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Tempeh: a tempting potential vitamin B₁₂ treat

An exploration of legumes and vitamin B₁₂

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ABSTRACT

Background

Legumes are beneficial for the environment, nutritious and coupled with various health benefits. However, they lack the essential vitamin B₁₂ and a vitamin B₁₂ deficiency can lead to hazardous health problems. Tempeh is a fermented legume product that has the potential of carrying vitamin B₁₂.

Aim

The aim of this thesis is to investigate consumers' awareness of vitamin B₁₂ and explore the indications for the possibility to introduce tempeh - a fermented legume product.

Method

A quantitative survey was carried out within four different areas of Stockholm using a clustering sample technique with 195 participants who were recruited outside supermarkets through convenience sampling.

Results

The majority of the subjects had moderate consumption of legumes and was open to trying tempeh even though they had never heard about it. While their knowledge of vitamin B₁₂ was limited, the biggest risk groups, vegans and the elderly, were aware that they were a risk group but only a minority of them knew the correct vitamin B₁₂ food sources.

Conclusion

The participants in this study had little knowledge about vitamin B₁₂ food sources, deficiencies or risk groups. Since their legume consumption was regular and they were positive towards trying tempeh even though they had never heard about tempeh before, there is potential for it in the market. However, more studies are needed to research the bioavailability of vitamin B₁₂ in tempeh even though new research gives hope that it can be a possibility.

Keywords

Tempeh, plant based vitamin B₁₂, environment, vegetarian, cobalamin, fermentation

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SAMMANFATTNING

Bakgrund

Baljväxter är fördelaktiga för miljön samtidigt som de är väldigt näringsrika och kopplade till en rad hälsofördelar. De saknar dock det essentiella vitaminet B₁₂. Brist på detta vitamin kan leda till en rad sjukdomar och komplikationer. Tempeh är en fermenterad baljväxtprodukt som möjligtvis kan innehålla vitamin B₁₂.

Syfte

Syftet under denna studie har varit att studera konsumenters kunskap gällande vitamin B₁₂ och utforska indikationer för en potentiell introduktion av tempeh.

Metod

En kvantitativ enkätundersökning har genomförts inom fyra skilda stadsdelar i Stockholms kommun. Stadsdelarna har valts utifrån klusterurval och 195 deltagarna har därefter rekryterats i matbutiker genom bekvämlighetsurval. Deskriptiv statistik har används för att analysera resultatet.

Resultat

En majoritet av deltagarna hade en måttlig konsumtion av baljväxter och var positivt inställda till att prova tempeh även om merparten av deltagarna tidigare inte hade hört talas om produkten. Det fanns en begränsad kunskap om vitamin B₁₂ även om de största riskgrupperna, äldre och veganer var medvetna om att de tillhörde en riskgrupp. Dock visste endast en minoritet av dessa i vilka livsmedel vitamin B₁₂ kunde hittas.

Slutsats

Deltagarna i denna studie hade begränsad kunskap angående vitamin B₁₂, dess källor och möjliga komplikationer vid brist. Då baljväxter visade sig vara något som de flesta av deltagarna konsumerar regelbundet samt att de flesta var positivt inställda till att prova tempeh, finns det potential att fortsätta studera dess marknad. Mer studier behövs dock för att kunna fastställa om tempeh kan vara en biotillgänglig vegetabilisk källa till vitamin B₁₂. Nyare studier ger dock hopp.

Keywords

Tempeh, vegetabiliskt vitamin B₁₂, miljö, vegetarian, cobalamin, fermentation

FOREWORD

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Introduction

The environment is a significant reason to steer towards a plant-based diet according to The Swedish Food Agency Livsmedelsverket (2015). Raising meat takes up substantial resources, so reducing meat consumption could decrease the carbon footprint by 920kgCO₂e, a vegetarian diet by 1,230kgCO₂e/year and a vegan diet by 1,560kgCO₂e/year (Scarborough, Appleby, Mizdrak, Briggs, Travis, Bradbury & Key, 2014). To be specific, cutting down on meat and dairy products by 50 percent could reduce nitrogen pollution in the air and water, greenhouse emissions could be reduced by about 40 percent, and large areas of farmland that are now being used as animal farms could become available for growing crops instead (Hawkes, 2014).

A study made by Laestadius, Neff, Barry & Frattaroli (2013) indicated that there is an active public outreach to reduce meat consumption in Sweden. The Swedish National Food Agency also mentioned an increased interest in vegetarian food (Livsmedelsverket, 2015) and Craig (2009) mentioned an increased popularity in vegetarian diets.

In the most recent Swedish National Food Consumption Survey, 2010-2011, three percent of the 2043 respondents ate different types of vegetarian diets (lacto-ovo-vegetarian, lacto-vegetarian or vegan) (Amcoff et al., 2012). This is a rise in the amount of vegetarians compared to The Swedish National Food Consumption Survey conducted in 1997-1998 where less than one percent of the 1170 respondents consumed a lacto-vegetarian diet and none of the respondents indicated a consumption of vegan diet¹(Pearson & Becker, 1998).

Using legumes can be a way to reduce meat intake especially as growing legumes (also called pulses) benefit the environment due to their nitrogen-fixing properties (Martín-Cabrejas, Chung, Amarowicz, Aryee, Domoney, Vaz Patto & Boye, 2015), and their protein quantity is similar to that of meat (Bouchenak and Lamri-Senhadjji, 2013). According to the United Nations, 2016 is the international year of pulses (FAO 2015), which makes it especially relevant to raise awareness of legumes and promote their use. Incidentally, the Swedish National Food Consumption Survey (Riksmaten 2010-2011) indicated that the consumption of legumes in Sweden had increased compared to prior food surveys made in the years 1989 and 1997-98 (Table1).

Table 1: Legume consumption per 10MJ in the Swedish National Food Consumption Survey: Riksmaten 2010-11, Riksmaten 1997-98 and Hulk 1989) (Amcoff et al., 2012).

	Swedish National Food Consumption Survey 2010-11	Swedish National Food Consumption Survey, 1997-98	Swedish National Dietary Survey (HULK), 1989
Female	16	10	7
Male	13	11	15
Total	29	21	22

¹ The Swedish National Food Consumption Survey (Riksmaten 1997-1998) defines vegetarians within two groups: vegans and lacto-vegetarians.

Although a plant-based diet with copious amounts of vegetables, fruits and legumes have been linked to a lower risk of acquiring diseases such as hypertension, cardiovascular disease and certain types of cancers in vegetarians, people abstaining from animal products could be at risk of consuming too little vitamin B₁₂ (Woo, Kwok & Celermajer, 2014; Livsmedelsverket, 2015).

Watanabe, Yabuta, Bito & Teng (2013) stated that there was a need for vitamin B₁₂ in plant-based sources as a deficiency in vitamin B₁₂ could lead to serious health conditions. They suggested that vegetarians and vegans should maintain an adequate intake of vitamin B₁₂ by consuming supplements or vitamin B₁₂-fortified foods, something that is also supported by the Swedish National Food Agency (Livsmedelsverket, 2015) and The Nordic Council of Ministers (2014). However, as many people may abstain from supplementation for various reasons and/or consume little or no fortified foods, there is a need to research plant-based vitamin B₁₂ sources. The introduction of new plant-based products containing natural vitamin B₁₂ could help counteract vitamin B₁₂ deficiency. Tempeh is a fermented legume product that has the potential of carrying vitamin B₁₂ if it is contaminated with the right bacteria (Watanabe et al. 2013). This paper explores the functionality of legumes, gives an overview of vitamin B₁₂ and its sources and researches tempeh and its nutritional benefits. In addition, identifying consumption of legumes and investigating knowledge regarding vitamin B₁₂ can give an indication of whether there is an interest in introducing tempeh, a fermented plant-based product that has the potential of carrying vitamin B₁₂. This study is meant to explore one of the options to prevent vitamin B₁₂ deficiency and therefore help fill a gap in the plant-based market by introducing tempeh, a nutritious product that can contain vitamin B₁₂.

Purpose and Research Questions

The aim of this thesis is to investigate consumers' awareness of vitamin B₁₂ and explore the indications for the possibility to introduce tempeh - a fermented legume product.

Research questions

1. What knowledge do consumers have regarding vitamin B₁₂?
2. How is the consumer's legume consumption?
3. Is there interest amongst consumers of trying tempeh with bioavailable vitamin B₁₂?

