (7) also stated that no label on a package containing any food shall bear a claim that such food is enriched, fortified, vitaminized, supplemented or strengthened, or shall contain any statement that may or is likely to convey the same meaning, unless a reference to the quantity of the food as specified in column (1) of Figure 1 provided not less than the amount of vitamin or mineral permitted. For example, calcium can only be fortified in any condensed milk not less than 190 mg. Moreover, in the same Regulations (10) stated about the food shall contain vitamin and mineral in an amount which exceeds the amount that has been specified in Figure 2. For calcium, it should not exceed 1.4 g which is the maximum amount in recommended daily serving.

NUTRIENT SUPPLEMENT																
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
Food	*Vitamin A, Vitamin A alcohol and esters, carotene (I.U. of Vitamin A)**	*Vitamin B_1, thiamine, thiamine hydrochloride, thiamine mononirate (milligrams of thiamine)*	*Vitamin B_2, riboflavin (milligrams of riboflavin)*	*Vitamin B_6, pyridoxine, pyridoxal, pyridoxiamine (milligrams of pyridoxamine)*	*Biotin (micrograms of biotin)*	*Pantothenic acid, pantothenyl alcohol (milligrams of pantothenic acid)*	*Niacin, niacinamide, nicotinic acid, nicotinamide (milligrams of niacin)*	*Vitamin C, ascorbic acid (milligrams of ascorbic acid)*	*Vitamin D, vitamin D_2, vitamin D_3 of (I.U. of vitamin D)**	*Vitamin E, alphatocopherol (I.U. of vitamin E)**	*Calcium (milligrams of of calcium)*	*Iodine (micrograms of iodine)*	*Iron (milligrams of iron)*	*Phosphorus (milligrams of phosphorus)*	*Folic acid (micrograms of folic acid)*	*Vitamin B_{12} (micrograms of vitamin B_{12})*
Reference Quantity: 100 grams																
Bread … … … … … … … … … …	500	0.21	0.33	0.42	40	1.46	2.3	6	83	4.2	150	20	2.1	150	8	0.3
Breakfast cereal (as purchased) … … … …	2,000	0.83	1.33	1.67	165	5.83	9.2	25	333	16.7	580	85	0.3	580	32	1.2
Condensed milk – sweetened and unsweetened; filled milk and condensed filled milk – sweetened and unsweetened	670	0.82	0.44	0.56	55	1.94	3.1	8	111	5.6	190	30	2.8	190	11	0.4
Dried milk powder (Full cream or skimmed) … …	2,000	0.83	1.33	1.67	165	5.83	9.2	25	333	16.7	580	85	4.3	580	32	1.2
Extract of meat or vegetable or yeast (modified or not)	12,000	5.00	8.00	10.00	1,000	35.00	55.00	150	2,000	100.0	3,500	500	50.0	3,500	192	7.2
Flour (wheat) … … … … … … … …	1,000	0.42	0.67	0.83	85	2.92	4.6	13	167	8.3	290	40	4.2	290	16	0.6
Malted milk powder … … … … … … …	4,000	1.67	2.67	3.33	335	11.67	18.3	50	667	33.3	1,170	165	16.7	1,170	64	2.4
Other solid food not specified above excluding canned food for infants and children and cereal based food for infants and children	1,000	0.42	0.67	0.83	85	2.92	4.6	13	167	8.3	290	40	4.2	290	16	0.6
Reference Quantity: 100 millilitres																
Liquid food including vegetable juice, fruit juice, fruit juice concentrate, fruit syrup, flavoured syrup (diluted according to directions)	600	0.25	0.40	0.50	50	1.75	2.8	8	100	5.0	180	25	2.5	180	9.6	0.4

NOTE : In places where the symbol "*" appears, it means that the substance may be expressed in milligrams or micrograms using the following conversion factor:
(a) In column (2) 1 I.U. Vitamin A is equivalent to 0.3 micrograms Vitamin A alcohol (retinol);
(b) In column (10) 1 I.U. Vitamin D is equivalent to 0.025 micrograms Vitamin D_2/Vitamin D_3; and
(c) In column (11) 1 I.U. Vitamin E is equivalent to milligram dl-alphatocopheryl acetate.

Figure 1: Minimum amount of vitamin and mineral permitted (Food Regulation, 1985)

Food shall not contain any of the added nutrient specified in column (1) of the Table below in excess of the amount specified against it in column (2) of the said Table.

(1) *Added Nutrient*	*(2)* *Maximum amount in recommended daily serving*
Vitamin A	5,000 I.U.
Thiamine	2.2 milligram
Riboflavin	3.2 milligram
Pyridoxine	4 milligrams
Biotin	400 micrograms
Pantothetic acid	14 milligrams
Niacin	22 milligrams
Ascorbic acid	100 milligrams
Vitamin D	800 I.U.
Vitamin E	50 I.U.
Calcium	1.4 grams
Iodine	200 micrograms
Iron	20 milligrams
Phosphorus	1.4 grams
Folic acid	400 micrograms
Vitamin B_{12}	4 micrograms

Figure 2: Maximum amount for added nutrient (Food Regulation, 1985)

On the other hand, studies have shown that calcium fortified foods are likely to play an increasingly important role in helping consumers achieve newer calcium requirements aimed at reducing the risk of diseases (Weaver, Proulx & Heaney, 2000; Bryant, Cadogan & Weaver, 2001). In fact, the fortificant of choice should be well absorbed and beneficial in building peak bone mass during growth and protecting against bone loss later in life. Example of calcium salts suitable for use as food fortificants are listed in Figure 3 below.

Compound	Calcium content (%)	Colour	Taste	Odour	Solubility (mmol/l)
Carbonate	40	Colourless	Soapy, lemony	Odourless	0.153
Chloride	36	Colourless	Salty, bitter	–	6712
Sulfate	29	Varies	–	–	15.3
Hydroxyapatite	40	–	–	–	0.08
Calcium phosphate dibasic	30	White	Sandy, bland	–	1.84
Calcium phosphate monobasic	17	Colourless	Sandy, bland	–	71.4
Calcium phosphate tribasic	38	White	Sandy, bland	Odourless	0.064
Calcium pyrophosphate	31	Colourless	–	–	Insoluble
Glycerophosphate	19	White	Almost tasteless	Odourless	95.2
Acetate	25	Colourless	–	–	2364
Lactate	13	White	Neutral	Almost odourless	0.13
Citrate	24	Colourless	Tart, clean	Odourless	1.49
Citrate malate	23	Colourless	–	–	80.0
Gluconate	9	White	Bland	Odourless	73.6
Hydroxide	54	Colourless	Slightly bitter	Odourless	25.0
Oxide	71	Colourless	–	–	23.3

Figure 3: Calcium fortificants (Physical characteristics) (Guidelines on Food Fortification with Micronutrient, WHO, 2000)

The range of foods that are fortified with calcium has steadily grown over the years as it became increasingly clear that intakes are low in many populations. According to the Guidelines on Food Fortification with Micronutrient (2000), the more soluble calcium salts, such as the citrate malate or the gluconate, are generally used to fortify juices and other beverages. The authors also mentioned that tribasic calcium phosphate, and sometimes calcium carbonate or lactate, is used to fortify milk, to which gums (e.g. carrageenan, guar gum) must also be added to prevent the calcium salt from sedimenting.

A study conducted by Chaiwanon et al. (2000) has shown that soy milk contains equivalent quantities of protein but has only one-fifth of calcium. In that study, calcium carbonate and tri-calcium phosphate salts were used as fortificants, among the two fortificants used, tri-calcium phosphate with the combination of sequestering and stabilizing agents, presented a satisfied suspension in fortified soybean milk with an optimum Ca:P ratio 1:3:1 in the final product. The author also found out that, calcium carbonate showed reduced calcium stability on fortification but the bioavailability was better. On the other hand, processing treatments such as flaking, blanching, hot grinding and ultra-high temperature treatment could cause the loss of nutrients especially vitamins and minerals (Chaiwanon et al., 2000). Hence, to meet nutritional requirements, enrichment of nutrients is done to restore nutrients inevitably lost during processing.

