Sveriges lantbruksuniversitet
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Department of Molecular Sciences
Master´s thesis • 30 hec • Second cycle, A2E
Agriculture Programme - Food Science
Molecular Sciences, 2017:10
Uppsala 2017

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Credits: 30 hec
Level: Second cycle, A2E
Course title: Independent project/degree project in Food Science - Master's thesis
Course code: EX0425
Programme/education: Agriculture Programme - Food Science

Place of publication: Uppsala
Year of publication: 2017
Title of series: Molecular Sciences
Part number: 2017:10
Online publication: http://stud.epsilon.slu.se

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Abstract

Legumes are nutritious and contribute to a sustainable food production system. In Sweden, brown beans (*Phaseolus vulgaris* L) are cultivated on Öland. Vitamin B_6 is one of many nutrients found in brown beans and it works as a coenzyme in the metabolism in the human body. Vitamin B_6 consist of pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM) and their phosphorylated forms PL-5-phosphate, PN-5-phosphate and PM-5-phosphate. Pyridoxine is in plants glycosylated (pyridoxine-5-β-D-glucoside) to 5-80 percent and this form has a reduced bioavailability in humans. The aim of this study was to determine the content of vitamin B_6 in brown beans from Öland and to find factors which influence the content. Consideration was taken to the factors cultivars, year of cultivation, geographical areas, and different types of fertilizer. The total-, free-, glycosylated vitamin B_6 as well as the water content were determined. In total 25 samples were analysed with HPLC with fluorescence detector. The content of vitamin B_6 in brown beans was 0.32 ± 0.02 mg/100g and on average 49 percent was glycosylated. There was no difference in the content of vitamin B_6 between cultivars, geographical areas or types of fertilizer, but there was a difference between years. Vitamin B_6 was higher 2008 compared to 2009. Glycosylated vitamin B_6 was higher 2008 and 2010 compared to 2009 meanwhile there was no significant difference in free PN (all vitamers except glycosylated PN). The meteorological weather conditions were less beneficial for the beans 2008 and 2010 compared to 2009. Thereby, during less beneficial meteorological weather conditions, the content of vitamin B_6 increased by increased content of glycosylated pyridoxine. The water content was significantly higher in Katja compared to Karin, indicating on better water holding capacity (WHC). For Katja, there was a significant difference between years and water content. The chemical composition between cultivars and in cultivars between years (affected by weather) must be elucidated for an explanation of the difference in WHC.

Keywords: Brown beans from Öland, vitamin B_6, glycosylated PN, cultivar, year and water content.

Sammanfattning

Baljväxter har ett högt näringsvärde och bidrar till en hållbar livsmedelsproduktion. I Sverige odlas bruna bönor (*Phaseolus vulgaris* L) på Öland. Vitamin B_6 är ett av många näringsämnen i bruna bönor och det fungerar som ett koenzym i metabolismen i kroppen. Vitamin B_6 består av pyridoxal (PL), pyridoxin (PN) och pyridoxamin (PM) och deras fosforylerade former PL-5-fosfat, PN-5-fosfat och PM-5-fosfat. Pyridoxin är i olika mängd glykosylerat i växter (pyridoxin-5-β-D-glukosid). Sett till totalhalten av vitamin B_6 är 5-80 procent glykosylerat i växter. Syftet med studien var att bestämma innehållet av vitamin B_6 i bruna bönor från Öland och hitta faktorer som kan påverka innehållet. Faktorer som inkluderades var sort (Karin och Katja), odlingsår (2008-2010), geografiskt område för odling (Borgholm, Färjestaden, Mörbylånga, Kastlösa, Degerhamn) och typ av gödsel (konst- eller naturgödsel). Den totala, fria och glykosylerade halten av vitamin B_6 bestämdes. Även vattenhalten analyserades. Totalt analyserades 25 prover med HPLC med flourescens som detektor. Bruna bönor innehöll 0.32 $\pm$ 0.02 mg/100g vitamin B_6 och 49 procent var glykosylerat. Det fanns ingen skillnad i innehåll av vitamin B_6 mellan sorter, odlingsområde och typ av gödsel. Det fanns en signifikant skillnad mellan år för total halt av vitamin B_6 respektive glykolyserat pyridoxin men det var ingen signifikant skillnad mellan år för fritt vitamin B_6 (alla vitamerer förutom glykosylerat PN). Total halt av vitamin B_6 var högre 2008 jämfört med 2009. Glykosyserat PN var högre 2008 och 2010 jämfört med 2009. Väderförhållandena (nederbörd och temperatur) var mindre gynnsamma 2008 och 2010 jämfört med 2009. Därav dras slutsatsen att under mindre gynnsamma odlingsförhållanden ökar vitamin B_6. Vattenhalten var högre i Katja än Karin vilket visar på en bättre vattenhållande kapacitet. Den kemiska sammansättningen måste kartläggas för att förklara skillnaden. Katja hade en högre vattenhalt 2009 jämfört med 2008 och 2010. Skillnaden beror troligtvis på att väderförhållanden påverkat den kemiska sammansättningen.

Nyckelord: Bruna bönor från Öland, vitamin B_6, glykosylerat PN, sort, år och vattenhalt.

Abbreviations

HPLC	High pressure liquid chromatography
PL	Pyridoxal
PM	Pyridoxamine
PN	Pyridoxine
PLP	Pyridoxal-5-phosphate
PMP	Pyridoxamine-5-phosphate
PNP	Pyridoxine-5-phosphate
WHC	Water holding capacity

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1 Introduction

The world encounters a challenge to ensure food security and combat malnutrition. There is a malnutrition paradox; around 800 million people suffer from chronic hunger meantime the rate of obese people increase. The term double burden is an increasing problem and refers to a co-existence of both undernutrition and overweight (FAO, 2015). There is also a need for more sustainable food production systems. In 2015 United Nations implemented Agenda 2030 which included 17 goals to obtain sustainable development (United Nations, 2015). These goals aimed at reducing hunger and malnutrition as well as to a sustainable food production, among other objectives.

To gain a sustainable food system and healthy diets FAO (Food and Agriculture Organization of the United Nation) and WHO (World Health Organization) concluded at the international conference of nutrition in 2014 that there is a need to diversify crops including underutilized traditional crops (FAO & WHO, 2014). Two years later the 68th UN General Assembly declared 2016 to be the international year of pulses (IYP). FAO was nominated to facilitate the implementation with collaboration with governments, organizations, nongovernmental organizations and relevant stakeholders. The aim was to highlight the nutritional and environmental benefits of pulses and to promote further global production (FAO, 2016). The IYP is in accordance with Agenda 2030, to focus on seeds for sustainability in order to end hunger while protecting the environment (FAO, 2016b).