Background

Types of diets

Clarys et al. (2014) classified five different types of diets that are used throughout this paper: omnivores, semi-vegetarians, pescetarians, vegetarians and vegans. Omnivores eat meat and fish on a daily basis. Semi-vegetarians are those who eat red meat, poultry or fish no more than once a week. Pescetarians (also sometimes called "pesco-vegetarian") choose to eat seafood but no other meat. Vegetarians do not eat meat or fish and vegans do not eat any animal products. According to The Swedish National Food Agency (Livsmedelsverket, 2015), there are three kinds of plant-based eaters: lacto-ovo-vegetarian, lacto-vegetarian and vegan. Lacto-ovo-vegetarians do not eat meat, poultry or seafood but still eat dairy products and eggs. Lacto-vegetarians, on the other hand, do not eat eggs but consume dairy products. Vegans exclude everything from the animal kingdom and rely solely on plant-based foods.

Legumes

Legumes are the sustainable answer to the environmental dilemma according to Araújo et al. (2015): “They provide an input-saving and resource-conserving alternative because they fix atmospheric nitrogen, thus reducing the need for chemical fertilizers while enhancing overall crop productivity. In farming systems, legumes are often used as an inter-crop (e.g., combined with cereals) or in crop rotation resulting in a decrease in pests, diseases and weed populations, while enhancing the overall farm productivity and income of smallholder farmers” (Araujo et al., 2015).

In terms of nutrition, when replacing meat with plant-based sources, legumes (also known as *pulses*) provide essential vitamins, minerals, antioxidants, fiber and other substantial nutrients according to Bouchenak et al. (2013). Lazarte, Carlsson, Almgren, Sandberg & Granfeldt (2015) specified that legumes are a great source of minerals such as iron, calcium and zinc. In addition, the protein content is comparable to that of meat (Bouchenak et al., 2013). Craig (2009) indicated that although legumes do not contain the same amino acids as meat, eating a varied diet throughout the day ensures sufficient protein-intake because the body collects and stores amino acids from various foods. Moreover, The Swedish Food Agency’s latest advice on protein stated that legumes do not need to be “completed” with cereals in the same meal in order to obtain adequate protein (Craig, 2009; Livsmedelsverket, 2015).

Dietary fibers in legumes “include resistant starch, nonstarch polysaccharides (cellulose, hemicellulose, pectin, gums, and β -glucans), nondigestible oligosaccharides, and lignin” (Bouchenak et al., 2013). Fiber has numerous health benefits such as “fecal bulking, alteration in transit time, prevention of colon cancer, lowering cholesterol and the rate of glucose absorption and postprandial plasma glucose concentrations” (Martín-Cabrejas et al., 2015). Moreover, Bouchenak et al. (2013) stated that legumes’ fiber is linked to improving insulin sensitivity and protecting against obesity. Messina (2014) found that the fiber in legumes could reduce the risk of ischemic heart disease in people with diabetes.

Tempeh is a legume product that, due to its fermentation, is more digestible and breaks down bioactive compounds (Mo et al., 2013), yielding in optimal nutrient absorption (Reyes-Bastidas, Reyes-Fernández, López-Cervantes, Milán-Carrillo, Loarca-Piña & Reyes-Moreno, 2010).

Bioactive compounds

Preparation is crucial in order to gain legumes’ nutrients as they contain various bioactive compounds such as lectins, phytates, oligosaccharides, trypsin inhibitors and phenolic compounds that can inhibit optimal absorption according to Bouchenak et al. (2013) and Curiel, Coda, Centomani, Summo, Gobetti & Rizzello (2015). Bouchenak et al. (2013) and Martín-Cabrejas et al. (2015) stated, however, that these bioactive compounds also carried various health benefits and therefore should not be called “anti-nutrients” as they often are. Various methods of preparation help break down the compounds to aid optimal nutrient absorption (Curiel et al., 2015).

Lectins

Gebrelibanos et al. (2013) indicated that lectins can prevent overall nutrient absorption as they reduce digestibility by binding to carbohydrates, but are mostly inactivated through heat and cooking. Bouchenak et al. (2013) found that they can treat or prevent obesity and hypertension.

Phytates

According to Bouchenak et al. (2013), phytates are involved with antioxidant activity in the body as well as protect DNA from damage. Messina (2014) also stated that phytates protect against certain cancers and kidney stones. However, they can also reduce digestion (Bouchenak et al., 2013) and prevent optimal mineral absorption (Lazarte et al., 2015). Soaking, germination (sprouting) and fermentation can reduce them (Lazarte et al., 2015; Messina 2014). Reyes-Bastidas et al. (2010) explained that the synthesis of phytase by the *Rhizopus* fungus decreased the phytic acid during solid-state fermentation.

Phenolic compounds

Antioxidants such as phenolic compounds include phenolic acids, tannins and flavonoids (Messina, 2014). Martín-Cabrejas et al. (2015) processed various types of legumes and found that dehulling (removal of the outer covering of the legume), germination, soaking, pressure-cooking, and/or cooking reduced the amount of phenolic acids. Interestingly, Reyes-Bastidas et al. (2010) noted an increase in phenolic content after solid-state fermentation as the fungal β -glucosidase helped release the aglycones from the bean. Curiel et al. (2015) noted its increased bioavailability² with lactic acidification and enzyme activity. According to Bouchenak et al. (2013), while phenolic acids can reduce protein digestibility and mineral bioavailability, they can also protect against cardiovascular disease, diabetes and metabolic stress and contain antioxidant properties.

Curiel et al. (2015) stated that tannins, which are also phenolic compounds, reduce nutrient absorption and digestibility as they bind enzymes, proteins and/or minerals. However, they informed that spontaneous fermentation reduces them. Martín-Cabrejas et al. (2015) noted that germination (releasing the seed coat where tannins are most prominent) also reduced tannins.

Oligosaccharides

Martín-Cabrejas et al. (2015) explained that bacteria in the large intestine, which with the formation of carbon dioxide can cause flatulence, ferment oligosaccharides. However, these prebiotics are food for gut bacteria, which maintains intestinal health (Martín-Cabrejas et al., 2015). Moreover, oligosaccharides promote colon health, decrease colon cancer and are linked to longevity (Messina 2014). They can be reduced in various ways: boiling unsoaked legumes, soaking legumes and discarding the water before cooking considerably reduces oligosaccharides up to 76 percent. “Cooking beans in alkaline water reduces oligosaccharide content even further. Germinating beans has been shown to reduce amounts of these carbohydrates as well” (Messina 2014). Curiel et al. (2015) noted that fermentation with lactobacilli and *Leuconostoc* also reduced oligosaccharides. During fermentation, the microorganisms that feed on legumes break down the legume cell walls that in turn diminish the walls between proteins and carbohydrates, therefore modifying polysaccharides and reducing soluble dietary fiber according to Martín-Cabrejas et al. (2015).

Trypsin inhibitors

Trypsin inhibitors prevent the body from digesting the protein found in legumes (Gebrelibanos, Tesfaye, Raghavendra & Sintayeyu, 2013). 80-90 percent of them can be deactivated through cooking (Messina 2014), but it should be noted that trypsin inhibitors could also be anti-

² The proportion of a drug or other substance, which enters the circulation when introduced into the body and so, is able to have an active effect (Oxford Dictionaries, n.d.).

carcinogenic (Martín-Cabrejas et al., 2015; Gebrelibanos et al., 2013). Curiel et al. (2015) noted that trypsin inhibitors were reduced through lactic acid fermentation with *Lactobacillus plantarum*.

In summary, various processes reduce bioactive compounds, fermentation being the most effective. During legume fermentation, tempeh, with the addition of *Klebsiella pneumoniae* or *Citrobacter freundii*, has the potential to contain the essential vitamin B₁₂ (Ayu, Suwanto & Barus, 2015).

Vitamin B₁₂: an essential vitamin for various bodily functions

Gröber, Kisters & Schmidt (2013) describe the structural framework of the essential water-soluble vitamin B₁₂ as a vitamin that is based on the corrin ring system comprising four reduced pyrrole rings and a central cobalt atom. Cobalamin, as vitamin B₁₂ also is called, is a generic term for six active forms; cyanocobalamin, hydroxycobalamin, aquocobalamin, methylcobalamin, nitrocobalamin and adenosylcobalamin (Gröber et al., 2013). Methylcobalamin and adenosylcobalamin are two biologically active forms of B₁₂. Other forms such as hydroxocobalamin, aquocobalamin and cyanocobalamin must be metabolized to either of the two active forms in order to be used in human cells (Pawlak, James, Raj, Cullum-Dugan & Lucus, 2013).

The vitamin is essential for a variety of bodily functions such as DNA synthesis and creation of red blood cells (Molina, Médici, Font de Valdez & Taranto, 2012; Sachdev, 2005; O’Leary & Samman, 2010). Furthermore vitamin B₁₂ is a principal component of fatty acid, carbohydrate and nucleic acid metabolism (Gröber et al., 2013).