2.3 Plant-based milk and cow's milk

Globally, cow's milk has been widely consumed around the world for hundreds of centuries and acts as an important source of protein. It also acts as a wholesome complete food providing all the major nutrients like calcium, fat, carbohydrates and proteins. Furthermore, researchers have shown that the consumption of bovine milk can help the human body by providing wide range of host-defense proteins (Hettinga et al., 2011; van Neerven et al., 2012). This is because various beneficial anti-microbial effects are observed in both human and bovine milks. Especially in the case of infants, it was observed that the consumption of raw cow's milk has reduced the risk of fever and respiratory infections considerable (Loss et al., 2015). Despite the considerable advantages with the consumption of cow's milk, there are various downsides. According to an estimate, 15 % of European consumers avoid dairy products for a variety of reasons, including medical reasons such as lactose intolerance (LI), cow's milk allergy (CMA), cholesterol issues and phenylketonuria, as well as lifestyle choices like a vegetarian or vegan diet or concerns about growth hormone or antibiotic residues in cow's milk (Jago, 2011; Leatherhead Food Research, 2011).

Besides, a study by Oliver et al. (2009) has proven that the presence of various pathogens like *Salmonella* spp. and *Escherichia coli* O157:H7 in milk has been associated with the of spread disease around the world. Globally, the cow's milk allergy is one of the most wide spread allergy among infants and children (Vanga et al., 2015). According to the latest reports, 2.2-3.5% of the infants are allergic to cow's milk followed by peanuts and tree nuts (Gray et al., 2014; Sicherer & Sampson, 2014; Vanga et al., 2015). However, studies conducted on a large scale have shown that about 35% of these infants out grow their allergenic potential towards milk by the age of 5-6 years; and this may further increase to 80% by the time they reach 16 years (Gray et al., 2014; Santos et al., 2010; Skripark et al., 2007). Another common issue widely associated with consumption of cows' milk is 'lactose intolerance'. The intolerance is due to the absence or deficiency of the enzyme lactase in the digestive tract and is widely observed in 15–75% of the adults (Bahna 2002; Scrimshaw and Murray 1988). Few studies showed that 80% of people from African origin and 100% people of Asian and American Indian origin are lactose intolerant (Swagerty Jr et al. 2002). In Malaysia, the rate of lactose intolerance is extremely high among adults in Malaysia with 88% of the Malays, 91% of the Chinese and 83% of the Indians (Asmawi, Seppo, Vapaatalo & Korpela, 2006). According to Boey (2001), children in Malaysia, ages 9 to 10 years old have a high prevalence of lactase deficiency (70.8%) with a recurrent abdominal pain after an intake of 2-20 gram of lactose (10% lactose solution) (Boey, 2001).

On the other hand, plant substitutes are often perceived as healthy, possibly due to negative perceptions about the nutritional properties of cow's milk and the health claims associated with soy (Bus & Worsley, 2003; Patisaul & Jefferson, 2010). A study has shown that the nutritional properties vary greatly, as they depend strongly on the raw material, processing, fortification and the presence of other ingredients such as sweeteners and oil (Outi, Viivi, Emanuele & Elke, 2015). Based on the same study, when the authors compare the products, it is evident that only soy milk has value comparable to cow's milk with protein contents ranging from 2.9-3.7%. All the other products are very low in protein, with only quiona, hemp and oat milk containing ≥1% protein. This may pose a risk if plant milk substitutes are used to replace cow's milk without knowledge about the differences, especially when given to young children; several cases of kwashiorkor, a protein-energy malnutrition typical for areas of famine, have been reported in Western countries as a result of using rice milk (0.1-0.2% protein) as a weaning food (Calvalho et al., 2001; Katz et al., 2005). Also, milk produced of legumes other than soy, such as peanut and cowpea can have a protein content as high as 4% (Rustom et al., 1991; Tano-Debrah et al., 2005). Although plant milk substitutes are low in saturated fats and most products have caloric counts comparable to skim milk, some products contain as much energy as full milk, originating mostly from sugars and other carbohydrates.

Besides that, study by Onning et al., (2000) stated that consumption of oat milk alternative, at a beta-glucan content of 0.5 g 100 g^{-1}, has been shown to reduce LDL cholesterol, as studies have shown that beta-glucans, a soluble-fiber, have been repeatedly shown to lower LDL level when consumed at or above 2.9 g per day (Othman et al., 2011; Whitehead, Beck, Tosh & Wolever, 2014). Thus, this would make oat milk alternatives a suitable bovine milk replacement in individuals who want to control their LDL cholesterol. Plant based milk also has beneficial fat content when compared with bovine milk. According to the National Institutes of Health (2018), hemp milk alternative contains alpha linoleic acid, an essential omega-3 fatty acid at 0.4 g 100 mL^{-1}, 25 % of recommended 1.6 g per day. Nevertheless, it does not contain other essential omega-3 fatty acids such as EPA and DHA. Many studies found out that despite of high saturated fat content in coconut milk, it contains medium chain triglycerides (MCT) that have beneficial effects on HDL and LDL cholesterol, body mass, waist circumference, insulin sensitivity, energy expenditure and overall adiposity (Cardoso et al., 2015; Han et al., 2007; St-Onge & Bosarge, 2008; St-Onge, Ross, Parsons & Jones, 2003). Moreover, there is also a study that has proven the health benefits of the MCTs in coconut oil. The author of the study has examined the effects of consuming 200 mL of coconut milk, reconstituted from a commercially sourced coconut powder, for an eight-week period has shown to show a significant decrease in LDL as well as increases in HDL (Ekanayaka, Perera & De Silva, 2013). Furthermore, bovine milk generally low in vitamin E, whereas as almond milk contains vitamin E content of 6.33 mg per 100 g, 42% of the 15 mg recommended daily amount (National Institutes of Health, 2016; USDA, 2018). It has been shown that vitamin E is a fat-soluble antioxidant that can protect cells from the deleterious, cancer-promoting and CVD-promoting effect of free radical (National Institutes of Health, 2018). There are also other anti-nutrients present in common plant-based milk include lectins and saponins. Lectins are prevalent in soy, peanuts and other beans, have been shown to significantly inhibit glucose absorption in the intestine, affecting total caloric intake while saponins present in soy, oats, peas and beans have been shown repeatedly to impact the digestion of proteins, especially soy proteins by creating insoluble saponin-protein complexes that are resistant to digestion (Francis, Kerem, Makkar & Becker, 2002).

Plant-based milks in general are more expensive substitutes to cow's milk that act as the major restraint to those who cannot afford it. However, to those who are allergic to cow's milk, nutritionally they are not comparable or equivalent to cow' milk (Swati, Tyaqi & Rahul, 2016). People who consume cereal milk in place of bovine milk are more prone to nutritional inadequacy. So, in order to use plant-based milk as substitute of cow's milk, fortification with protein, essential vitamins and minerals is generally performed. Besides protein, calcium is another essential nutrient required for growth and is a limiting nutrient in cereals such as rice and oats. Therefore, to assure the consumers in order to meet their nutritional supplementation, calcium fortification is generally adopted in preparation of plant-based milk substitutes.

CHAPTER 3

METHODOLOGY

3.1 Sample selection and sampling method

There were two different types of calcium fortified milk, which were plant-based milk and animal-based milk and there were 20 different types of samples were analyzed in this study in which 13 of them were from plant-based milk and seven were from animal-based milk. The plant-based milk samples are Brand A (Almond milk), Brand B (Soy milk), Brand C (Cashew milk), Brand J (Oat milk), Brand K (Almond milk), Brand L (Coconut milk), Brand M (Unsweetened oat milk), Brand N (Unsweetened almond milk), Brand O (Coconut milk), Brand P (Almond milk), Brand Q (Oat milk), Brand R (Hazelnut milk) and Brand T (Almond milk). While for the animal-based milk samples are Brand D (Full cream milk), Brand E (Low-fat milk), Brand F (Low-fat milk), Brand G (Malt milk), Brand H (HL milk), Brand I (High calcium milk) and Brand S (Low-fat high-calcium milk). All of the samples were purchased using purposive sampling from Jaya Grocer, Bangi. The samples chosen in the market were based on the ingredient list that contain calcium in it which it showed that the milk was fortified with calcium. The fresh cow's milk was used as positive reference to compare the calcium content between calcium fortified in plant-based milk and animal-based milk. The experiments were conducted in Nutrition Laboratory in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia. The samples of each milk sample were prepared and data were recorded in triplicates based on two independent experiment.