Sweden has formulated a food strategy which aims to increase food production with focus on sustainability and to support the native food production (Proposition 2016/17:104). Legumes are nutritious and more sustainable to grow than many other crops (FAO, 2016). In Sweden, different type of legumes are grown such as broad beans, peas, lupins and the brown bean from Öland

(Fogerfors, 2015). The brown bean from Öland has by EU a protected geographical indication (PGI) ‘Bruna bönor från Öland’. The designation is for products where either the production, processing or preparation occurs within the geographical area (Livsmedelsverket, 2017b). The areal for producing the brown bean from Öland in Sweden is increasing. There are also test cultivations of other types of beans in Sweden (Fogelberg, 2008). Vitamin B_6 is one of many important nutrients in legumes. Vitamin B_6 is an important coenzyme in the metabolism for humans, it is especially involved in the conversion of amino acids (Combs, 2008).

Thereby, there are both political and health implications to investigate the content of vitamin B_6 in brown beans on Öland. Previously, there are no studies investigating how factors such as cultivars, year of cultivation, geographical area and type of fertilizer can affect the content of vitamin B_6.

2 Background

2.1 Legumes

The family Leguminosae or Fabaceae is defined by its podded fruit and its ability to form nodules with *rhizobia*. Leguminosae is divided in sub-families; Caesalpinioideae, Papilionoideae and Mimosoideae. Edible legumes are mainly found in Papilionoideae (Maloy, 2013). The term pulse refers to the dried seed. Crops harvested green are instead classified as vegetable crops (green bean, green peas). Also excluded are crops mainly used for oil extraction e.g. groundnuts and soy beans and crops solely used for sowing e.g. seeds of clover (FAO, 2015b).

Scientific and common names for commonly consumed pulses are (FAO, 2017):

Phaseolus vulgaris L	Beans
Vicia faba L	Broad bean
Vigna sinensis L	Cow peas
Cicer arietinum L	Chickpea
Lens esculenta Moench	Lentils
Cajanus spp.	Pigeon pea
Vicia sativa L	Vetch
Vigna spp.	Black gram
Pisum spp.	Peas

Phaseolus vulgaris L is considered the most widely grown bean (Siddiq & Mark, 2013). It has the most varied growth habits considering adaptation, maturation and seed characteristics (size, shape and color) (Graham & Ranalli, 1997). Due to the diverse species and cultivars with abilities to grow in different environments, pulses are produced all over the world and the largest producer is India (FAO, 2014b). Beans are sensitive to both too high and low temperatures. There are cool-season crops as lentils, dry peas and chickpeas and warm-season crops as dry bean and soy bean. The cool-season crops can during seed germination handle temperatures down to 0 °C, meanwhile warm-season crops need 5-10 °C (Miller *et al.*, 2002). Beans need 300-500 mm water during their growing period (FAO, 2017b). Legumes can grow in poor soils due to its nitrogen fixation capacity. The optimal pH is 6.5-7.0 but some cultivation areas has a pH of 5.0 (Belay, 2006).

The production of different grains increased with 200 to 800 percent between 1961 to 2012, meanwhile pulses only increased with 59 percent. Production has risen with ~1 percent since the 1960s but the trade of the production has risen significantly, which is indicating an integration of pulses in the world trade (FAO, 2014b). The global production of pulses was in 1961-1963 43.8 million tons and in 2011-13 71.6 million tons (FAO, 2016c).

2.2 Nutritional and health aspects of legumes

Legumes are an important source of food supply with high content of proteins (20-30 percent), carbohydrates (50-60 percent), high content of dietary fibre, low levels of fat and favorable composition of unsaturated fatty acids, and they are low in salt. They have a good composition of proteins, carbohydrates, vitamins, minerals and polyphenolic compounds (Hayat et al., 2014; Reyes-Moreno C & Paredes-López O, 1993; Singh, 2017. The digestibility of bean carbohydrates is reduced due to viscous soluble dietary fibre and high amylose and resistant starch content (Campos-Vega, Loarca-Piña & Oomah, 2010; Zhou, Hoover & Liu, 2004). This prevents elevated glucose levels and beans are considered as low glycemic index (Chung *et al.*, 2008). Legumes contain a great part of essential amino acids but are deficient in sulfur amino acids. Cereals are meanwhile deficient in the amino acid lysine. Since these foods commonly are consumed together, they provide adequate amounts of essential amino acids (Broughton *et al.*, 2003; Siddiq & Mark, 2013). Legumes have been considered a poor man's eat

(Tharanathan & Mahadevamma, 2003) showing their importance in low-income countries where protein malnutrition is a great concern (FAO, 2015). Diets with legumes show beneficial physiological effects in the prevention of chronic and degenerative disease such as cardiovascular disease, diabetes, obesity and cancer (Díaz-Batalla *et al.*, 2006; Jenkins *et al.*, 2002; Key *et al.*, 1999). The health effects are related to the diverse nutrient content, phytochemicals and dietary fibre (Marlett, McBurney & Slavin, 2002; Tharanathan & Mahadevamma, 2003).

However, beans also have undesirable attributes as being enzyme inhibitors, contain lectins and phytates. These factors affect the bioavailability of nutrients in a negative way. Other drawbacks with beans are that they cause flatulence and they require long cooking times (Reyes-Moreno C & Paredes-López O, 1993). Examples of enzymes being inhibited are trypsin, chymotrypsin, substilisin and α-amylase. Lectins are glycoproteins resistant to digestive enzymes and commonly found in beans. They interact with the epithelial cells and reduce nutrient uptake. They can also bind to red blood cell surface proteins causing agglutination. The heat stability of enzyme inhibiting substances and lectins vary but the majority is inactivated by cooking. Phytates can bind with metal ions which decrease the bioavailability of minerals. (Siddiq & Mark, 2013). Germination lead to less loss in nutrients and large loss in phytic acid compared to other processes (Mubarak, 2005; Vidal-Valverde *et al.*, 1998).

2.3 Environmental aspects of legumes

Nitrous oxide is a strong greenhouse gas and the concentration in the atmosphere has since 1750 increased with 17 percent. The increase is attributed to the use of fertilizers, nitrogen fixing crops, manure and fossil fuel combustion (IPCC, 2001). Legumes have a symbiotic relationship with the bacteria *Rhizoba* which fixate nitrogen from the air to the soil. Although the use of nitrogen fixating crops contribute to increased nitrous oxide, it leads to need of less fertilizers compared to other crops, and there will be a lower net emission of nitrous oxide. Since a great amount of the greenhouse gas emission in crop production comes from nitrogen fertilizers, legumes have a lower carbon footprint compared to other crops (Reckling *et al.*, 2014). Legumes also have a positive effect on the soil by contributing with carbon, humus, nitrogen and phosphorus (Stagnari *et al.*, 2017). Legumes have two main roles in cropping systems, fixation of nitrogen (Peoples *et al.*, 2009) and to work as rotation of the cropping system. The benefit to rotate crop cultivation are to prevent diseases, increase microbial diversity and improvement of soils (Stagnari *et al.*, 2017). Pulses have lower water use than many other foods. This is beneficial because the world's freshwater resources are under pressure. The water footprint per gram of protein is for egg, milk and chicken 1.5 times more than for pulses, for beef it is 6 times larger than for pulses (Mekonnen & Hoekstra, 2010).