Vitamin B₁₂ is important for the central nervous system as the vitamin is involved in the methionine-homocysteine metabolism (Gröber et al., 2013). Folate, vitamin B₁₂ and vitamin B₆ convert homocysteine to either cysteine or methionine, which is an important function since hyperhomocysteinemia, an abnormally high level of homocysteine in the blood, might be a risk factor for vascular disease, brain atrophy, cognitive impairment, Alzheimer disease, depression, and several neuropsychiatric diseases (Sachdev, 2005). Furthermore homocysteine concentration has been shown to increase with age (Fuh, 2010). Individuals 65–72 years of age have been shown to have higher levels of homocysteine (Obeid, Schorr, Eckert & Herrmann, 2004). Sachdev (2005) indicated that homocysteine levels can be lowered by about seven percent with a vitamin B₁₂ dose of 0.02– 1.0 mg/day.

Vitamin B₁₂ deficiency is mainly associated with megaloblastic anaemia³ and/or neurological symptoms due to the degeneration of the spinal cord, brain and optic and peripheral nerves (Nordic Council of Ministers, 2014). In addition, literature reviews also mentioned megaloblastic anaemia, vascular diseases (Chatthanawaree, 2011; Pawlak et al., 2013; O’Leary & Samman, 2010), cognitive decline and neuropsychiatric diseases such as Alzheimer disease and depression (Pawlak et al., 2013; O’Leary & Samman, 2010; Gröber et al., 2013). In addition neurological damage (Pawlak et al., 2013; O’Leary & Samman, 2010; Gröber et al., 2013) according to Chatthanawaree (2011) also could be irreversible as a result of vitamin B₁₂ deficiency. What is more, a longitudinal cohort study performed by Clarke et al. (2007), which studied elderly over

³ Abnormalities in the red blood cells that result in many large immature and dysfunctional red blood cells (megaloblasts) in the bone marrow (Pontes et al., 2009).

65 years old, found that low vitamin B₁₂ status was associated with more rapid cognitive decline. Furthermore Brescoll & Daveluy (2015) stated that vitamin B₁₂ deficiency also is associated with dermatological manifestations such as hyperpigmentation, oral changes and acne. Acne can on the other hand also result from excess of the vitamin (Brescoll et al., 2015). A case report by Pontes et al. (2009) demonstrated how oral changes like tongue inflammation can be seen and a case report by (Chiang, Hung, Wang, Lee & Yang, 2013) addresses the issue of hyperpigmentation due to vitamin B₁₂ deficiency.

Women with very low status of vitamin B₁₂ during pregnancy and/or lactation risk giving birth to infants with severely delayed development causing neurological symptoms in infants (Wagnon et al., 2005; Borkowska et al., 2007; Roed, Skovby & Lund, 2009; Lücke et al., 2007). Benbir et al. (2007) have also identified involuntary movements and seizures in infants that was able to be treated with oral and intramuscular vitamin B₁₂.

Absorption

Watanabe et al. (2013) explain that in order to be absorbed, vitamin B₁₂ has to be bound to a protein in the stomach, re-bound to haptocorrin (transcobalamin, TC I) and then bound to the intrinsic factor in the proximal ileum. The TC II-vitamin B₁₂ complex, also called holotranscobalamin (holoTC), enters the blood circulation and is rapidly taken up by the liver, bone marrow, and other tissue (Nordic Council of Ministers, 2014). About half of the body stores of vitamin B₁₂ (2-5 milligram) are stored in the liver and with a minor daily loss of 0,1 percent, so deficiency generally develops only after several years of insufficient dietary intake or decreased absorption (Nordic Council of Ministers, 2014).

Bioactive or bioavailable vitamin B₁₂ is the kind that humans can absorb, distinguishing itself from other vitamin B₁₂ sources that contain inactive corrinoids, which are not absorbable (Chamlagain et al. 2015). Vitamin B₁₂ bioavailability decreases significantly with increasing intake of vitamin B₁₂ per meal (Allen, 2010; Watanabe, 2007).

Deficiency and Risk groups

Vitamin B₁₂ deficiency can be caused either by dietary deficiency or malabsorption (Gröber et al., 2013). According to Gröber et al (2013) alcoholics, vegetarians, vegans and elderly over 65 years old are a risk group for dietary deficiency. Moreover they also stated that elderly usually have malabsorption due to lack of intrinsic factor, gastric acid deficiency or drug interaction. The prevalence of cobalamin deficiency increases with age (Chatthanawaree, 2011).

Gröber et al (2013) also mentioned people with Crohn's disease as a risk group amongst other gastrointestinal diseases that inhibit vitamin B₁₂ absorption. Pawlak et al (2013) also identified people with Celiac disease as a risk group. Sachdev (2005) explained that clinical studies showed that chronic alcoholism leads to hyperhomocysteinemia.

The elderly and vegetarians/vegans have been identified as the major risk groups according to the Nordic Council of Ministers (2014). A systematic literature search conducted by Pawlak et al. (2013) supports this claim. Additionally, they found that vegans have higher deficiency prevalence than vegetarians.

The Nordic Council of Ministers (2014) has estimated a lower daily intake level and an average daily requirement of 1 microgram and 1.4 microgram of vitamin B₁₂ for both men and women respectively. The Swedish National Food Consumption Survey, 2010-2011, indicated a daily

average intake of 5 microgram for women and 6 µg men based on 95 percent omnivore respondents (Amcoff et al., 2012).

Fortified products and supplementation

Fortifying vitamin B₁₂ into vegan products is a way to prevent deficiency (Clarys, 2014; Watanabe et al., 2013) although fortifying products with vitamin B₁₂ is not mandatory in Sweden (Livsmedelsverket, 2015). Woo et al. (2014) have shown that vitamin B₁₂ supplementation can help alleviate deficiency. The Swedish National Food Agency (Livsmedelsverket, 2015) and The Nordic Council of Ministers (2014) recommend vegetarians and vegans to either supplement or consume vitamin B₁₂-fortified foods. The latest Swedish National Food Consumption Survey indicated that 21 percent of the respondents used different types of dietary supplements and of these, three percent were vitamin B₁₂ supplements (Amcoff et al., 2012).

Dietary sources

The common dietary sources of vitamin B₁₂ are animal foods such as meat, fish, shellfish, dairy products and eggs (Watanabe, 2007). Vitamin B₁₂ may also be found in plant foods fermented by certain bacteria (Madhu, Giribhattanavar, Narayan & Prapulla, 2010; Watanabe et al., 2013). Tables 2-4 indicate the amount of vitamin B₁₂ found in different sources. The Swedish nutrition-calculation program Dietist XP indicates that the amount of vitamin B₁₂ in different sources varies with different preparation techniques. Data on *Chlorella* sp, dried green (*Enteromorpha* sp.) and purple (*Porphyra* sp.) lavers (nori) or black trumpet mushroom (*Craterellus cornucopioides*) was only found in the study by Watanabe et al. (2013). There was no data for tempeh in the Swedish Food Agency database.

Table 2: Amounts of vitamin B12 in different sources (Watanabe et al., 2013)

Source	µg/100g
<i>Chlorella</i> sp	200
Nori	133
Shellfish	52.4
Meat	9.4
Fish	8.9
Tempeh	8.0-0.7
Black trumpet mushroom	2.65-1.09
Egg	1.3
Milk	0.4

Table 3: Amounts of vitamin B12 in different sources (DietistXP)

Source	µg/100g
Raw/Fried salmon	6.2/6.09
Deep fried fish	2.32
Organic raw/fried/boiled egg	2.17/2.0/1.95
Shellfish	2.06
Conventional raw/fried/boiled egg	1.47/1.36/1.32
Fried cod	1.46
Raw/fried beef	1.4/0.7
Milk (1.5% fat)	0.59
Fried chicken breast	0.28
Tempeh	0.08

Table 4: Amounts of vitamin B12 in different sources (Livsmedelsverket, n.d)

Source	µg/100 g
Raw/Fried salmon	6.2 / 5.86
Deep fried fish	2.37
Organic raw/fried/boiled egg	2.17/2.0/1.95
Shellfish	2.01
Conventional raw/fried/boiled egg	1.47/1.36/1.32
Fried cod	1.46
Raw/fried beef	1.4/1.31
Milk (1.5% fat)	0.59
Fried chicken breast	0.28

Food processing and vitamin B₁₂ content

Watanabe et al. (2013) stated that vitamin B₁₂ can be heated up to 120 °C without significant loss, however that microwave heating resulted in appreciable vitamin B₁₂ loss due to degradation of the vitamin molecule. Furthermore they indicated that milk has a very low content of vitamin B₁₂ (approximately 0.3–0.4 microgram per 100 grams), and appreciable losses of vitamin B₁₂ occur during the processing of milk. According to Pawlak et al (2013) boiling milk can destroy 30 percent to 50 percent of B₁₂ depending on the duration of cooking and pasteurization destroys 5-10 percent of B₁₂. However, approximately 20–60 percent of the vitamin B₁₂ that is initially present in milk is recovered in cottage cheese, hard cheese, and blue cheese (Watanabe et al., 2013).