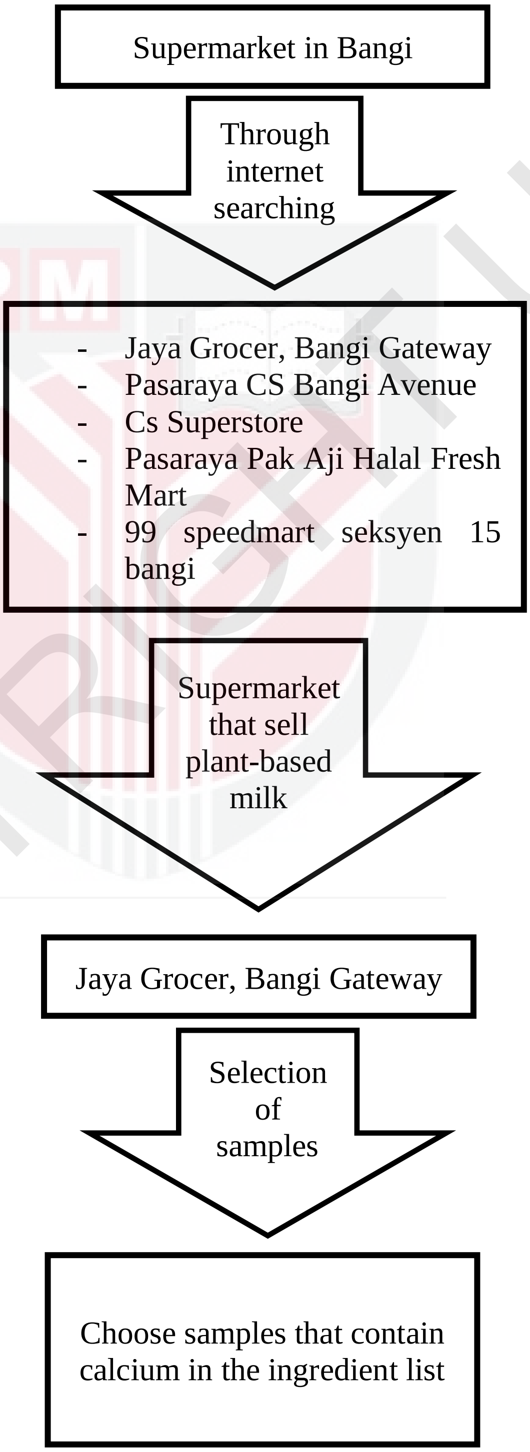


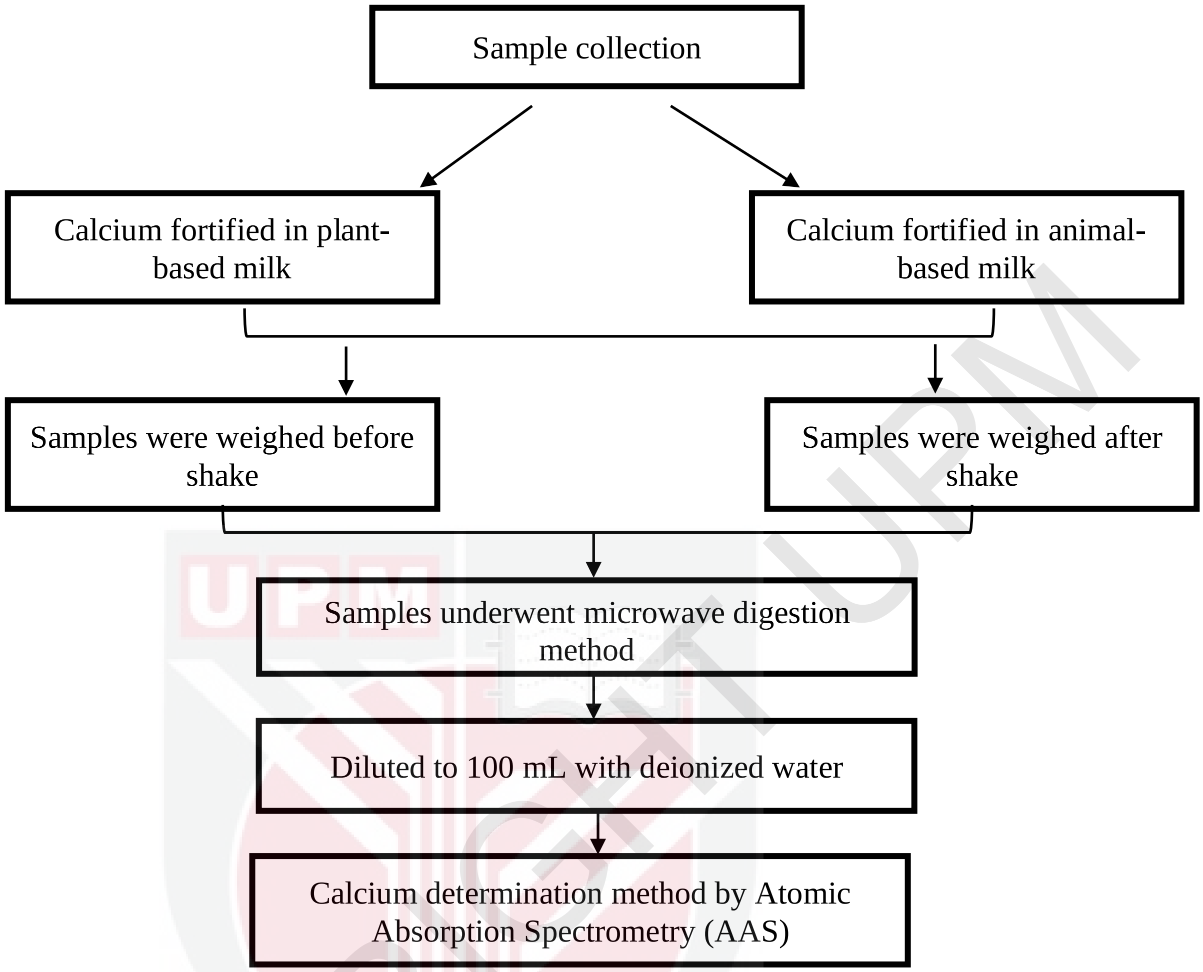
Figure 4 : Flowchart of sample selection

3.2 Reagents and chemicals

The reagents used in this experiment were 70% nitric acid (HNO) Sigma-Aldrich, 30% hydrogen peroxide (H_2O_2) Sigma-Aldrich, and deionized water.

3.3 Experimental design

For the sample preparation which was done in the laboratory, the samples were purchased in the same supermarket in Jaya Grocer, Bangi. The samples were kept in the chiller before being analyzed. The samples were weighed before and after being shake. The samples were then undergone microwave digestion method and the mixture were diluted to 100 mL with deionized water. After that, the samples were analyzed by Atomic Absorption Spectrometry (AAS) for determination of calcium content. Calibration curve was plotted with various concentration of calcium standard and calcium content in each sample was extrapolated from the standard curve.



(Association of Official Analytical Chemist, 1984; AAnalyst 400 Atomic Absorption Hardware Guide, 2004)

3.4 Determination of calcium content

Calcium content from the samples were determined by an atomic absorption spectrometry (AAS). Samples were subjected to microwave digestion before the determination of calcium. Firstly, 3.0 mL of unshaken and shaken samples were transferred to digestion vessel. For the shaken sample, all of the samples were shaken by using belly dancer shaker for 10 minutes. Next, 12 mL of 70% nitric acid and 2 mL of 30% hydrogen peroxide were added into the digestion vessel. The mixture was shaken and kept for 10 minutes before closing the vessel and the samples were then subjected to closed vessel microwave digestion for 40 minutes.