2.4 Brown beans from Öland (*Phaseolus vulgaris* L.)

Brown beans have been cultivated on Öland since the end of the 19th century. The cultivated types are Bonita, Karin, Katja and Stella I (Föreningen för bruna bönor på Öland, 2017). Karin and Katja are cultivated to largest extent (Lundberg[1]). Brown beans from Öland require well-drained warm soil with a pH 6.5-7.5. Sandy soil and light clay soil are suitable for cultivation. The beans are relatively freeze sensitive and should not be sown when the soil temperature is <10 °C. The time for sowing is normally between 15th of May to 10th of June (Fogelberg, 2008; Fogerfors, 2015). An early spring and a late autumn with high average temperature and low levels of rain in the autumn is optimal for the beans. It is important with ample access to water during germination and flowering. There is little problem with plant rotation diseases (Fogerfors, 2015) and the present system is to grow beans every fifth year. The beans are harvested in September or in the beginning of October (Föreningen för bruna bönor på Öland, 2017). In 1995-2011 the average harvest of brown beans from Öland was 1616 kilo per hectare/year and the average production was 1400 tons per year (Jordbruksverket, 2012). The average areal for production of brown beans in 1996-2013 was 500-100 hectare (Fogerfors, 2015). The beans are dried with different methods which can range between drying on the field to drying in special facilities. They are dried until they have a water content of at most 18 percent and they should be stored with this water content to not crack (Föreningen för bruna bönor på Öland, 2017).

The soil at Öland predominantly consists of moraine, sedimentary rock and sand. Beneath Färjestaden the main soil type is sedimentary rock with limestone. Gotland has the same soil characteristics as Öland (Sveriges geologiska undersökning, 2017). In Skåne there are moraine soils. There have been successful test cultivations of brown beans from Öland both in Gotland and in Kristianstad (Skåne) (Lundberg[1]). Since the beginning of the 21th century in Sweden, there have been

[1] Stefan Lundberg, Föreningen för bruna bönor på Öland, 2017-05-03

successful test cultivations of other type of beans. The possible geographical area for cultivation and increased type of bean cultivation bring possibilities to increase the cultivation of beans in Sweden (Fogelberg, 2008).

2.5 Nutrients in pulses

Nutrients in commonly consumed pulses and brown beans from Öland are shown in Table 1. All pulses are presented as dried except for brown beans from Öland where both the nutritional quantity of boiled and raw beans is presented. The content of energy, water, carbohydrates, dietary fibre, protein, fat, fatty acids (saturated, monounaturated, polyunsaturated), iron, vitamin B_6, folate and salt is presented. The beans included are broad beans, pigeon beans, chickpeas, lentils and brown beans from Öland.

Table 1. *Nutrients in commonly consumed pulses and brown beans from Öland. All values are reported as dried beans except for the brown beans from Öland which is also reported as cooked*

Per 100g	Broad beans[1]	Chickpeas[2]	Lentils[2]	Pigeon peas[2]	Red beans[1]	Brown beans from Öland[1]	Brown beans from Öland (cooked with salt)[1]
Energy (kcal)	318	378	352	343	324	317	137
Water (g)	11.90	7.68	8.26	10.59	10.80	11.20	58.80
Carbohydrates (g)	41.9	62.95	63.35	62.78	48.00	45.10	16.80
Dietary fibre (g)	16.40	12.2	10.7	15.0	14.00	16.4	13.20
Protein (g)	25.0	20.47	24.63	21.70	22.00	22.0	8.75
Fat (g)	1.70	6.04	1.06	1.49	1.50	1.50	0.9
Fatty acids (FA), saturated (g)	0.23	0.603	0.154	0.33	0.22	0.18	0.22
FA, monounsaturated (g)	0.33	1.377	0.193	0.012	0.12	0.11	0.06
FA, polyunsaturated (g)	0.72	2.731	0.526	0.814	0.90	0.87	0.43
Iron (mg)	7.1	4.31	6.51	5.23	6.9	5	2.2
Vitamin B_6 (mg)	0.56	0.535	0.54	0.283	0.56	0.30	0.1
Folate (µg)	423	557	479	456	394	394	59.3
Salt (mg)	30	24	6	17	30	60	470

[1]Values derived from the Swedish National Food Agency (National Food Agency, 2017)

[2]Values derived from the United States Department of Agriculture (US department of agriculture, 2016)

2.6 Vitamins

It was believed that building blocks for animal growth solely consist of carbohydrates, proteins and lipids. In 1912, a famous paper was published with feeding experiments on animals observing growth rates. Based on differences in growth rate it was concluded there were other important substances essential to life than carbohydrates, proteins and lipids (Hopkins, 1912). Another famous paper was published 1912 by Casimir Funk presenting the first isolation of what would later be called thiamine. For years, there had been efforts trying to understand diseases as beri-beri and after many hypotheses they were believed to be due to deficiency in essential substances in food. These substances were by Funk (1912) for the first time mentioned as vitamins.

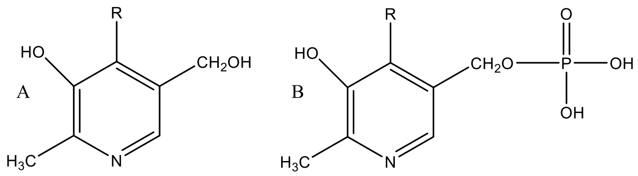
Today vitamins are defined as organic compounds different from carbohydrates, proteins and lipids, a natural component of food, essential for humans to maintain normal physiological functions, they are not synthesized by the host in sufficient amount and they cause deficiency syndrome when not consumed in sufficient amount (Combs, 2008).

After the discovery of thiamine the vitamins were discovered one by one. Today 13 compounds are considered as vitamins. Four fat soluble; vitamin A, D, E and K. Nine water soluble, vitamin C and eight B vitamins; thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folate (B_9) and cobalamins (B_{12}). B-vitamins are coenzymes or precursors to coenzymes for metabolic processes. All B-vitamins are found in a variety of foods except for cobalamins which are mainly found in fermented foods since it is only synthesized in bacteria. It is also found in animal tissue obtained by the intestinal ruminal microflora (Combs, 2008).

2.7 Vitamin B_6

2.7.1 Chemical properties

Vitamin B_6 was discovered in 1934 (Preedy, 2013). The three forms of vitamin B_6 are the vitamers pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their phosphate esters pyridoxal-5-phosphate (PLP), pyridoxine-5-phosphate (PNP) and pyridoxamine-5-phosphate (PMP) (Figure 1). They are soluble in water and the solubility decreases with decreasing polarity. The vitamers' stability fluctuates with pH. They are relatively heat stable in acidic environments but heat labile in alkaline medium (Preedy, 2013). Vitamin B_6 is sensitive to light and especially UV-light (Ball, 2006).