Bioavailability in food sources

Although several dietary sources may contain vitamin B₁₂, their bioavailability varies. The bioavailability of vitamin B₁₂ in healthy humans from fish meat, sheep meat and chicken meat averaged 42 percent, 56-89 percent, and 61-66 percent respectively (Watanabe, 2007). Watanabe et al. (2013) mentioned that the vitamin B₁₂ content in eggs had an average bioavailability of 3.7-9.2 percent. Pawlak et al (2013) mentioned an absorption rate of vitamin B₁₂ from milk of about 65 percent. Bioavailability of vitamin B₁₂ from eggs depends on the preparation method ranging from 4 -9percent (Pawlak et al., 2013).

Vitamin B₁₂ found in plant-based foods are limited and can contain inactive compounds (Watanabe et al., 2013). Chamlagain et al. (2015) explain that some mushrooms and tempeh can contain bioavailable vitamin B₁₂ if contaminating bacteria produces them. Chamlagain et al. (2015) were able to synthesize bioavailable vitamin B₁₂ in cereals with the *Propionibacteria freudenreichii* subspecies *shermanii*.

Certain dried algae like *Spirulina* sp. can contain substantial amounts of vitamin B₁₂ according to Watabane et al (2013), however they reported that it may not be bioavailable. On the other hand, nori eaten by rats and humans has shown bioavailable vitamin B₁₂, which suggests that regular nori consumption can prevent B₁₂ deficiency (Watanabe et al., 2013). Furthermore, Watanabe et al. (2013) have identified that “consumption of approximately 100 g of dried black trumpet (approximately 1 kg of fresh mushroom with 90 percent moisture content) could provide the RDA for adults (2.4 µg/day).

Tempeh

Tempeh is a fermented legume product originating from Indonesia most commonly made from soybeans fermented with the fungus *Rhizopus oligosporus* or *Rhizopus microsporus* (Ayu et al., 2015; Mo et al., 2013). During fermentation, the fungus produces mycelium that binds to the legumes into a compact cake that holds together (Starzyńska-Janiszewska, Stodolak & Mickowska, 2014). Tempeh can also be made with various other legumes, and cereals (Alminger, Eklund-Jonsson, Kidman, & Langton, 2012; Starzyńska et al., 2014), has an increased protein and fiber content compared to unfermented legumes, and is lower in fat and carbohydrates (Ahmad, Nassar, Mubarak & El-Beltagy, 2008). Its high protein content makes it a popular meat substitute amongst vegetarians according to Chang, Hsu, Chou, Chen, Huang, Chung (2009). In addition, tempeh contains phytochemicals, bioactive substances and a reduced amount of oligosaccharides, which in turn reduce flatulence and increase digestibility (Soka, Suwanto, Sajuthi & Rusmana, 2014). Due to its high folate content, tempeh can also help with memory

preservation (Mo et al., 2013). Ahmad, Ramasamy, Jaafar, Majeed & Mani (2014) supported this claim but added that tempeh consumption also helped reverse amnesia, improved cholinergic activities and reduced neuro-inflammation in the brain. Starzyńska-Janiszewska et al. (2014) stated that in addition to the reduction of oligosaccharides and tannins, antioxidants and protein bioavailability were improved because the bean is broken down during the fermentation process. In addition, Chang et al. (2009) indicated that it could carry vitamin D2 while Craig (2009) noted that it can also contain bioavailable iron.

During tempeh fermentation, there is interaction between molds, yeasts and bacteria (Soka et al., 2014). In addition to the fungus *Rhizopus oligosporus*, the bacteria *Klebsiella pneumoniae* can also be present and is responsible for creating vitamin B₁₂ according to Ayu et al. (2015). Mo et al. (2013) indicated that *Citrobacter freundii* can also be a vitamin B₁₂-forming bacteria. Therefore, contaminating bacteria [*Klebsiella pneumoniae* or *Citrobacter freundii*] is necessary to synthesize vitamin B₁₂ in tempeh (Chamlagain et al., 2015; Ayu et al., 2015). Researchers have noted that tempeh can contain between 0.7-8.0 microgram per 100 grams (Watanabe et al., 2013) and 0.04-0.13 microgram per 100 grams (Mo et al., 2013).

Creating bioavailable plant-based vitamin B₁₂ sources

In a recent study produced by Madhu et al. (2010), lactic acid bacterial strains contaminated by vitamin B₁₂ produced bioavailable vitamin B₁₂. Another study published by Molina et al. (2012) also created vitamin B₁₂ using lactic acid bacteria (*Lactobacilli*) but in soymilk. They indicated that this was able to prevent symptoms of vitamin B₁₂ deficiency in mice. Chamlagain et al. (2015) have developed a technique that can determine bioavailable vitamin B₁₂ quality from pseudovitamin B₁₂. They claim that “a nutritionally relevant amount of active vitamin B₁₂ was produced by *Propionibacterium freudenreichii* in rye and barley malt matrices.”

Method

A quantitative survey was carried out in the municipality of Stockholm. Surveys were handed out instead of sent online in order to reach a broader audience and receive immediate data. This study was conducted between March 30th, 2015 and May 29th, 2015. The authors contacted the legume company GoGreen to provide samples of beans for the subjects responding to the surveys.

The surveys were handed out on weekdays at the same exact time, between the hours of 16 and 20 in all four locations. The inclusion criteria was all adults over 18 years of age residing within the municipality of Stockholm. The exclusion criteria included individuals under the age of 18 and individuals not residing within the targeted geographic area. Surveys from individuals under the age of 18 years were rejected when sorting out the data. A sample of 195 subjects was selected using a cluster sampling technique to reflect the heterogeneity of the total population. Four districts within Stockholm were selected: *Östermalm*, *Södermalm*, *Hässelby-Vällingby* and *Rinkeby-Kista* and were chosen based on income and foreign-to-national inhabitant ratio to represent a variety of consumers in the Stockholm municipality (Figure 1 and 2).

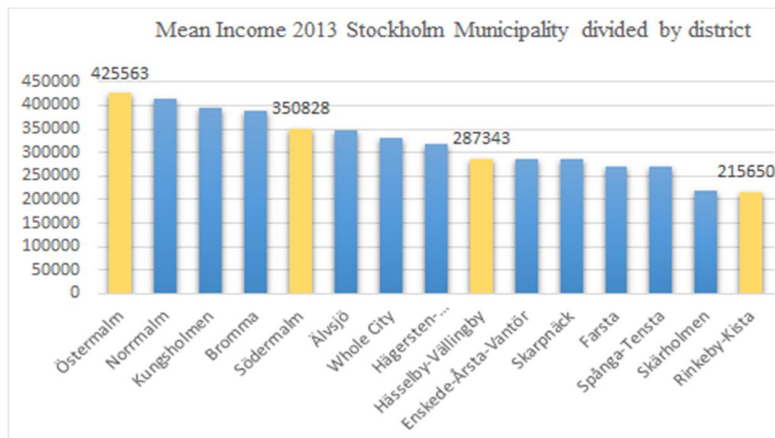


Figure 1: Mean Income in the districts of Stockholm (Statistik om Stockholm, 2014)

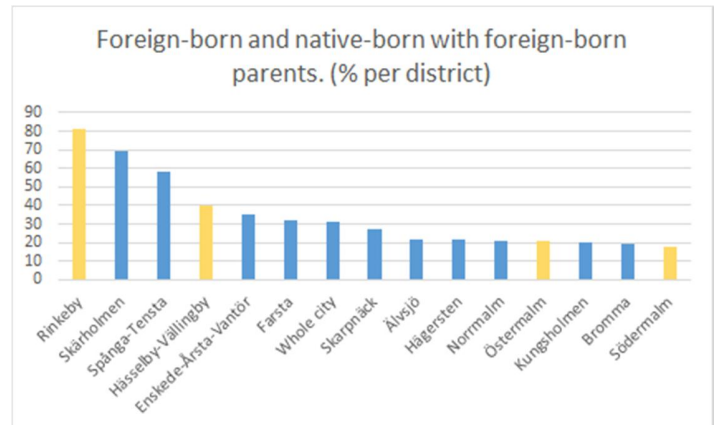


Figure 2: Foreign-born and native-born with foreign-born parents (Statistik om Stockholm, 2014)

Östermalm was found to have the highest mean income and one of the lowest amounts of foreign inhabitants, Södermalm relatively high income and the lowest amount of foreign inhabitants. On the other hand, Hässelby-Vällingby had a middle-low income and fairly high foreign inhabitants while Rinkeby-Kista had the lowest mean income and had the highest amount of foreign inhabitants.