Calibration curve was plotted with various concentration of calcium and calcium content in each sample was extrapolated from the standard curve.

3.5 Statistical analysis

Data entry and analysis of all the nutrient content were carried out by using Statistical Package for the Social Sciences (SPSS) Version 24.0. The purpose of the analysis was to determine and compare the calcium content in calcium fortified plant-based and animal-based milk before and after shake. Independent sample t-test was used to compare calcium content in each of the samples and compare between calcium fortified plant-based and animal-based milk with the positive reference which was the fresh cow's milk. The accepted level of significance was set at $p<0.05$.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Calcium content in unshaken and shaken calcium fortified milk

The concentration of calcium content in calcium fortified milk was determined by using atomic absorption spectrometry (AAS). All of the samples were measured 3 ml each, before and after shake, then each of them was added with 12 ml of hydrogen peroxide (H_2O_2) and 12 ml of nitric acid (HNO_2). After that, each of the samples was subjected into closed vessel microwave digestion for 40 minutes before they were transferred to AAS for determination of calcium content. Triplicate analysis was run for all samples. Overall, there were 20 samples of calcium fortified milk from different brands had been chosen as the samples, in which the calcium content was labeled ranging from 100 to 278 mg per 100 ml calcium.

Table 1: Results of independent t-test of the calcium content of unshaken and shaken calcium fortified milk for each brand

Sample	Mean (mg/ml) (n=3)	SD	*t*-value	*p*-value
Unshaken Brand A (almond milk)	27.95	1.14	-0.383	0.747
Shaken Brand A (almond milk)	28.59	2.04		
Unshaken Brand B (soy milk)	35.77	0.70	-1.00	0.500
Shaken Brand B (soy milk)	36.36	0.00		
Unshaken Brand C (cashew milk)	27.14	1.03	-1.434	0.314

Shaken Brand C (cashew milk)	29.17	1.73		
Unshaken Brand D (full cream milk)	30.35	0.45	-5.693	0.042*
Shaken Brand D (full cream milk)	33.69	0.70		
Unshaken Brand E (low-fat milk)	32.65	1.28	-2.633	0.122
Shaken Brand E (low-fat milk)	35.77	1.08		
Unshaken Brand F (low-fat milk)	22.42	1.05	-8.866	0.057
Shaken Brand F (low-fat milk)	29.21	0.25		
Unshaken Brand G (malt milk)	25.47	0.45	-1.255	0.401
Shaken Brand G (malt milk)	26.78	1.41		
Unshaken Brand H (HL milk)	32.30	0.13	-3.074	0.194
Shaken Brand H (HL milk)	34.68	1.09		
Unshaken Brand I (High calcium milk)	51.66	0.96	-1.298	0.377
Shaken Brand I (High calcium milk)	52.61	0.38		
Unshaken Brand J (oat milk)	30.68	0.21	-4.387	0.114
Shaken Brand J (oat milk)	32.97	0.71		
Unshaken Brand K (almond milk)	33.64	0.00	-9.444	0.067
Shaken Brand K (almond milk)	35.77	0.32		
Unshaken Brand L (coconut milk)	31.92	0.13	-4.926	0.119
Shaken Brand L (coconut milk)	34.86	0.83		
Unshaken Brand M (unsweetened oat milk)	38.93	0.83	-2.231	0.268
Shaken Brand M (unsweetened oat milk)	40.23	0.00		
Unshaken Brand N (unsweetened almond milk)	29.67	0.00	0.431	0.741
Shaken Brand N (unsweetened almond milk)	29.13	1.79		
Unshaken Brand O (almond milk)	26.33	1.53	-1.297	0.338
Shaken Brand O (almond milk)	28.81	2.23		
Unshaken Brand P (coconut milk)	29.03	0.13	-3.004	0.115
Shaken Brand P (coconut milk)	29.53	0.20		

Unshaken Brand Q (oat milk)	10.05	2.71	0.774	0.569
Shaken Brand Q (oat milk)	8.52	0.65		
Unshaken Brand R (hazelnut milk)	7.28	0.00	-1.218	0.438
Shaken Brand R (hazelnut milk)	8.85	1.82		
Unshaken Brand S (low-fat high-calcium milk)	26.69	0.90	3.268	0.189
Shaken Brand S (low-fat high-calcium milk)	28.76	0.00		
Unshaken Brand T (almond milk)	17.06	0.00	-47.968	0.013*
Shaken Brand T (almond milk)	47.28	0.89		

*$p<0.05$

Independent t-test analysis was done to compare calcium content between unshaken and shaken calcium fortified milk for each brand and it was reported in Table 1. It can be seen that shaken Brand I (High calcium milk) had the highest calcium content while shaken Brand Q (oat milk) had the lowest calcium content which were 52.61 mg/ml and 8.52 mg/ml respectively. There were only two samples with significant difference ($p < 0.05$) between the unshaken and shaken calcium fortified milk whereas another 18 samples were not significantly difference ($p > 0.005$). Moreover, there were two of the samples showed inconsistent result where, the calcium content was higher in unshaken samples compared to shaken samples. Two of them were from Brand N (unsweetened almond milk) and Brand Q (oat milk) which shown that the calcium content in unshaken samples were higher than in shaken samples. This may be due to the calcium residue stick at the wall of the bottle during shaking which causing the amount of calcium content to be lower. However, overall analysis showed that the calcium content was higher for the shaken samples compared to unshaken samples.

Generally, plant-based milk has known to have lower calcium content compared to animal-based milk. Therefore, people who consume plant-based milk or who practice vegetarian or vegan diet are more prone to calcium deficiency. In order to meet the calcium requirement, food manufacturer fortified the products with calcium such as calcium carbonate and tricalcium phosphate. Although, such fortification is beneficial, the added calcium might be sedimented as calcium has low solubility. Therefore, shaking the product prior the consumption is often labelled in the food package as an instruction.

This finding was consistent with previous study by Heaney and Rafferty (2006) which reported that the calcium content in the shaken milk was higher than in unshaken milk. This might be due to the sedimentation of added calcium in calcium fortified milk. It is possible that without shaking the products prior use, the added calcium will remain as precipitate in the bottom of the container and the consumer will be not get adequate calcium from the product. Hence, it is important to emphasize on the shaking instruction in the label to the consumer in order to provide them with maximum amount of calcium provided in the milk.

Other than that, calcium is insoluble in water so it has to be in form of salt such as phosphate, carbonate and gluconate to become soluble in water. Therefore, lower calcium content in unshaken milks may be also due to the calcium salts used in the calcium fortified milk. Tricalcium phosphate and calcium carbonate were often used for fortification of milk (Guidelines on Food Fortification with Micronutrient (2000). However, these types of calcium salts have been reported to have low solubility which will result in precipitation of calcium (Chaiwanon et al., 2000). Thus, in order to prevent the calcium salts from sedimentation, gums such as carrageenan or guar gum must also be added during fortification (Guidelines on Food Fortification with Micronutrient, 2000).

4.2 Calcium content in calcium fortified milk vs in Nutritional Information Panels (NIP)

The comparison of calcium content between analyzed calcium fortified milk and Nutrition Information Panels (NIP) was reported in Table 2. The discrepancies between the calcium contents found by analysis and those stated on the label by the manufacturer deserve mention. From the table, it can be seen that Brand E (low-fat milk) had the smallest difference between the calcium content found by analysis and calcium stated on the label which was 0.2% while Brand Q (oat milk) had the large disparity with 74.25%. A recovery experiment was also done in this study in order to see the validation of the experiment. As for the recovery experiment, calcium tablet with concentration of 39 mg was analyzed and the recovery rate was calculated. According to the Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens, it stated that the recovery rate should be reproducible to within ±15% (United Nations Office on Drugs and Crime, 2009). Therefore, the calculated recovery rate was 91.2% and this showed that the discrepancies between calcium content measured and those stated on the label were not because of experimental procedure. Additionally, the analyzed calcium had been corrected based on the recovery rate 91.2%.