R	Vitamers
CHO	PL, PLP
CH_2OH	PN, PNP
CH_2NH_2	PM, PMP

Figure 1. The structure of vitamin B_6 compounds, the unphosphorylated vitamers (A) the phosphorylated vitamers (B).

Vitamin B_6 is found in small amounts in a variety of foods but especially good sources are meats, whole grain products, nuts and vegetables (Allen, 2006). The most stable form of vitamin B_6 is PN hydrochloride and is the vitamer used in fortification (Combs, 2008). The chemical profile of the vitamin varies in animal and plant foods. PN is the most prevalent vitamer in plant foods and PL and PM are the most common in animal foods. PN is glycosylated in plant foods (pyridoxine-5-glucoside) in a range of 5-80 percent of the total vitamin B_6 content. Other glycosylations of PN has been found, but pyridoxine-5-β-D-glucoside is the most prevalent glycosylated form in plant based foods (Gregory & Ink, 1987; Gregory & Sartain, 1991). It is believed that glycosylated PN maintain the intracellular pool of vitamin B_6 by increasing the stability against stress as light, UV-radiation and heat (Gregory & Ink, 1987; Nishimura *et al.*, 2008). There is no evidence of PL and PM being glycosylated (Gregory, 1998). The bioavailability of glycosylated PN is ~50 percent relative to PN in humans (Nakano, McMahon & Gregory, 1997).

2.7.2 Stability in food

Vitamin B_6 is more sensitive to heat treatment in animal foods compared to plant foods. Losses in plant foods are mainly a result of leaching. Pyridoxal and pyridoxamine are dominant in animal foods and pyridoxine is the main vitamer in plant foods (Eitenmiller & Laden, 1999). Due to enhanced stability because of the glycosylation, pyridoxine has higher retention during food processing (Lešková *et al.*, 2006). Losses of vitamin B_6 in vegetables vary between 10-47 percent and in foods of animal origin 40-58 percent (Lešková *et al.*, 2006). There is greatest loss when cooking compared to steaming, least loss was found when braising (Bognár, 1993). Vitamin B_6 is also affected by storage. For fish, cereals and milk there were 25-30 percent loss after 10 months of storage, and 35-52 percent loss after 40 months (Kirchgessner & Kösters, 1977).

2.7.3 Metabolism in humans

The physiological active form of vitamin B_6 in the human body is PLP and to a less extent PMP. It works as a coenzyme to over 100 reactions, most involving amino acids. All the forms of vitamin B_6 have similar vitamin activity because in vivo they can be converted to PLP (Preedy, 2013). The vitamers are absorbed via passive diffusion in jejunum and ileum. The vitamers with phosphates are dephosphorylated by alkaline phosphatase and when absorbed rephosphorylated by pyridoxal kinase. Pyridoxal cross membranes easier than pyridoxal phosphate and is the form absorbed by tissues. After absorption, some of the pyridoxal is released in the blood, but most of the vitamers goes go the liver which is the organ where all the interconversions of the vitamin take place. Storage of vitamin B_6 is limited, only small quantities of pyridoxal phosphate and to a less extent pyridoxamine phosphate are stored in the body (Caballero, Allen & Prentice, 2012). Highest levels are found in liver, kidney, spleen and muscle where the vitamin is bound to proteins. The pool of vitamin B_6 in the human body is estimated to be 40-150 mg, which constitute the supply for 20-75 days (Combs, 2008). Approximately 80 percent is stored in the muscle, bound to glycogen phosphorylase. Vitamin B_6 is oxidized in the liver and excreted in the urine mainly as 4-pyridoxic acid (Caballero, Allen & Prentice, 2012).

The role of pyridoxal phosphate in amino acid metabolism is in α-decarboxylation, racemization of amino acids, transamination and side-chain elimination and replacement reactions. The vitamin is also involved in steroid hormone action, glycogen utilization, neurotransmitter synthesis, haemoglobin synthesis and function, lipid metabolism and gene expression (Combs, 2008).

2.7.4 Requirements, deficiency and health implications

The estimated average requirement of vitamin B_6 is for women 1.1 mg and for men 1.3 mg (Nordic Council of Ministers, 2012). The average intake in the Swedish adult population is for women 1.8 mg and for men 2.3 mg (Amcoff *et al.*, 2012).

Deficiency is rare and symptoms can be diffuse since vitamin B_6 is involved in many processes in the metabolism. Symptoms range from sleepiness, weakness, loss of appetite to more severe symptoms such as skin leisure's, anaemia, arteriosclerosis, and neurological symptoms such as peripheral neuropathy. Severe vitamin B_6 deficiency leads to homocysteinemia because of lost capacity to catabolize homocysteine due to malfunction of the pyridoxal phosphate enzyme cystathionine β-synthase. High levels of homocysteine are associated with cardiovascular disease (Combs, 2008). Deficiency is more likely to occur in alcoholics, elderly, people with certain diseases and it can be induced by certain drugs (Caballero, Allen & Prentice, 2012).

Toxicity of vitamin B_6 is not associated with food intake but with high intake of vitamin B_6 supplements. Symptoms mainly have a neurological character with neuronal damage and motor and sensory effects. The EU Scientific Committee on Food has set an upper intake level of 25 mg/day for adults (EFSA, 2006).

2.7.5 Vitamin B_6 in foods

European Food Information Resource Network (EuroFIR) is a nonprofit organization collecting food composition data in Europe. The vitamin B_6 content in brown beans in different databases in European countries varies between

0.3-0.415 mg/100g (Table 2).

A survey of food consumption in Sweden (Amcoff *et al.*, 2012) shows the range of vitamin B_6 to be in 0.04-0.78 mg/100g in some of the commonly consumed foods (Table 3). Vitamin B_6 is ~ 75 percent bioavailable in an omnivorous diet (Tarr, Tamura & Stokstad, 1981).