Furthermore, an expert interview with a marketing manager of one of the biggest grocery retail companies in Sweden identified Hässelby-Vällingby as a district with a high percentage of families with children and the district of Södermalm with a lot of individuals with “alternative styles” and vegetarians (personal interview 16 April 2015). To include a variety of supermarkets for the sample, two out of the four suggested supermarkets were chosen in addition to two other competing supermarkets within the same districts. In total, four food stores were chosen for the sample: Coop Vällingby, Coop Östermalm, Hemköp Hornstull Galleria, Rinkeby Matcenter.

The questionnaires were distributed within the supermarkets using convenience sampling technique to collect as many participants as possible. In total 195 questionnaires were gathered (Coop Vällingby - 48 subjects, Coop Östermalm - 57 subjects, Hemköp Hornstull Galleria - 54 subjects, Rinkeby Matcenter - 36 subjects). In collaboration with the brand GoGreen (Lantmännen), samples of dry black beans and white beans were distributed to people responding the questionnaires.

Questionnaire design

All of the questions were constructed as multiple-choice questions except demographic questions. Beforehand a pilot study with fifteen respondents was conducted using the convenient sample method, which provided comments and insights regarding the formulation of the questions and the outline of the questionnaire. Adjustments were thereafter made until the questionnaire reached its final form (Appendix 1). The questionnaire included 15 questions regarding choice of diet, consumption of legumes, knowledge of vitamin B₁₂ and knowledge and interest in tempeh.

The results were collected, analyzed and graphed using Microsoft Excel and Statistical Package used for Social Sciences (SPSS) The data was analyzed using descriptive analysis.

Ethical aspects

Ejlertsson & Axelsson (2005) mention that according to the Swedish Research Council, there are four research principles that need to be addressed when conducting a study: information, consent, confidentiality and use. The information principle requires informing the participants about the study, its purpose and that it is voluntary. The consent principle states that answering the survey is a way to show respondents' consent to participate and that participants under the age of 15 would require their guardian's consent. The confidentiality principle ensures that the respondents' identity will not be revealed or used for other purposes. Lastly the use principle states that the information collected in the study should be used only for the study and for no other purpose. In the introduction part of the questionnaire the respondents are informed of the purpose of the study and about the confidentiality of their answers. Respondents were participated voluntarily and no guardian consent was needed as everyone was over the age of 18.

The results gathered throughout the questionnaire were strictly used for the utilization of this thesis, which aims to provide public accessible information (Ejlertsson & Axelsson, 2005). The respondents could include contact details (e-mail address) in order to receive the study results.

Literature

Background research was conducted with the help of the search tools Uppsala University library, PubMed, Scopus and Google Scholar with a 10 year search criteria in order to find the most recent studies. Six keywords were used (vitamin B₁₂, cobalamin, fermentation, legumes, tempeh, Sweden) in different combinations. Relevant references from these publications where also used, despite some being older than 10 years.

RESULTS

Of the 195 subjects in this study, 68 were men and 127 were women. Their average age was 44 and there was a normal distribution among the ages. Ages ranged from 18 - 85. All lived in the municipality of Stockholm.

Omnivores are still the majority

A majority (74 %) of the subjects were omnivores. A complete list of the different diets can be seen in Figure 3 and more specific results regarding diets and geographical area can be seen in Table 5. One subject did not answer this question and therefore his/her diet cannot be specified.

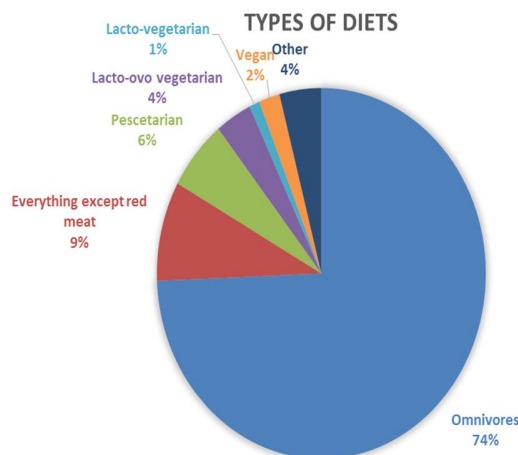


Figure 1: Types of diets among 194 respondents in Stockholm city

Table 5: Types of diet among the 194 respondents within the four areas of Stockholm

Type of Diet	Östermalm	Södermalm	Rinkeby-Kista	Hässelby-Vällingby	Total	n=(%)
Omnivore	47	39	24	35	145	(74.7)
Everything except red meat	4	5	1	7	17	(8.8)
Pescetarian	3	5	2	2	12	(6.2)
Lacto-ovo-vegetarian	2	4	0	1	7	(3.6)
Lacto-vegetarian	0	0	0	1	1	(0.5)
Vegan	1	0	2	1	4	(2.1)
Other	0	0	7	1	8	(4.1)
Total	57 (29.4)	53(27.3)	36 (18.6)	48 (24.7)	194	(100)

Vitamin B₁₂ awareness

Almost half of the subjects (48%) responded that they thought about obtaining vitamin B₁₂ in their diet. Of these, seven lacto-ovo-vegetarians, one lacto-vegetarian and three vegans answered while one vegan indicated that he/she did not think about it. Out of the 30 elderly subjects (65 years and older) 16 thought about obtaining vitamin B₁₂ in their diet while 12 did not. Two elderly subjects did not answer this question.

Little knowledge regarding vitamin B₁₂

The majority of the subjects answered incorrectly regarding which food groups contained vitamin B₁₂ (see Figure 4). Either they responded legumes and vegetables (54 %), both food groups (6%), or that they did not know (12%). 27 percent answered correctly that vitamin B₁₂ can be found in meat, eggs and dairy products. Most of the omnivores and people that abstained from eating red meat answered incorrectly that legumes and vegetables contained vitamin B₁₂. The lacto-ovo-vegetarians, lacto-vegetarian and vegan claimed that they knew what sources contained vitamin B₁₂ although their answers varied. Five out of the twelve vegetarians and vegan answered correctly (3 lacto-ovo- vegetarians, 1 lacto-vegetarian, and 1 vegan). Six out of 28 (21.4%) elderly knew the correct vitamin B₁₂ food group.

Which of the following groups contains vitamin B₁₂?

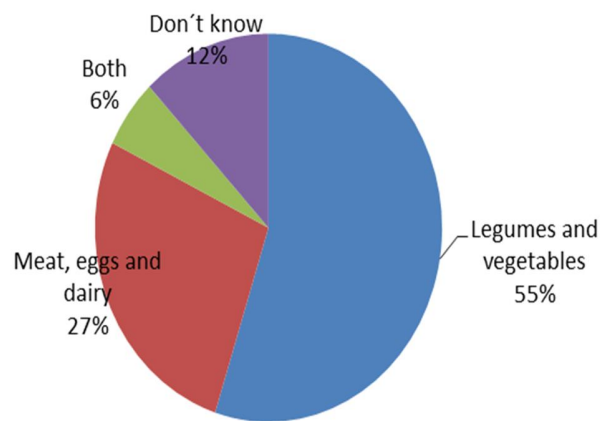


Figure 2: Respondents' responses of the food group that they think contain vitamin B₁₂

Little knowledge regarding vitamin B₁₂ deficiency and risk groups

Although all the choices in the multiple choice questions regarding vitamin B₁₂ deficiency could be marked, only eight subjects identified all the deficiencies. 44 percent did not know the consequences of vitamin B₁₂ deficiency. Answers varied amongst depression (36%), changes in the skin (22%), permanent nerve damage (18%) and late development in children (17%). 35 subjects (18%) stated that vitamin B₁₂ deficiency resulted in permanent nerve damage. Amongst these 35 subjects two were vegans, three were lacto-ovo-vegetarians and seven were elderly (over 65 years old).

The question regarding vitamin B₁₂ deficiency was also a multiple-choice question where more than one answer could be marked. Although all the answers were applicable, most subjects' responses varied regarding the risk groups for vitamin B₁₂ deficiency: alcoholics (33%), vegans (29%), individuals with Celiac disease (15%) elderly (33%). 30 percent answered that they did not know. Eight respondents (4%) chose all the correct alternatives for B₁₂ deficiency risk groups. Three of the eight vegetarians answered that vegans were a risk group. All the 4 vegans answered correctly. 15 subjects (68%) out of the 22 elderly (aged 65+) answered that elderly were one of the risk groups. Ultimately five subjects (2.5%) in the sample answered correctly all the vitamin B₁₂ deficiency consequences and all the vitamin B₁₂ deficiency risk groups.