Table 2: The comparison of calcium content between analyzed calcium fortified milk (shake) and Nutrition Information Panels (NIP)

Sample	Analyzed Ca (mg per 100 mL)	Ca in NIP (mg per 100 ml)	% Difference
Brand A (Almond milk)	103.66	120	13.67
Brand B (Soy milk)	131.51	160	17.81
Brand C (Cashew milk)	105.79	120	11.84
Brand D (Full cream milk)	122.17	130	6.02
Brand E (Low-fat milk)	129.71	130	0.2
Brand F (Low-fat milk)	105.62	160	33.99

Brand G (Malt milk)	97.10	130	25.31
Brand H (HL milk)	125.77	150	16.15
Brand I (High calcium milk)	190.80	278	31.37
Brand J (Oat milk)	117.74	120	1.88
Brand K (Almond milk)	118.82	120	0.98
Brand L (Coconut milk)	115.55	120	3.71
Brand M (Unsweetened oat milk)	113.27	120	5.66
Brand N (Unsweetened almond milk)	105.62	120	11.98
Brand O (Coconut milk)	98.39	100	1.61
Brand P (Almond milk)	97.95	103	4.90
Brand Q (Oat milk)	30.90	120	74.25
Brand R (Hazelnut milk)	32.07	120	73.28
Brand S (Low-fat high-calcium milk)	104.31	130	19.76
Brand T (Almond milk)	158.41	160	0.99

*The analyzed Ca was corrected based on the recovery rate of 91.2%

From the table, it showed that the calcium content found by analysis and those stated on the label were not equally the same, in which the calcium content stated on the label was higher than the calcium content measured. The result was consistent with the previous study by Heaney and Rafferty (2006), which reported that shaken calcium fortified soy beverages have lower calcium content compared to the label claim. This may be because of the added calcium that remains insoluble during milk storage and this would explain the difference between the actual calcium contents found and the content stated by the manufacturer (Perales, Barbera, Lagarda & Farre, 2006). The discrepancies between calcium measured and in label have also been reported by De la Fuente et al. (2004) in skimmed milk enriched with calcium when compared with the label. More accurate results can be obtained if calcium content in the residue was measured where it can be compared with the calcium content in calcium fortified milk to see which one have the higher calcium content. As a study by Perales et al. (2006) mentioned that the calcium content of the residue was three times higher in fortified milks than in nonfortified milks thus, it may be significant if for calcium content in calcium residue was identified to see whether it can made up for what was missing from the shaken samples. However, it is not been identified in this study.

Besides that, the findings also found that eight out of twenty products showed calcium difference more than 15% between the calcium content in analyzed calcium and calcium stated on the label. All of the products used different types of added calcium. Therefore, a greater difference between calcium analyzed and calcium stated on the label might be due to sedimentation of added calcium. Several studies have found out that adding calcium salts to the milks will increased the sedimentation (Lewis, Grandison, Lin & Tsioulpas, 2011) whereas sedimentation can be markedly reduced by raising pH and/or adding calcium chelators (Gaur, Schalk & Anema, 2018). Additionally, the results found can also be due to the calcium sticks inside the package during the shaking process of the package. A study by Heaney & Rafferty (2006), stated that it was observed that the presence of the residue at the bottom of the empty cartons although, the package had been vigorously shaken. Therefore, it showed that the insolubility of added calcium still remains a problem in calcium fortified milk which lead to discrepancies between the amount of calcium analyzed in calcium fortified milk and the amount of calcium indicated on the label.

Calcium is an essential mineral for bone and teeth development, blood clotting, muscle contraction and nerve transmission. However, not all calcium that is present in food is absorbed by the body. The bioavailability of calcium (fraction that is absorbed into the body) depends on the type of food. For example, 30% to 32% of the calcium naturally present in dairy products is absorbed, compared to 20% to 30% of the calcium from plant sources, such as almonds and beans (Wardlaw & Byrd-Bredbenner, 2013). Differences in bioavailability also occur in fortified beverages. A study comparing the bioavailability of calcium citrate malate and of a combination of tricalcium phosphate and calcium lactate found that equivalent calcium contents on a nutritional label do not guarantee equivalent nutritional value (Heaney, Rafferty, Dowell & Bierman, 2005). Thus, the bioavailability of added calcium is also important to ensure maximum calcium absorption for consumer. However, the calcium bioavailability was not investigated in this study.

4.3 Calcium content in plant-based and animal-based calcium fortified milk

Table 3 shows the result of independent t-test of calcium content between plant-based and animal-based calcium fortified milk. Based from the table, it can be seen that animal-based milk contained higher calcium content compared to plant-based milk when fortified with calcium which were 34.50 mg/ml and 30 mg/ml respectively. There was no significant difference ($p > 0.05$) in calcium content between plant-based and animal-based calcium fortified milk. However, the mean difference showed very small different between plant-based and animal-based calcium fortified milk where, the slight difference may be due to calcium loss during experimental procedure or calcium sedimented at the bottom of the packaging. Additionally, the higher calcium content of calcium fortified in animal-based milk may be also due to animal-based milk itself contain high amount of calcium, therefore when it has been fortified the calcium content in the milk become higher than calcium content in plant-based milk which is known to have low calcium content.

Table 3: Results of independent t-test of calcium content between plant-based and animal-based calcium fortified

Samples	Mean (mg/ml)	SD	t-value	p-value

	(n=20)			
Plant-based calcium fortified milk	30.00	10.71	-1.363	0.181
Animal-based calcium fortified milk	34.50	8.35		

Besides that, higher calcium content in both calcium fortified in plant based and animal-based milk has shown to increase calcium level compared to fresh cow's milk as reported in Table 4. Therefore, calcium fortified milk is a recommended for those who are having calcium deficiency in which, it can help them to increase their calcium level. Several studies have shown that calcium fortified foods or beverages are likely to play an increasingly important role in helping consumers achieve their calcium requirements aimed at reducing the risk of disease (Weaver, Proulx & Heaney, 2000; Bryant, Cadogan & Weaver, 2001). Therefore, fortification of calcium in foods and beverages are crucial especially for adolescence in order for them to have strong bone structure as it is a critical stages of rapid growth and during this stages calcium is absorbed more effectively than at any other time in life. Hence, calcium deficiency can be prevented during the early life. Moreover, the Malaysian Dietary Guidelines also recommend consumer especially women to obtain adequate calcium from low-fat milk and its products and other calcium-rich food sources to prevent calcium deficiency, which can lead to osteoporosis among pre- and postmenopausal women (Malaysian Dietary Guidelines, 2010). Consequently, the range of foods or beverages that are fortified with calcium has steadily grown over the years as it became increasingly clear that intakes are low in many populations.

4.4 Calcium content in plant-based calcium fortified milk with fresh cow's milk

Table 4 shows the results of independent sample t-test of calcium content between analyzed plant-based calcium fortified milk and fresh cow's milk. It can be seen in Table 4, the highest calcium content that was analyzed in plant-based calcium fortified milk was Brand T (almond milk) and the lowest calcium content was Brand Q (oat milk) when compared to the fresh cow's milk which is 47.28 mg/ml and 8.52 mg/ml respectively. The fresh cow's milk was chosen as a positive reference to compare the calcium content in plant-based calcium fortified milk and fresh cow's milk itself. The mean calcium content for fresh cow's milk is 25.29 mg/ml with the standard deviation of 0.45. Overall, the result showed there was a significant difference ($p > 0.05$) between most of the plant-based calcium fortified milk and fresh cow's milk where, calcium content was higher in plant-based calcium fortified milk compared to fresh cow's milk.