Table 2. Determination of the content of vitamin B_6 in brown beans (mg/100g). Values are collected from EuroFIR (food composition data in Europe) (fresh weight)

	Vitamin B_6 mg/100g	Method
Swedish National Food Agency	0.3	Microbiological assay
Denmark	0.415	Microbiological assay
The Netherlands	0.35	Not defined
Norway	0.3	Not defined

Table 3. The content of vitamin B_6 in a selection of commonly consumed foods in Sweden (National Food Agency, 2017) (Fresh weight)

	Raw mg/100g	Processed (mg/100g)
Salmon	0.78 (cured)	0.45 (fried)
Beef Entrecôte	0.37	0.22 (fried)
Pork	0.48	0.31 (fried)
Potato	0.21	0.19 (boiled)
Milk 3%	0.04	
Apple	0.04	
Banana	0.58	
Tomato	0.1	

2.7.6 Methods to analyse vitamin B_6

Initially microbiological assays (MA) were used to determine the total amount of vitamin B_6 (Leklem & Reynolds, 1981). Today high pressure liquid chromatography (HPLC) with fluorescence is the most common method and offers the possibility to analyze all the vitamers separately. The vitamers are relatively weak UV absorbers and fluorescence is generally preferred as a detector due to its sensitivity and specificity (Nollet & Toldrá, 2013). There are several different methods for HPLC with fluorescence and one is developed by Kall (2003). In the method by Kall (2003), the glycosylated PN is determined by calculating the difference between free PN and total PN after using/not using β-glucosidase in the same sample extract. All the phosphorylated forms of the vitamers are converted and analysed as the free forms by treatment with acid phosphatase. There have been attempts to create methods to analyse several water-soluble vitamins at once, some of them are the methods by Kakitani *et al.* (2014), Lebiedzińska *et al.* (2007) and Leporati *et al.* (2005) but none is in commercial use. To the author's knowledge it is at the Swedish National Food Agency currently a process of developing a UHPLC-MSMS (ultra high pressure liquid chromatography- tandem mass spectrometry) method with the ability to, with the same extraction procedure, analyse several of the B vitamins at once.

2.8 Water content in foods

The water content in foods can affect its properties as for example the structure. It can as well affect its durability because water is essential for microbial growth and enzymatic reactions. Water can in different ways be bound to food material. Vicinal water is water held at hydrophilic sides at a molecule, multilayer water is water forming layer around the vicinal water. Entrapped water is water with normal solvent capability but due to capillary forces or gel structures, it is prevented from flowing freely. Free water is water trapped in a matrix due to capillary forces or trapped in layers of fatty materials and it is easily squeezed out (Damodaran, Parkin & Fennema, 2008). Water in food not bound to molecules can support growth of microorganisms as bacteria, yeast and mould. The durability of foods is determined by its water activity. The water activity is determined by comparing the vapor pressure of the food and the vapor pressure of pure water. The ratio is multiplied by 100 and the equilibrium relative humidity of the food is determined. The calculations are based on foods enclosed with air in a sealed lid with constant temperature. Water activity is measured between 0-1.0. Normal bacteria grow at a water activity of 0.91, mould and yeast grown down to 0.65 respectively 0.60 (Coultrate, 2009).

Water holding capacity is a term which describes the ability of a matrix (mostly macromolecules) to entrap water. It is the maximal bound water a material can contain. Charged groups interact with water mainly through electrostatic forces. The more charged groups, the more water is bound (Damodaran, Parkin & Fennema, 2008).

3 Aim

The aim of the study was to determine the content of vitamin B_6 in brown beans from Öland and to find factors which influence the content. Consideration was taken to the factors cultivars (Karin and Katja), difference between year of cultivation (2008-2010), geographical areas (Borgholm, Färjestaden, Mörbylånga, Kastlösa and Degerhamn) and different types of fertilizer (artificial/natural). If there would be differences between years, they would be related to meteorological conditions. The variables total-, free- and glycosylated form of pyridoxine was determined as well as the water content and related to the factors cultivars, years, geographical areas and different types of fertilizer.

4 Materials and methods

Brown beans from Öland were during the years 2008, 2009 and 2010 collected from different farmers in the southern part of Öland; Borgholm, Färjestaden, Mörbylånga, Kastlösa and Degerhamn. The beans were collected by the National Food Agency of Sweden and stored in freezers. Each sample in this study is from one farmer and is either the brown bean Karin or Katja and has since collection been stored in -70 °C. All work in this study was performed in environment free from UV-light. The method for analysis is based on Kall (2003) and further developed and validated by the Danish Fødevareinstituttet (Jakobsen, 2005). The method is also validated by the National Food Agency of Sweden (Livsmedelverket, 2016a). The method in this study is, with minor modifications, performed by a manual from the Swedish National Food Agency (Livsmedelsverket, 2016b).

4.1 Meteorological conditions on Öland

The weather conditions concerning precipitation (mm) and temperature (°C) are collected from SMHI (2017). Consideration was taken for difference between the years 2008-2010 and between the months May-October. The years included for comparison, except for the selected years for this study, is total precipitation (mm) and mean temperature (°C) from 1994-1990 and 2002-2016.

4.2 Sample preparation and extraction

A batch of mixed brown beans from Öland was milled to serve as control sample during further analysis. The beans were in two steps milled with a Retsch ZM100 to avoid excessive heating, first to more course pieces and then to a powder. The size of the sieves was 4.0 mm and 0.5 mm. The milled beans were vacuumpacked in individual samples and stored in -70 °C. The time between milling and analysing of beans was up to four weeks.

In total, 25 samples were analysed in a randomized order in five batches. The number of samples for cultivars, years, geographical areas and type of fertilizer is illustrated in Table 4. The number of samples for Karin and Katja for years, geographical area and type of fertilizer is as well presented in Table 4. Each milled bean sample (2.5g) was weighed in each of two 200 ml E-flasks, analysed as duplicates to assure precision. Extraction media (50 ml 0.1 M HCl) was added and the sample was mixed to a homogenous dispersion by a magnetic stirrer. The sample was autoclaved for 5 minutes at 121 °C and cooled to room temperature in water. The pH was adjusted to 4.5 ± 0.1 with 2 M sodium acetate. The sample was quantitatively transferred to a 100 ml volumetric flask and diluted with MQ® water. The extract was filtered (0.45 μm Munktel V120H) and two aliquots of 2.5 ml of the extract were transferred to two 10 ml volumetric flasks. Acid phosphatase (25 units/ml, 200 μl) was added to one of the volumetric flasks. Acid phosphatase (25 units/ml, 200 μl) and beta-glucosidase (45 units/ml, 600 μl) were added to the other volumetric flask. The flask was closed and incubated for 18 h at +45 °C. The sample was cooled by adding 1 ml 1 M HCl and further with 0.01 M HCl to 10 ml. It was finally filtered with Whatman Rotrand 0.2 μm (cellulose acetate membrane and polycarbonate housing) and transferred to a HPLC-vial.

Table 4. *The table shows the total number of samples for the cultivars, years, geographical areas and type of fertilizer. Number of the cultivars Karin and Katja is presented for years, geographical area and fertilizer*

	Karin	Katja	Total
2008	1	7	8
2009	7	7	14
2010	1	2	3
Total	9	16	25
Borgholm	2	-	2
Degerhamn	2	9	11
Färjestaden	3	1	4
Kastlösa	1	4	5
Mörbylånga	1	2	3
Total	9	16	25
Natural fertilizer	4	4	8

Artificial	2	12	14
Other fertilizier	3	-	3
Total	9	16	25

Stock solutions of pyridoxal hydrochloride, pyridoxine hydrochloride and pyridoxamine dihydrochloride served as calibration samples and were prepared with the concentration 10 µg/ml and stored up to two months. The concentration was controlled by UV absorption (λ_{max} 288 nm pyridoxal, λ_{max} 290 nm pyridoxine and λ_{max} 293 nm pyridoxamine) and the expected absorbance for pyridoxal is 0.445 AU, pyridoxine 0.425 AU and pyridoxamine 0.374 AU. The correction factor is determined by $\frac{\text{Measured absorbance}}{\text{Expected absorbance}}$ and must be $\geq$ 0.95 for the calibration solution to be fit for use. The calibration samples were diluted with 0.01 M HCl to concentrations of 5, 25 and 100 ng/ml and transferred to HPLC-vials.