Moderate legumes consumption amongst the majority within the sample

Almost the entire sample (193 subjects) indicated their legume consumption. Responses varied as can be seen in Figure 5. The figure shows legume consumption frequency and the total amount of subjects grouped by diets. Most answered between 2-3 times a month (44 subjects) to 2-3 times a week (42 subjects). All pescetarians, lacto-ovo-vegetarians, lacto-vegetarians or vegans answered more than 2-3 times a month.

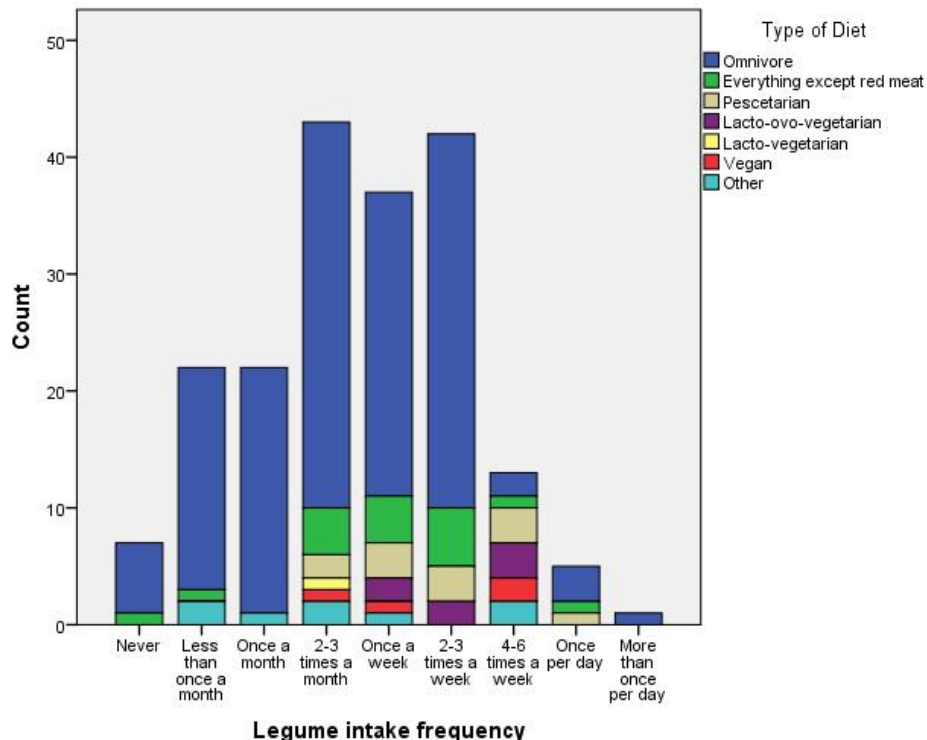


Figure 3: Legume intake frequency divided into subjects consuming different diets

81 of the 193 subjects indicated a medium consumption of legumes (2-3/month to once a week). 61 subjects displayed a high consumption of legumes (2-3/week to several times a day) with Östermalm displaying the highest amount of legume consumers within this group (Table 6). When taking consideration that the amount of subjects in the different areas varied, the percentage of subjects in each area having a low, medium and high consumption of legumes were similar.

Table 6: Consumption of legumes in the studied areas

Area	Low Consumption	Medium Consumption	High Consumption	n= (%) Total
Hässelby-Vällingby	12	21	13	46 (24)
Rinkeby-Kista	10	16	10	36 (19)
Södermalm	15	21	18	54 (28)
Östermalm	14	23	20	57 (29)
Total	51 (26)	81(42)	61(32)	193 (100)

Lentils, chickpeas and kidney beans

Out of 195 respondents, 127 (65 %) ranked their favorite legumes from one through three, the top choice being lentils, second chickpeas, and third kidney beans. Although the remaining 68 subjects did not rank the legumes, lentils, chickpeas and kidney beans were still the most popular legumes amongst the sample, lentils being selected 100 times, chickpeas 98 times and kidney beans 81 times without any order of preference. The popular legumes can be seen in Figure 6.

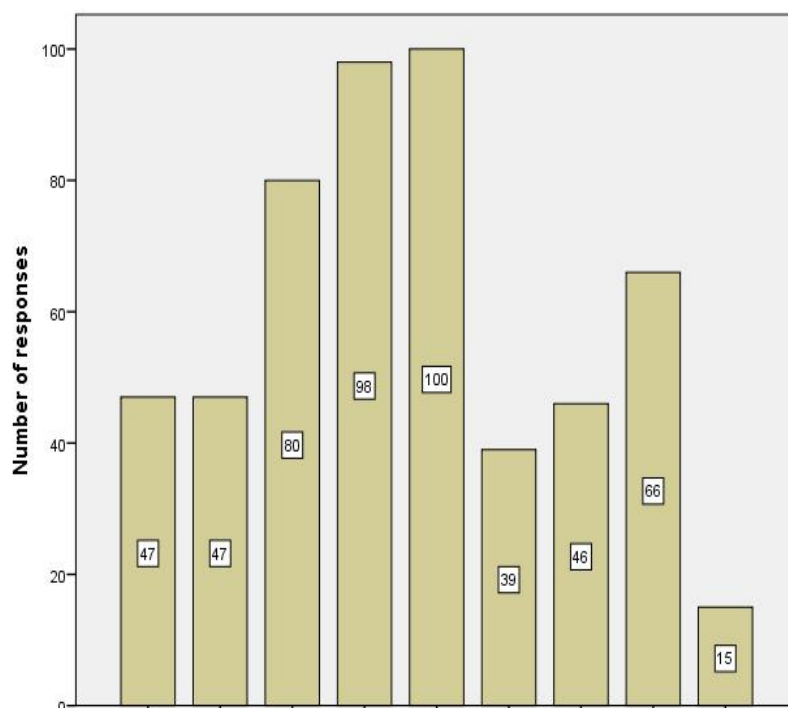


Figure 4: Popular legumes according to the subjects without any order of preference. Several choices could be selected

Local versus organically produced legumes

The subjects were asked to gage the most important factors when purchasing legumes. Of the 109 subjects that ranked the most important factor, 51 percent chose organically grown legumes (see Figure 7). Of the 100 subjects that ranked their second most important factor, 25 percent ranked locally produced beans and 28 percent of the 94 subjects that chose their third most important factor mentioned price.

The most important factors when purchasing legumes

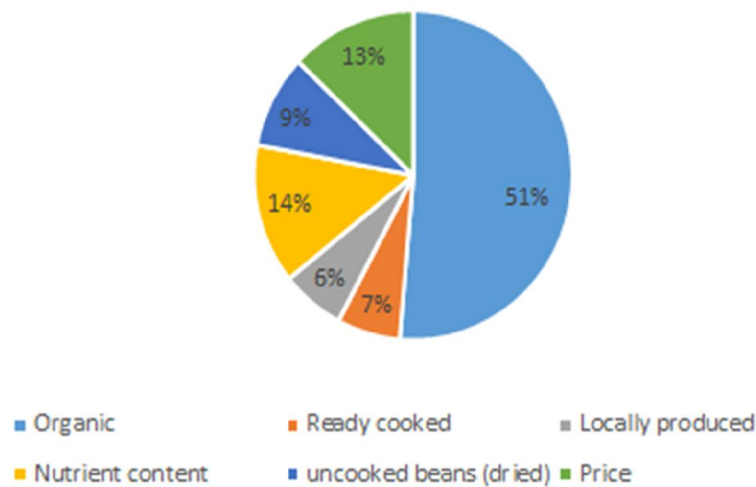


Figure 7: The most important factors when purchasing legumes

When including the subjects that did not rank but instead just marked their choices, organically grown legumes were still the most important factor when purchasing legumes. In the question requesting their preference for local or imported organic legumes, some interesting results were found: 34 out of the 56 subjects that ranked organic legumes as their most important choice ended up preferring locally produced *non-organic* legumes over imported organic legumes. The most popular brands were GoGreen (20%) and store brands (19%).

Positive attitudes towards tempeh

Overall, the subjects were interested in trying tempeh despite not having heard of it before. Out of the 194 subjects that responded the question, 162 (83.5%) had never heard of tempeh before and of them, 130 (80%) were interested in trying tempeh with vitamin B₁₂. The remaining 32 that previously heard about tempeh were mostly positive towards trying it (87.5%).

DISCUSSION

This paper explores the functionality of legumes, gives an overview of vitamin B₁₂ and its sources and researches tempeh and its nutritional benefits. This study investigated knowledge about vitamin B₁₂, legume consumption, and interest in tempeh for indications to introduce tempeh with vitamin B₁₂ in the city of Stockholm. The majority of the subjects had a moderate consumption of legumes and was open to trying tempeh even though they had never heard about it. Although their knowledge of vitamin B₁₂ was limited, the biggest risk groups, vegans and the elderly, were aware that they were a risk group, however, only a minority of them knew the correct vitamin B₁₂ food sources.