Table 4: Results of independent sample t-test of calcium content between analyzed plant-based calcium fortified milk and fresh cow's milk

Samples	Mean (mg/ml) (n=3)	SD	t-value	p-value
Brand A (Almond milk)	28.59	2.04	2.231	0.251
Brand B (Soy milk)	36.26	0.00	38.84	0.018
Brand C (Cashew milk)	29.17	1.73	3.083	0.176
Brand J (Oat milk)	32.97	0.71	13.00	0.011
Brand K (Almond milk)	35.77	0.32	27.07	0.002
Brand L (Coconut milk)	34.86	0.83	14.32	0.013
Brand M (Unsweetened oat milk)	40.23	0.00	47.44	0.013
Brand N (Unsweetened almond milk)	29.13	1.79	2.946	0.186

Brand O (Coconut milk)	28.59	0.20	12.315	0.022
Brand P (Almond milk)	28.81	2.23	2.188	0.258
Brand Q (Oat milk)	8.52	0.65	-30.07	0.002
Brand R (Hazelnut milk)	8.85	1.82	-12.43	0.039
Brand T (Almond milk)	47.28	0.89	31.23	0.005

*The mean calcium content for fresh cow's milk is 25.29 mg/ml with the standard deviation of 0.45

This result was consistent with a study by Sebastian, Chloe & Benjamin (2018), which reported that calcium content of milk and plant-based milk, although when fortified, often contained higher total calcium amounts compared to the bovine milk. The same study also found out that one serving of 2% bovine milk contains 120 mg of calcium, while plant-based milk when fortified had higher levels of calcium than bovine milk (Sebastian, Chloe & Benjamin, 2018). Another study by Heaney et al. (2000) and Zhao et al. (2005), compare between cow's milk and soy milk fortified with tricalcium phosphate revealed a 75% absorption in soy milk compared to cow's milk, while no differences have been observed when calcium carbonate was used. However, the finding also showed inconsistent result for Brand A (almond milk), Brand C (cashew milk), Brand N (unsweetened almond milk) and Brand P (almond milk) here, it showed statistically no significant different between plant-based calcium fortified milk and fresh cow's milk. There was also a small difference found between the amount calcium measured and calcium stated on the label in which, the amount of calcium analyzed was only 110.99 mg per 100 ml while in the label it stated that the calcium content was 120 mg per 100 ml with 7.5% difference.

The findings also showed that Brand Q (oat milk) had the lowest calcium content when compared to cow's fresh milk. This may be due to the shaking process during the sample preparation. All of the samples were shaken using the belly dancer vortex for 10 minutes with 100 ppm to ensure the calcium sediment at the bottom of the package mix well with the milk before the samples were subjected into the microwave digestion. However, the results still showed lower amount of calcium content after being shake. Furthermore, it may also be attributed to calcium availability of the added calcium used during fortification. A study by Chaiwanon et al. (2000), reported that calcium carbonate exhibited higher calcium availability than tricalcium phosphate fortified soybean milk and cow's milk although, it showed less calcium stability. Since calcium bioavailability is the critical factors in choosing calcium salts to be used in fortification, calcium carbonate could be considered as the calcium fortifying agent (Chaiwanon et al., 2000). Moreover, the addition of phosphate salts has been reported to increase the phosphorus content, which has been associated with the calcium salts failing to stay in solution (Fuente, Belloque & Juarez, 2004). Therefore, it is crucial for the manufacturer to carefully choose added calcium to be used in fortification in order to produce nutritionally useful calcium content for consumer hence, produce comparable calcium content with cow's fresh milk.

Based on the findings of this study, it showed that plant-based calcium fortified milk can be an alternative source of calcium especially for those who are vegetarian or vegan. Plant-based milk has also showed a lot of benefit. A study by Onning et al. (2000) mentioned that, the consumption of oat milk has been shown to reduce LDL cholesterol, as studies have shown that beta-glucans, a soluble-fiber, have been repeatedly shown to lower LDL level when consumed at or above 2.9 g per day (Othman et al., 2011; Whitehead, Beck, Tosh & Wolever, 2014). Hence, this would make oat milk, a suitable bovine milk replacement in individuals who want to control their LDL cholesterol. Additionally, several studies also found out that despite of high saturated fat content in coconut milk, it contains medium chain triglycerides (MCT) that have beneficial effects on HDL and LDL cholesterol, body mass, waist circumference, insulin sensitivity, energy expenditure and overall adiposity (Cardoso et al., 2015; Han et al., 2007; St-Onge & Bosarge, 2008; St-Onge, Ross, Parsons & Jones, 2003). Furthermore, bovine milk generally low in vitamin E, whereas as almond milk contains vitamin E content of 6.33 mg per 100 ml, 42% of the 15 mg recommended daily amount (National Institutes of Health, 2016; USDA, 2018). It has been shown that vitamin E is a fat-soluble antioxidant that can protect cells from the harmful, cancer-promoting and cardiovascular disease-promoting effect of free radical (National Institutes of Health, 2018). Thus, plant-based milk fortified with calcium may be a valuable source of calcium for individuals with medical conditions that prevent the consumption of dairy products.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, this study found that the shaken Brand I (high calcium milk) contained the highest calcium content while shaken Brand Q (oat milk) contained the lowest calcium content which were 52.61 mg/ml and 8.52 mg/ml respectively. Furthermore, in terms of the discrepancies between the calcium contents found by analysis and those stated on the label, this study found that Brand E (low-fat milk) has the smallest difference between the calcium content found by analysis and calcium stated on the nutrition labelling which was 0.2% while Brand Q (oat milk) has the large disparity with 74.25%. The results also showed that animal-based milk contained higher calcium content which is 34.40 mg/ml compared to plant-based milk when fortified which was 30.00 mg/ml. Finally, for the comparison between the calcium content in the plant-based calcium fortified milk with fresh cow's milk, this study found that Brand T (almond milk) contained the highest calcium content and Brand Q (oat milk) contained the lowest calcium content which were 47.28 mg/ml and 8.52 mg/ml respectively.

From the results, first hypothesis of the study can be rejected as the results showed that there was no significant difference in the calcium content between calcium fortified milk in plant-based and animal-based milk. However, the mean difference was small between plant-based and animal-based calcium fortified milk where, such difference may be due to calcium loss during experimental procedure or calcium sedimented at the bottom of the packaging. Moreover, the results also found that calcium content was higher in both animal-based and plant-based calcium fortified milk compared to the fresh cow's milk. It is found that calcium content of animal-based milk and plant-based milk when fortified often contained higher total calcium amounts than cow's fresh milk. Hence, plant-based milk fortified with calcium may be a valuable and alternative source of calcium for individuals who are avoiding the consumption of dairy products due to medical condition such as lactose intolerance and cow's milk allergy. This is also can be significant to consumer who are in need to consume adequate amount of calcium especially among pre and postmenopausal women who want to prevent certain chronic disease such as osteoporosis. Thus, it is important that food manufacturer to consider in fortification other products with added calcium in order to help consumer with inadequate calcium intake.

On the other hand, the second hypothesis of the study can also be rejected as the results have shown that there was no significant difference in the calcium content between calcium fortified milk in plant-based and animal-based milk before and after being shake except for two samples which were Brand T (almond milk) and Brand D (full cream milk) which showed significantly difference. However, overall analysis showed that the calcium content was higher for the shaken samples compared to unshaken samples. This may due to the fact that the added calcium remains soluble during milk storage which explain the difference of calcium content between unshaken and shaken samples. Therefore, it is important for the consumer to shake the products prior use in order to get the amount of calcium that is stated on the label. It is also important for the food manufacturer to emphasize on the shaking instruction in the label to the consumer in order to provide them with maximum amount of calcium provided in the milk.

5.2 Recommendations

There are several limitations that can be pointed out in this study. Throughout the study, the calcium content between the plant-based and animal-based calcium fortified milk before and after shake were discussed. However, the bioavailability of the added calcium used in the calcium fortified milk is not identified. Bioavailability refers to how well a nutrient can be absorbed and used by the body. The bioavailability of calcium from different foods and sources often varies depending on food matrix. Bioavailability of calcium can be affected by many factors such as the presence of anti-nutrients, for instance, phytates, oxalates and polyphenols as well as competition with other nutrients. Moreover, literature reviews have shown that the calcium deficiency is commonly due to its low bioavailability. Thus, the determination of bioavailability of added calcium is also crucial to ensure maximum calcium absorption for consumer in order to meet their requirement needs.