4.3 HPLC

Isocratic HPLC was carried out on a C18 reverse phase column (Phenomerex Kinetex 2.6 µ 150x 4.6 mm) with an auto sampler to inject 50 µl. The samples were kept in dark at 5 °C during analysis. The column was equipped with a column saver, 0.5 µm. The HPLC buffer (pH 2.75) consisted of 2.2 mM octansulfonic acid, 81 mM di-potassium hydrogen phosphate, 19 mM o-phosphoric acid and 4 mM trimethylamine, the buffer was filtered through a 0.45 µm Millipore filter. The mobile phase was prepared with 4 percent (v/v) acetonitrile-grade HPLC in HPLC buffer. The mobile phase was run at 0.7 ml/min. The column oven was held at 25 °C. To improve the detection, a post-column buffer was prepared by regulating pH in a 0.5 M di-potassium hydrogen phosphate solution to 7.5 ± 0.1 with 0.5 M potassium dihydrogen phosphate, the buffer was added post-column at 0.3 ml/min. The vitamers were detected by a florescence detector-, with excitation at 333 nm and emission at 375 nm. Each sample was run 20 minutes.

4.4 Calculations of results

Vitamin B_6 is presented as total pyridoxine, including the vitamers PL, PN and PM. Glycosylated pyridoxine is calculated as a difference between free pyridoxine (all vitamers except the glycosylated pyridoxine) and total pyridoxine. Duplicates were calculated as an average. To compensate for the difference in molecular weight between the vitamers, it was calculated according to PN= [PN] + (0.85 x [PM]) + (1.01 x [PL]), thereby presented as pyridoxine hydrochloride. Pyridoxine is calculated as 0.825 mg corresponding to the molecular weight of 1 mg pyridoxine hydrochloride.

4.5 Dry weight calculations

The dry weight was determined for each bean sample according to the Commissionregulation (273/2008). A bowl and its lid was heated in the oven at 102 °C for 1 hour and cooled to room temperature in a desiccator and then weighed. The sample was weighed in the dried bowl, heated for 2-3 hours in 102 °C, cooled to room temperature and then again weighed. The procedure was repeated with heating 1 hour in the oven at 102 °C, cooled to room temperature and weighed until the weight had reduced with 1 mg or less, or increased between two successive weigh-ins.

4.6 Quality control

Repeatability and reproducibility were assured by analysing four replicates of the control sample in two batches before starting the analyses of the beans. A control chart was generated from these analyses. The control sample was included in all batches in this study. Accepted variation in the control chart for total PN was ± 6 percent between batches, the same applies for the duplicates within the batches. The allowed variation between batches and within duplicates have been determined by the Swedish Food Agency according to NMKL (2003) and NMKL (2016), respectively. Repeatability, reproducibility, accuracy, homogenization and stock standard solution have been considered. A warning and action limit was set for ± 2 and ± 3 standard deviations, respectively.

The accuracy was determined by certified reference material (CRM 485), participation in the FAPAS proficiency test program (Fapas, 2017) and as recovery by spiking four samples with the B_6 vitamers. The method (Livsmedelsverket, 2016c) allows for the yield to be in the range of 80-110 percent. Recovery was calculated according to the formula:

$$\mathrm{R}(\%) = \frac{(\text{Measured amount} - \text{ original amount of the sample})\ x\ 100}{\text{added amount}}$$

4.7 Statistics

Principal component analysis (PCA) was used to reduce the dimensionality of the data. Difference between factors were assessed by ANOVA with Generalized Linear Model (GLM), Tukey method with 95 percent confidence interval. Model was set to the factors cultivar (Karin and Katja were one factor as well as two separate factors), year of cultivation, geographical area or fertilizer. Responses were total pyridoxine, free pyridoxine, glycosylated pyridoxine and water content. $p<0.05$ was considered significant. The R square (adjusted) is presented together with the p-value of significant factors. Statistical analyses were performed in The Unscrambler version 10.1 (CAMO software A/S, Norway) and Minitab version 2016.

5 Results

5.1 Vitamin B_6 in brown beans from Öland

The average content of vitamin B_6 was 0.32 ± 0.02 mg/100g (Table 5). The range was 0.28 mg/100g to 0.35 mg/100g. In average, 49 percent of vitamin B_6 was glycosylated and 0.16 ± 0.01 mg/100g was free PN. The average water content was 13 percent (Table 5). No significant difference in content of vitamin B_6 was found between cultivars (p=0.49). No significant differences were found for type of fertilizer and geographical area with any variable. There were no significant differences for free PN with any factor. There was a significant difference in total PN respectively glycosylated PN between years. There was a significant difference in water content between the cultivars. For the cultivar Katja, there was a significant difference in water content between years. Significant variables for the factors are presented in Table 6.

Table 5. *Average vitamin B_6 content (fresh weight) and water content in all beans*

All beans	Average
Total pyridoxine (mg/100g)	0.32 ± 0.02
Free pyridoxine (mg/100g)	0.16 ± 0.01
Glycosylated pyridoxine (% of total)	49 ± 0.40
Water content (%)	13 ± 0.03

Table 6. *Significant variables for the factors years and cultivars*

Factor	Variable	R-sq (adj) (%)[1]	p-value
Year	Total PN (dry weight) (mg/100g)	21.4	0.03
Year	Glycosylated PN (%)	34.8	0.00
Cultivars	Water content (%)	46.3	0.00
The cultivar Katja and year	Water content (%)	57.2	0.00

1 How many percent of the variance which is explained by the model

5.2 Vitamin B$_6$ content in 2008-2010

Total PN content was highest 2008 and significantly different from 2009. There was no significant difference between 2010 and the other years (Table 7, Figure 2). For 2008, 2009 and 2010 the average proportion of glycosylated PN (% of total) was 51 percent, 47 percent respectively 52 percent (Table 8, Figure 3), it was significantly higher 2008 and 2010 compared to 2009. There was no significant difference in free PN between years (p=0.18) (data not shown).