Methodological discussion

The sample used within this study was chosen from four different geographical areas within the city of Stockholm using a cluster sampling technique to reflect the heterogeneity of the total population. Participants were selected in various grocery stores using convenience sampling technique which means that the study is not as representative as it could have been if random sampling technique was used instead. The city of Stockholm was chosen as the particular geographical area since, being a capital city, has the potential entry market for new products such as tempeh. The pilot study involving 15 individuals was composed of family members, friends and acquaintances using the convenience sample technique. The study would have been more reliable if it was comprised of randomly chosen individuals since it would have reflected the knowledge level of the population more accurately in order to create a more reader-friendly and valid survey.

The questionnaire in detail

The first three questions were intended to give demographic information. An exact age was requested instead of the age range to make sure that no inappropriate intervals were chosen (Ejlertsson & Axelsson, 2005). A literature overview concluded that it was interesting to further analyze elderly people over 65 years of age (Gröber et al., 2013; Obeid et al., 2004).

The fourth question used a nominal scale to classify the respondents in different groups based on their diet choices. A seven-point scale was used to mimic the survey carried out in 2010-2011 by the Swedish National Food Consumption Survey (Amcoff et al., 2012). However, the definition of Clarys et al. (2014) was used for a pescetarian (no meat, but eats fish and seafood) instead of a lacto-vegetarian that sometimes eats fish, which is a definition originally used in the Swedish National Food Consumption Survey 2010-2011 (Amcoff et al., 2012).

Questions five through eight were used to measure knowledge of vitamin B₁₂. While question five and six only have one possible answer, the remaining questions seven and eight have several answer possibilities. The questions requesting more than one answer seemed to have confused the subjects. Many of them marked only one of the options even though they could have answered all the choices that applied, which suggest that the instructions could have been clearer. In addition, it is difficult to know whether their answers were guessed or whether they genuinely measured their knowledge, which also questions the validity of the study. Question five asked the subjects if they thought about obtaining vitamin B₁₂ in their diet, which revealed itself to be confusing. The authors had wanted to find out if the subjects consciously thought about vitamin B₁₂ in their daily life; this question could have been formulated more precisely.

The ninth question aims to investigate the respondents' consumption of legumes with an ordinal scale. Inspired by prior studies measuring consumption of products, a nine grade scale was used (Fred Hutchinson Cancer Research Center, n.d; Amcoff et al., 2012). A higher grading scale has been found to provide better results (Wärneryd, 1990). The response options were designed to be mutually exclusive and not overlap (Trost & Hultåker, 2007; Wärneryd, 1990). The consumption choices were grouped into three categories: low (never to once a month), medium (2-3 times a month to 2-3 times a week) and high (4-6 times a week to more than once a day) for a more efficient analysis. The subjects that answered that they never ate legumes were asked to continue onto question 14 since questions 10-13 were designed to measure attitudes and preferences regarding legume consumption. A question asking why they did not eat legumes could have been asked for those that never ate legumes, as tempeh contains more digestive properties than regular legumes.

Question 10 and 11 were ranking questions about legume and purchasing preferences where up to three answers were requested. It was interesting to know the subjects' legume preferences since tempeh can be produced with various legumes (Ahmad et al., 2008). The amount of requested ranks were limited to three as multiple choices could have been difficult for the subjects (Ejlertsson & Axelsson, 2005). However the question seemed to be misunderstood by many of the subjects. Many of them failed to rank the questions and marked them instead, which prevented seeing a clear ranking amongst the entire sample. That the question was ambiguous challenges the reliability of the question. However, most of the subjects marked their preferred choices regardless of ranking, which still provided plausible data.

An even amount of choices could have prevented the subjects from choosing the middle alternative in question 10, which is an odd number scale (Ejlertsson & Axelsson, 2005). Lentils were the most popular legume and were in fact in the middle of the scale. This could be due to the odd scale or that they did indeed prefer them.

Finally question 14 and 15 investigated the knowledge and interest for tempeh. Even though most of the subjects had not heard about tempeh before, they answered that they were willing to try it. While this reflects their openness in trying new products, offering tempeh samples would have provided a sensory experience and a better indication of their preference. There were some participants who expressed that they did not understand the word "fermentation," which expresses that the question could have been more simplified to provide more reliability.

Limitations

Since bean samples were handed out to the subjects answering the questionnaire, most of the participants in the study could have been interested in legumes or complementary goods. This could have affected the results as it probably included more legume consumers than otherwise. Seeing the bean samples could have influenced the subjects' legume intake where they could have indicated consuming legumes more often than they actually did. This might not measure their actual legume consumption. Since the survey was written in Swedish, there could have been language barriers for people that did not have Swedish as their native tongue. Even though every effort was made to translate the survey in those instances, there could have been misunderstandings and misinterpretations. Ultimately, these factors could have attracted mostly native Swedish speakers that could have reacted more positively towards legumes. The fact that the survey took place in a supermarket during rush hour when people possibly did not have substantial time to answer the questions can have provided questionnaires with random answers

and the same type of age group. On the other hand, the distribution of surveys during rush hour allowed for a bigger variation of subjects and provided an even distribution of ages. As the questionnaire consisted of 15 extensive questions, it could have been tedious for the subjects to answer. In addition, memory, or lack thereof, could have played a role in how they responded to various questions such as legume frequency. Based on the short timeframe of the study, validity and reliability cannot be assessed.

Literature overview

The references used were predominantly journal articles published within the recent ten years. A snowball effect occurred especially when certain topics provided limited results. Therefore the search criteria became more flexible revealing relevant results from older studies. However, the articles that were most recently published were most preferred especially in regards to researching plant-based vitamin B₁₂ sources as they measure the bioavailability of vitamin B₁₂ values most accurately (Chamlagain et al., 2015).

Results discussion

The majority (74%) of the sample were omnivores although the amount of vegetarians (vegans, lacto-vegetarians and lacto-ovo-vegetarians) in the sample were doubled (6 %) compared to the numbers presented by the latest Swedish National Food Consumption Survey, 2010-11 (Amcoff et al., 2012). In Sweden, the daily average intake of vitamin B₁₂ is 5 micrograms for women and 6 micrograms for men, however, these figures are based predominantly on omnivores (Amcoff et al., 2012) and an increase in vegetarians could change these numbers. Most of the subjects did not know the food groups where vitamin B₁₂ could be found nor the risks or deficiencies concerning the vitamin. While it may be a problem to not know the source of an essential nutrient, it can be argued that people do not need to know it if they eat meat and/or fish regularly. It is predominantly when they age that they become a risk group due to malabsorption (Gröber et al., 2013). However, vegans and the elderly answered correctly that they were considered a risk group for vitamin B₁₂ deficiency. This is important as vegans and the elderly are considered to be the largest risk groups (Nordic Council of Ministers, 2014). Legume consumption was moderate with most subjects consuming them from two to three times a month to two to three times a week. These figures could have been affected by the bean samples that were given away when answering the survey. Most of the subjects chose organically grown legumes to be the most important aspect when purchasing legumes but the majority ended up selecting locally grown legumes in the question comparing them with imported organically grown ones. This indicates that there can be an interest for more locally produced legume products in the future.

The majority was positive towards trying tempeh regardless if they had heard of tempeh. It is difficult to judge the sample's acceptance of the product and consider its reliability since they did not receive a taste test. On the other hand, if resources had permitted it, a simple sensory analysis would have administered a more valid response.

Since tempeh can be produced by a variety of legumes (Ahmad et al., 2008), the favorites can become potential products. The most preferred legumes were lentils, chickpeas and kidney beans. The most popular brands were GoGreen (20%) and store brands (19%). This could reflect the fact that GoGreen bean samples were given away to those responding to the survey. The bean samples attracted subjects and provided additional responses but they could have also influenced the results.

Tempeh

The Swedish Food Agency, Livsmedelsverket has no mention of tempeh's nutritional implications on their website and this study displays that most people in Stockholm have never heard about tempeh. However, most of them were interested in trying it and also displayed that they consume a moderate amount of legumes. Given this study's results, tempeh production in Sweden could be produced with Swedish legumes to meet the consumers' demands for locally produced legumes.