Besides that, it is also important to determine and discuss the calcium content in the calcium residue, which is not done in the present study. Moreover, literature reviews have shown that the calcium content of the residue was three times higher in fortified milks than in nonfortified milks. Hence, it is vital to identify the calcium content in the calcium residue as it can be used to compare with the calcium content in calcium fortified milk as well as to find reasons to support the discrepancies between the unshaken and shaken calcium fortified milk observed in this study.

Lastly, this study only focuses on the calcium content in the calcium fortified milk, where other nutrients are not being investigated. It is important to identify and compared other essential nutrients analyzed and nutrients stated in the label, for example, protein and fat content. As it is also mentioned in the literature reviews that plant-based milk contains lower fat content than animal-based milk. Even though plant-based milk contains lower fat content, some products contain as much energy as full milk, originating mostly from sugars and others carbohydrates. Hence, it is vital to do further analysis to analyze other macronutrients content in the plant-based calcium fortified milk.

REFERENCES

Akyilmaz, E., & Kozgus, O. (2009). Determination of calcium in milk and water samples by using catalase enzyme electrode. *Food Chemistry*, *115*(1), 347–351. https://doi.org/10.1016/j.foodchem.2008.11.075

Appleby, P. N., & Key, T. J. (2016). The long-term health of vegetarians and vegans. *Proceedings of the Nutrition Society*, *75*(3), 287–293. https://doi.org/10.1017/S0029665115004334

Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods*, *70*(December 2019), 103975. https://doi.org/10.1016/j.jff.2020.103975

Baroni, L., Goggi, S., & Battino, M. (2019). Planning Well-Balanced Vegetarian Diets in Infants, Children, and Adolescents: The VegPlate Junior. *Journal of the Academy of Nutrition and Dietetics*, *119*(7), 1067–1074. https://doi.org/10.1016/j.jand.2018.06.008

Beto, J. A. (2015). The Role of Calcium in Human Aging. *Clinical Nutrition Research*, *4*(1), 1. https://doi.org/10.7762/cnr.2015.4.1.1

Binti Ida Umaya. (2017). No Titleالتواصل« طفرة على تتغذى جرائم ..الإلكتروني الابتزاز. *Universitas Nusantara PGRI Kediri*, *01*, 1–7. Retrieved from http://www.albayan.ae

Boniface, B., & Umberger, W. J. (2012). Factors influencing Malaysian consumers ' consumption of dairy products. *Australia Agricultural and Resource Economics Society*, *1*, 1–30.

Bonjour, J. P., Kraenzlin, M., Levasseur, R., Warren, M., & Whiting, S. (2013). Dairy in Adulthood: From Foods to Nutrient Interactions on Bone and Skeletal Muscle Health. *Journal of the American College of Nutrition*, *32*(4), 251–263. https://doi.org/10.1080/07315724.2013.816604

Bridges, M. (2018). Moo-ove Over, Cow's Milk: The Rise of Plant-Based Dairy Alternatives The Rise of Plant-Based Milks. *Practical Gastroenterology*, *171*(January), 20–27. Retrieved from https://med.virginia.edu/ginutrition/wp-content/uploads/sites/199/2014/06/January-18-Milk-Alternatives.pdf

Chaiwanon, P., Puwastien, P., Nitithamyong, A., & Sirichakwal, P. P. (2000). Calcium fortification in soybean milk and in vitro bioavailability. *Journal of Food Composition and Analysis*, *13*(4), 319–327. https://doi.org/10.1006/jfca.1999.0854

Chalupa-Krebzdak, S., Long, C. J., & Bohrer, B. M. (2018). Nutrient density and nutritional value of milk and plant-based milk alternatives. *International Dairy Journal*, *87*, 84–92. https://doi.org/10.1016/j.idairyj.2018.07.018

CHARLES, P. (1992). Calcium absorption and calcium bioavailability. *Journal of Internal Medicine*, *231*(2), 161–168. https://doi.org/10.1111/j.1365-2796.1992.tb00519.x

Chee, W. S. S., Suriah, A. R., Chan, S. P., Zaitun, Y., & Chan, Y. M. (2003). The effect of milk supplementation on bone mineral density in postmenopausal Chinese women in Malaysia. *Osteoporosis International*, *14*(10), 828–834. https://doi.org/10.1007/s00198-003-1448-6

Constantine, G., Seth, N., Chokshi, N., Minard, C. G., Guffey, D., Olive, A. P., & Davis, C. M. (2017). Combination Steroid and Test-based Food Elimination for Eosinophilic Esophagitis: A Retrospective Analysis. *Journal of Pediatric Gastroenterology and Nutrition*, *64*(6), 933–938. https://doi.org/10.1097/MPG.0000000000001584

Das, J. K., Salam, R. A., Lassi, Z. S., Bhutta, Z. A., Kumar, R., Moin, A., … Zamora, G. (2018). Food fortification with calcium and vitamin D: Impact on health outcomes. *Cochrane Database of Systematic Reviews*, *2018*(8). https://doi.org/10.1002/14651858.CD010201.pub2

De La Fuente, M. A., Belloque, J., & Juárez, M. (2004). Mineral contents and distribution between the soluble and the micellar phases in calcium-enriched UHT milks. *Journal of the Science of Food and Agriculture*, *84*(13), 1708–1714. https://doi.org/10.1002/jsfa.1868

De La Fuente, M. A., & Juárez, M. (1995). Rapid determination of calcium, magnesium, sodium and potassium in milk by flame atomic spectrometry after microwave oven digestion. *The Analyst*, *120*(1), 107–111. https://doi.org/10.1039/AN9952000107

Fernandez-Hernandez, A., Mateos, R., Garcia-Mesa, J. A., Beltran, G., & Fernandez-Escobar, R. (2010). Determination of mineral elements in fresh olive fruits by flame atomic spectrometry. *Spanish Journal of Agricultural Research*, *8*(4), 1183. https://doi.org/10.5424/sjar/2010084-1206

Food Act 1983. (2006). *Food Law 1983*.

Gan, W. Y., Boo, S., Seik, M. Y., & Khoo, H. E. (2018). Comparing the nutritional status of vegetarians and non-vegetarians from a Buddhist Organisation in Kuala Lumpur, Malaysia. *Malaysian Journal of Nutrition*, *24*(1), 89–101.

Gatehouse, B. M., & Willis, J. B. (1961). Performance of a simple atomic absorption spectrophotometer. *Spectrochimica Acta*, *17*(7), 710–718. https://doi.org/10.1016/0371-1951(61)80137-9

Gaur, V., Schalk, J., & Anema, S. G. (2018). Sedimentation in UHT milk.

International Dairy Journal, *78*, 92–102. https://doi.org/10.1016/j.idairyj.2017.11.003

Guéguen, L., & Pointillart, A. (2000a). The Bioavailability of Dietary Calcium. *Journal of the American College of Nutrition*, *19*(May), 119S-136S. https://doi.org/10.1080/07315724.2000.10718083

Guéguen, L., & Pointillart, A. (2000b). The Bioavailability of Dietary Calcium. *Journal of the American College of Nutrition*, *19*(October), 119S-136S. https://doi.org/10.1080/07315724.2000.10718083

Haas, R., Schnepps, A., Pichler, A., & Meixner, O. (2019). Cow Milk versus Plant-Based Milk Substitutes: A Comparison of Product Image and Motivational Structure of Consumption. *Sustainability*, *11*(18), 5046. https://doi.org/10.3390/su11185046

Heaney, R. P. (2000). Calcium, Dairy Products and Osteoporosis. *Journal of the American College of Nutrition*, *19*(August), 83S-99S. https://doi.org/10.1080/07315724.2000.10718088

Heaney, R. P., Dowell, M. S., Rafferty, K., & Bierman, J. (2000). Bioavailability of the calcium in fortified soy imitation milk, with some observations on method. *American Journal of Clinical Nutrition*, *71*(5), 1166–1169. https://doi.org/10.1093/ajcn/71.5.1166

Heaney, R. P., & Rafferty, K. (2006). The Settling Problem in Calcium-Fortified Soybean Drinks. *Journal of the American Dietetic Association*, *106*(11), 1753. https://doi.org/10.1016/j.jada.2006.08.008