Table 7. *Mean total PN ± standard deviation (dry weight) in 2008-2010.*

Year	n	Mean total PN (mg/100g)
2008	8	0.37 ± 0.02^{A}
2009	14	0.35 ± 0.02^{B}
2010	3	0.35 ± 0.01^{AB}

1Means not sharing the same letter are significantly different (A and B)

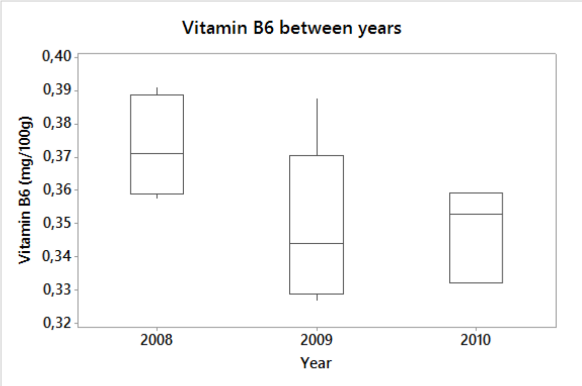


Figure 2. Total PN content between the years. The line in the boxplot illustrates the median. The bottom of the box is the lower quartile, 25% of the data has less than this value. The top of the box is the upper quartile, 25% has values above this value. The end of the line above and under the box are where the maximum and minimum value can be found.

Table 8. *Mean glycosylated PN (% of total) ± standard deviation in 2008-2010.*

Year	n	Glycosylated PN (% of total)[1]
2008	8	51 ± 0.03^{A}
2009	14	47 ± 0.03^{B}
2010	3	52 ± 0.06^{A}

1Means not sharing the same letter are significantly different (A and B)

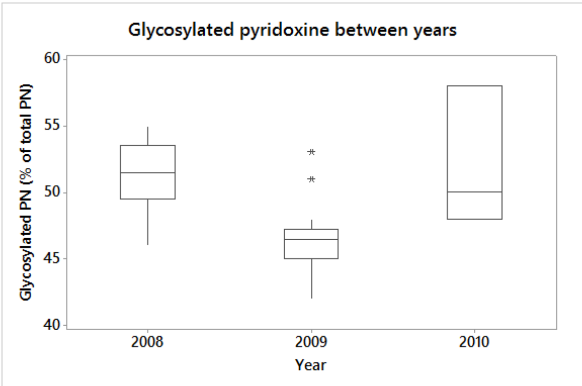


Figure 3. Mean glycosylated PN (% of total) in 2008-2010. The boxplot is read as explained in Figure 2. The dots above

the boxplot in 2009 figure are outliers, values more than 3/2 times above the upper quartile.

The precipitation (mm) and mean temperature °C in 2008-2010 as well as the average in 1944-1990 and 2002-2016 is presented in Table 9. There was least precipitation during 2008 and most 2010. 2010 was the coldest year (Table 9). In Appendix I (Table 15), precipitation (mm) and temperature °C for the years 2002-2016 are presented. The different weather conditions from May to October during 2008-2010 are presented in Figure 4 and 5. There was less rain in May-July 2008 compared to 2009. During August to October it rained less in 2008 compared to 2009. The precipitation 2010 did not follow a similar pattern to neither of the other years. It was nearly 5 times more rain in July 2010 compared to 2008 and around two times more than 2009. It was colder in May 2010 compared to 2008 and 2009. October was warmer 2008 compared to 2009 and 2010. The precipitation and temperature (°C) in May-October 2002-2016 are presented in Appendix I, Table 14 and 15. They show great variations between years for the same month.

Table 9. *Total precipitation (mm) and mean temperature (°C) at the southern cape of Öland 2008-2010*

	Precipitation (mm)	Temperature (°C)
2008	427	8.9
2009	439	8.0
2010	490	6.6
Mean 1944-1900	400	7.0
Mean 2002-2016	459	8.1

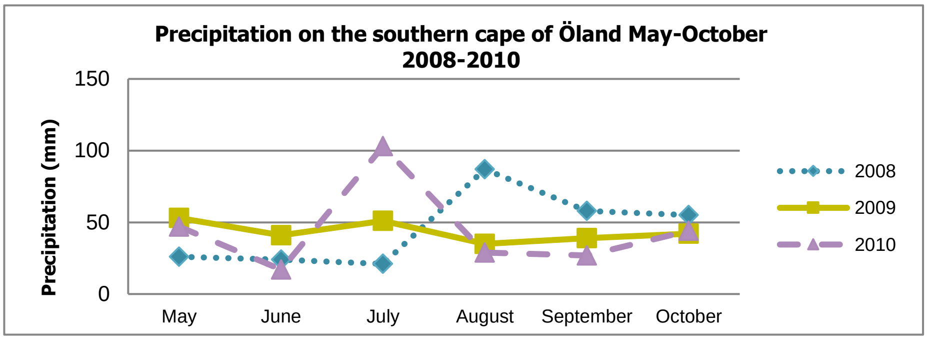


Figure 4. Precipitation (mm) on the southern cape of Öland May-October 2008-2010.

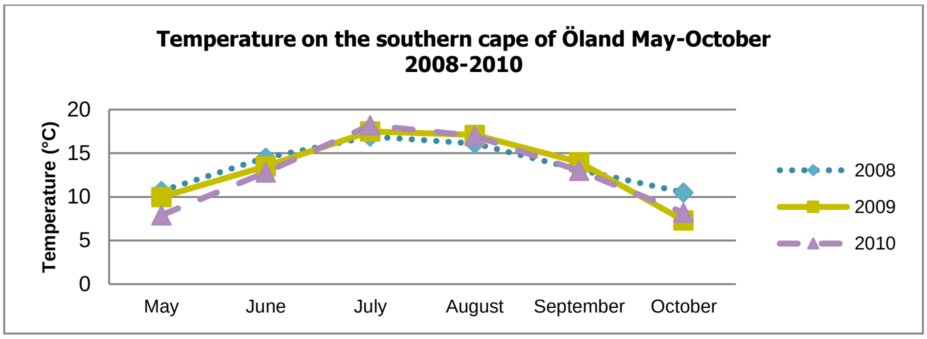


Figure 5. Temperature (°C) on the southern cape of Öland May-October 2008-2010.

5.3 Water content in the cultivars

A significant difference between the cultivars for the water content was found (table 6). The water content for Karin was 9.6 percent and for Katja 14.4 percent (Table 10, Figure 6). In the brown beans from Öland, there was no difference in water content between years (p=0.18), geographical area (p=0.56) or fertilizer (p=0.22). Even if there was no difference in water content between years for cultivars, the cultivar Katja had a difference in water content between years (p=0.00), with highest content 2009 (Table 11, Figure 7). For Karin, no such difference was found (p=0.28).