New techniques in measuring the bioavailability of vitamin B₁₂ have recently surfaced (Chamlagain et al., 2015). There has been considerable focus on finding sources that indicate the bioavailability of tempeh for this paper, but the results have been limited. Watanbe et al. (2013) and Mo et al. (2013) appeared to indicate contrasting vitamin B₁₂ figures in tempeh: Watanbe et al. (2013) found that it can contain 0.7-8.0 micrograms per 100 grams, stating that a higher amount can be achieved only if the right bacteria is present. While their study reviews plant-based bioavailable sources, it appears that they have not investigated the bioavailability of tempeh. Mo et al. (2013) analyzed tempeh and found that its bioavailability was 0.016-0.072 microgram per 100 grams, which is considerably less than what Watanbe et al. (2013) indicated. This could be because Mo et al. (2013) did not use the vitamin B₁₂ producing bacteria in their study. Admittedly these results display an uncertain amount of vitamin B₁₂. Ultimately, more studies are needed to gage a more accurate picture of the bioavailability of vitamin B₁₂ in tempeh, ferment it with *Klebsiella pneumoniae* or *Citrobacter freundii* to ensure that vitamin B₁₂ will be present, and also attempt to increase the vitamin B₁₂ quantity to make it a reliable plant-based vitamin B₁₂ source. In addition, a sensory analysis, consumer test and production efficiency also need to be addressed in the future to bring tempeh with vitamin B₁₂ onto the market.

Regardless of whether tempeh can be fermented with substantial amounts of vitamin B₁₂ bacteria or not, its overall digestibility (Mo et al., (2013), abundance in nutrients and fiber. This can make it a potential meat alternative, especially as the mycelium (bacteria mold) binds the legumes into a cake that holds together (Starzyńska-Janiszewska et al., 2014) which can then be treated, among other things, as a slab of meat, crumbled into a burger or sliced into "bacon."

An important factor to consider is that growing legumes are beneficial to the environment. In Sweden, USA and Canada, non-governmental organizations have attempted to reduce meat consumption due to the environmental dilemma concerning meat (Laestadius et al., 2013). In addition, as there is an increased interest in vegetarian food (Livsmedelsverket, 2015; Craig, 2009) means that there is a necessity to address essential vitamins like vitamin B₁₂, which are usually lacking in plant-based diets. Although fortified foods and supplements are recommended (Nordic Council of Ministers, 2014) plant-based products with essential nutrients, such as vitamin B₁₂, should be highlighted since not all people prefer taking supplements or fortified products.

A shortcoming of our study was the negligence in asking the subjects if they wanted to reduce their meat intake, as this is an actual concern for the environment today (Laestadius et al., 2013; Hawkes, 2014). This question could have helped link the fact that tempeh can be treated as a meat substitute due to its essential nutrients such as protein, iron and potential to carry vitamin B₁₂. Moreover, in regards to legume consumption, a question could have asked why the subjects do not consume them more frequently. Many people avoid legumes due to stomach discomfort (Messina 2014) which is another factor that tempeh can address due to its reduction in

oligosaccharides (Soka et al., 2014). Future studies could focus on whether people would consume more legumes if they could digest them better.

Thesis Results in relation to the Dietetic Profession

The results of this study indicate a moderate level of legume consumption. Due to legumes' environmental and health benefits, they can be recommended as a meat alternative for those who want to reduce their meat consumption. The fact that the majority of the subjects did not know where vitamin B₁₂ could be found or what its deficiencies led to expresses that there is a need for educating the public on essential nutrients, especially as vegetarian food is becoming more popular. Furthermore, while most academic studies claim that vitamin B₁₂ can only be found in animal products, new research indicates progress in plant-based vitamin B₁₂ sources (Chamlagain et al., 2015; Molina et al., 2012). Additional research is needed to investigate them and also experiment with vitamin B₁₂ in tempeh made from different legumes. While this may take considerable time, legumes eaten after fermentation, like tempeh, can break down bioactive compounds, increase nutrient absorption and relieve digestive discomfort more than eating normally prepared legumes and should therefore be promoted to the public.

CONCLUSION

The aim of this thesis was to investigate consumers' awareness of vitamin B₁₂ and explore the indications for the possibility to introduce tempeh, a fermented legume product. Knowledge regarding vitamin B₁₂ was limited among the subjects. Legume consumption was fairly regular, which displays opportunities to introduce new products with legumes, especially as the participants appeared to be positive towards trying tempeh. While there are limited studies on tempeh with bioavailable vitamin B₁₂, new research on bioavailable plant-based vitamin B₁₂ provides promise that vitamin B₁₂ can be potentially bioavailable in tempeh and/or other plant-based products. There is a need for them especially when considering the environmental dilemma surrounding meat and also simultaneously the increase in vegetarian diets. Regardless of whether tempeh can be inoculated with vitamin B₁₂ or not, it is more digestible than unfermented legumes, which means that it can be suitable for a broader population than just vegans or vegetarians.

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APPENDIX 1: Work distribution

The authors evenly distributed the work for planning, performing the study, literature research, data collection, analysis and in writing the paper.

Planning of the study and the the thesis

50/50

Literature research

50/50

Data collection

50/50

Analysis

50/50

Writing of the paper

50/50

APPENDIX 2: Questionnaire

En studie om kostvanor & vitamin B12

Uppsala Universitet, Kostvetarprogrammet.

Hej! Vi uppskattar att du tar några minuter för att svara på 15 frågor om mat. Resultatet kommer att användas för att genomföra en kandidatuppsats om kostvanor och baljväxtkonsumtion vid Institutionen för Kostvetenskap, Uppsala Universitet. Alla svar är anonyma och kommer att behandlas konfidentiellt. Om du vill ta del av studien var god fyll i din e-mail adress nedan:

1. Kön

Kvinna

Man

Annat

2. Bor du i Stockholms Län?

Ja

Nej

3. Ange din ålder: _____

4. Vilken typ av mat äter du? (Kryssa i det alternativ som passar dig bäst)

All sorts mat (Allätare) <input type="checkbox"/>	Allt utom rött kött <input type="checkbox"/>	Pescetariskt, dvs. inget kött men äter fisk och skaldjur <input type="checkbox"/>	Lakto-ovo-Vegetariskt, dvs. inte kött, fågel, fisk <input type="checkbox"/>	Lakto-Vegetariskt, dvs. inte kött, fågel, fisk eller ägg <input type="checkbox"/>	Vegankost, dvs. inte kött, fågel, fisk, ägg eller mejeriprodukter <input type="checkbox"/>	Annat <input type="checkbox"/>
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5. Tänker du på att få i dig tillräckligt mycket vitamin B12 i din kost?

Ja

Nej

6. Vilka av följande produkter tror du innehåller vitamin B12?

Baljväxter och grönsaker

Kött, Mjök och Ägg

Vet ej

7. Vad tror du att vitamin B12 brist kan leda till? *Flera alternativ är möjliga. Välj det du tycker bäst överensstämmer.*

Depression

Hudförändringar

Permanenta nervskador

Sen utveckling hos barn

Vet inte

8. Vilka grupper tror du har risk för att få vitamin B12 brist? *Flera alternativ är möjliga.*

Alkoholmissbrukare Personer med Celiaki (Glutenintolerans) Veganer Äldre Vet inte

9. Hur ofta äter du baljväxter (Bönor, linser, kikärtor, gula ärter, etc.)

Aldrig Mindre än 1 gång i månaden 1 gång per månad 2-3 gånger per månad 1 gång per vecka 2-3 gånger per vecka 4-6 gånger per vecka 1 gång per dag Mer än 1 gång per dag

Om du svarat "aldrig", var god hoppa över till fråga nr 14

10. Vilka av följande baljväxter gillar du bäst? *Välj max 3 stycken.* Rangordna dom efter preferens från 1-3, där 1 är den du tycker mest om.

Bruna bönor Gula ärter Kidneybönor Kikärtor Linser Sojabönor Svarta bönor Vita bönor Annan sort

11. Vad är viktigt för dig när du handlar baljväxter? *Välj de tre viktigaste för dig.* Rangordna dom 1-3, där 1 är den *viktigaste* och 3 är den minst viktigaste.

Ekologiskt producerad	Färdigkokta	Lokalt producerad	Näringsinnehåll	Okokta, dvs. torkade	Pris	Varumärke	Vet ej <input checked="" type="checkbox"/>
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12. När du köper baljväxter, vilket varumärke väljer du?

Egna märkesvaror (ex. ICA (ICA, ICA Basic), COOP (COOP, xtra, Änglamark)) GoGreen Risenta Saltå Kvarn Zeta Annat Vet ej

13. Vilket av följande två alternativ skulle du föredra? (markera ett alternativ)

Importerade *Ekologiska* baljväxter Lokalt producerade baljväxter

14. Här du hört talas om Tempeh (fermenterad sojabönskaka)?

Ja Nej

15. Skulle du vara intresserad av att prova en fermenterad produkt gjord på baljväxter som även innehåller vitamin B12?

Ja Nej

Tack för din medverkan!