Heaney, R. P., Rafferty, K., Dowell, M. S., & Bierman, J. (2005). Calcium fortification systems differ in bioavailability. *Journal of the American Dietetic Association*, *105*(5), 807–809. https://doi.org/10.1016/j.jada.2005.02.012

Hettinga, K., van Valenberg, H., de Vries, S., Boeren, S., van Hooijdonk, T., van Arendonk, J., & Vervoort, J. (2011). The host defense proteome of human and bovine milk. *PLoS ONE*, *6*(4), 2–9. https://doi.org/10.1371/journal.pone.0019433

Iguacel, I., Miguel-Berges, M. L., Gómez-Bruton, A., Moreno, L. A., & Julián, C. (2019). Veganism, vegetarianism, bone mineral density, and fracture risk: A systematic review and meta-analysis. *Nutrition Reviews*, *77*(1), 1–18. https://doi.org/10.1093/nutrit/nuy045

Jeske, S., Zannini, E., & Arendt, E. K. (2018). Past, present and future: The strength of plant-based dairy substitutes based on gluten-free raw materials. *Food Research International*, *110*, 42–51. https://doi.org/10.1016/j.foodres.2017.03.045

Kapaj, A., & Deci, E. (2017). World milk production and socio-economic factors effecting its consumption. In *Dairy in Human Health and Disease across the*

Lifespan. https://doi.org/10.1016/B978-0-12-809868-4.00007-8

Kruger, M. C., Gallaher, B. W., & Schollum, L. M. (2003). Bioavailability of calcium is equivalent from milk fortified with either calcium carbonate or milk calcium in growing male rats. *Nutrition Research*, *23*(9), 1229–1237. https://doi.org/10.1016/S0271-5317(03)00100-3

Makbul, I. A. A., Daud, N. M., Aziz, N. A. A., & Yahya, N. F. S. (2016). Lactose intolerance among Malay and Orang Asli female children in selected rural Selangor and its effect on bone mineral density. *AIP Conference Proceedings*, *1784*. https://doi.org/10.1063/1.4966784

Mäkinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for Special Dietary Needs: Non-dairy Plant-based Milk Substitutes and Fermented Dairy-type Products. *Critical Reviews in Food Science and Nutrition*, *56*(3), 339–349. https://doi.org/10.1080/10408398.2012.761950

McCarthy, K. S., Parker, M., Ameerally, A., Drake, S. L., & Drake, M. A. (2017). Drivers of choice for fluid milk versus plant-based alternatives: What are consumer perceptions of fluid milk? *Journal of Dairy Science*, *100*(8), 6125–6138. https://doi.org/10.3168/jds.2016-12519

McKee, R. F. (2015). Artificial nutrition and nutritional support and refeeding syndrome. *Medicine (United Kingdom)*, *43*(2), 119–123. https://doi.org/10.1016/j.mpmed.2014.11.013

Miller, G. D., Jarvis, J. K., & McBean, L. D. (2001). The Importance of Meeting Calcium Needs with Foods. *Journal of the American College of Nutrition*, *20*(2), 168S-185S. https://doi.org/10.1080/07315724.2001.10719029

Nickel, K. P., Martin, B. R., Smith, D. L., Smith, J. B., Miller, G. D., & Weaver, C. M. (1996). Calcium Bioavailability from Bovine Milk and Dairy Products in Premenopausal Women Using Intrinsic and Extrinsic Labeling Techniques. *The Journal of Nutrition*, *126*(5), 1406–1411. https://doi.org/10.1093/jn/126.5.1406

Norhaizan, M. E., & Nor Faizadatul Ain, A. W. (2009). Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malaysian Journal of Nutrition*, *15*(2), 213–222.

Perales, S., Barberá, R., Lagarda, M. J., & Farré, R. (2006). Fortification of milk with calcium: Effect on calcium bioavailability and interactions with iron and zinc. *Journal of Agricultural and Food Chemistry*, *54*(13), 4901–4906. https://doi.org/10.1021/jf0601214

Schedule, F., Requiring, F., & Warranty, W. (2011). *Colouring substance Flavouring substance Full cream milk powder Infant formula Skimmed milk powder Tea , tea dust , tea extract and scented tea FOOD ACT 1983 FOOD REGULATIONS 1985*. *2011*(December), 1–92.

Scholz-Ahrens, K. E., Ahrens, F., & Barth, C. A. (2020). Nutritional and health attributes of milk and milk imitations. *European Journal of Nutrition*, *59*(1), 19–34. https://doi.org/10.1007/s00394-019-01936-3

Sethi, S., Tyagi, S. K., & Anurag, R. K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: a review. *Journal of Food Science and Technology*, *53*(9), 3408–3423. https://doi.org/10.1007/s13197-016-2328-3

Sousa, A., & Bolanz, K. A. K. (2017). Nutritional Implications of an Increasing Consumption of Non-Dairy Plant-Based Beverages Instead of Cow's Milk in Switzerland. *Advances in Dairy Research*, *05*(04). https://doi.org/10.4172/2329-888x.1000197

Trudeau, D. L., & Freier, E. F. (1967). Determination of calcium in urine and serum by atomic absorption spectrophotometry (AAS). *Clinical Chemistry*, *13*(2), 101–114.

Tuohimaa, P. (2012). Vitamin D, aging, and chronic diseases. *Vitamin D: Oxidative Stress, Immunity, and Aging*, *8*(3), 385–406. https://doi.org/10.1201/b13714

United Nations Office on Drugs and Crime. (2009). *A commitment to quality and continuous improvement*.

Van Der Velde, R. Y., Brouwers, J. R. B. J., Geusens, P. P., Lems, W. F., & Van Den Bergh, J. P. W. (2014). Calcium and vitamin D supplementation: State of the art for daily practice. *Food and Nutrition Research*, *58*, 1–12. https://doi.org/10.3402/fnr.v58.21796

Vanga, S. K., & Raghavan, V. (2017). Processing effects on tree nut allergens: A review. *Critical Reviews in Food Science and Nutrition*, *57*(17), 3794–3806. https://doi.org/10.1080/10408398.2016.1175415

Vanga, S. K., & Raghavan, V. (2018). How well do plant based alternatives fare nutritionally compared to cow's milk? *Journal of Food Science and Technology*, *55*(1), 10–20. https://doi.org/10.1007/s13197-017-2915-y

Vanga, S. K., Singh, A., Harish Vagadia, B., & Raghavan, V. (2015). Global food allergy research trend: a bibliometric analysis. *Scientometrics*, *105*(1), 203–213. https://doi.org/10.1007/s11192-015-1660-0

Vanga, S. K., Singh, A., & Raghavan, V. (2015). Effect of thermal and electric field treatment on the conformation of Ara h 6 peanut protein allergen. *Innovative Food Science and Emerging Technologies*, *30*, 79–88. https://doi.org/10.1016/j.ifset.2015.03.003

Vavrusova, M., & Skibsted, L. H. (2014). Calcium nutrition. Bioavailability and fortification. *LWT - Food Science and Technology*, *59*(2P2), 1198–1204. https://doi.org/10.1016/j.lwt.2014.04.034

Weaver, C. M., Heaney, R. P., Martin, B. R., & Fitzsimmons, M. L. (1992). Extrinsic vs intrinsic labeling of the calcium in whole-wheat flour. *American Journal of*

Clinical Nutrition, *55*(2), 452–454. https://doi.org/10.1093/ajcn/55.2.452

Weaver, Connie M. (1998). Calcium in food fortification strategies. *International Dairy Journal*, *8*(5–6), 443–449. https://doi.org/10.1016/S0958-6946(98)00067-3

Wong, Y. S., Shalini, S., Farah Liana, M. R., Wan Nurul Amera, W. M. S., Mohd Naqiuddin, M. Y., Norhazlina, A. W., & Zaleha, M. I. (2013). Nutrient intake pattern of vegetarians and non-vegetarians in Kuala Lumpur, Malaysia. *Pakistan Journal of Nutrition*, *12*(4), 371–376. https://doi.org/10.3923/pjn.2013.371.376