Table 10. *The water content in the cultivars Karin and Katja*

Cultivar	n	Water content (%)
Karin	9	9.6 ± 1.01
Katja	16	14.4 ± 2.9

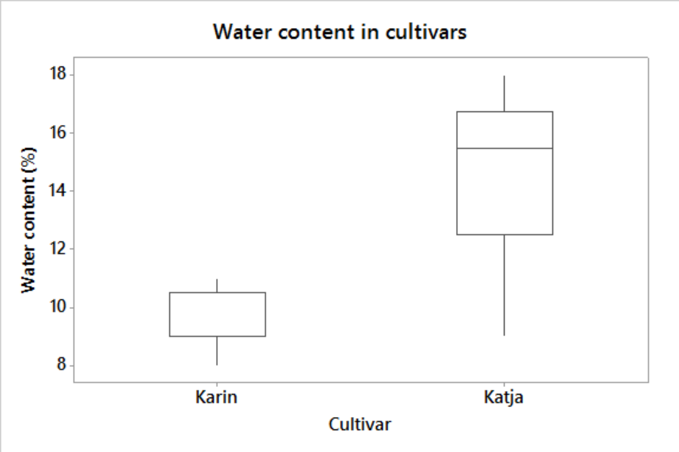


Figure 6. Water content in Karin and Katja.

Table 11. *Water content for Katja in 2008-2010*

Year	n	Water content (%)[1]
2008	7	13.7 ± 2.5 [A]
2009	7	16.4 ± 1.27 [B]
2010	2	9.5 ± 0.71 [C]

[1]Means not sharing the same letter are significantly different (A, B and C)

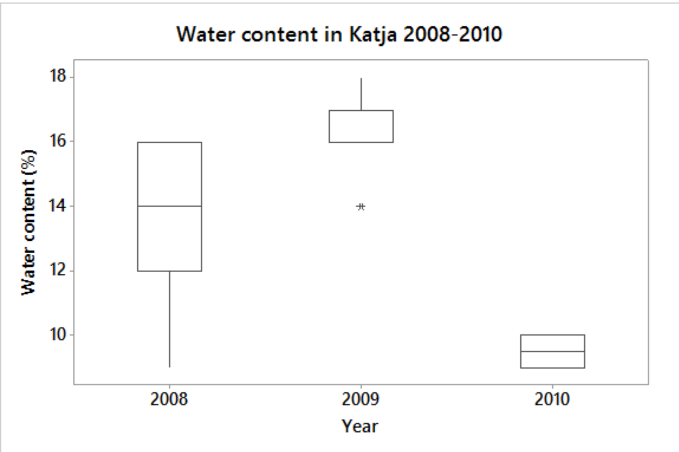


Figure 7. Water content in Katja 2008-2010.

5.4 Quality control tests

The quality control samples in the batches and the duplicates were held within the measurement uncertainty, assuring reproducibility and repeatability. The recovery of analytes in spiked samples is presented in Table 12. The result of the FAPA's test is satisfying with a z score of 0.5, allowed z score variation $\pm$ 2 (Fapas, 2017b). However, the total PN-HCl result of CRM 485 gave higher result, 0.77 mg/100g than the certified value (data not shown), 0.48 mg/100g (Finglas *et al.*, 1998) .

Table 12. *Recovery of the analytes pyridoxal-HCl, pyridoxine- HCl and pyridoxamine-2HCl*

Number	Pyridoxal-HCl (%)	Free pyridox-ine-HCl (%)	Total pyridoxine-HCl (%)	Pyridoxamine-2HCl (%)
2	87.5	97	94.3	97

5.5 β-Glucosidase treatment

Figure 8 illustrates chromatogram of bean samples treated and not treated with β-glucosidase. The peak with retention time 10.53 minutes disappeared after treatment with β-glucosidase. The area of pyridoxine simultaneously increased with equal amount. The unlabeled peaks in Figure 8 were not identified in this study but earlier studies have shown that the peak that disappear after treatment with β-glucosidase correspond to the increased area in the pyridoxine peak and has been verified as pyridoxine-5-β-D-glycoside (Gregory & Ink, 1987; Gregory & Sartain, 1991).

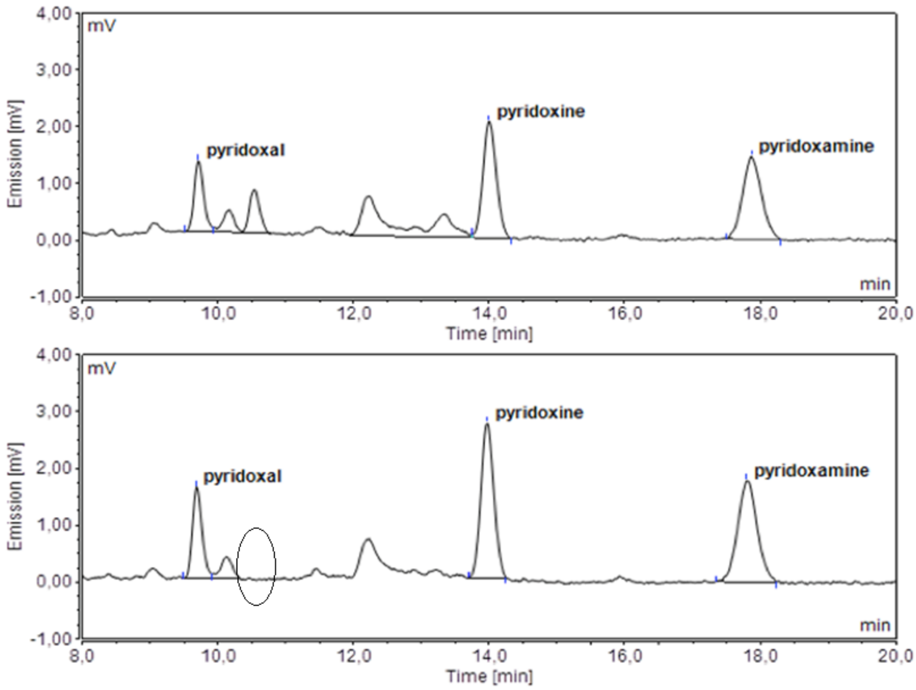


Figure 8. Chromatogram of a bean sample not treated with β-glucosidase (A). Chromatogram of a bean sample treated with β-glucosidase (B), a peak disappears with retention time 10.53 minutes meanwhile pyridoxine simultaneously increases with equal area.

5.6 Choice of filter

During the laboratory analysis, different filters were tested before put into HPLC vials. The filters were Whatman Rotrand 0.2 μm with celluloseacetate membrane and polycarbonate housing, Whatman with polyvinylidene difluoride (PVDF) filter membrane and polypropylene housing, the third filter was Whatman mini – Uni-prep 0.45 μm with polypropylene filter membrane and polypropylene housing. The filters with polypropene resulted in a peak after 76 minutes which made it impossible to interpret the pyridoxal peak in the fourth sample (each sample was run for 20 minutes) (Figure 9). No such peak was found with the filter Whatman Rotrand 0.2 μm with celluloseacetate. Each filter was used in different batches to rule out contamination. They were used both for bean samples and for calibration samples. Furthermore, four bean sample extracts were tested with each of the three filters in the same batch.

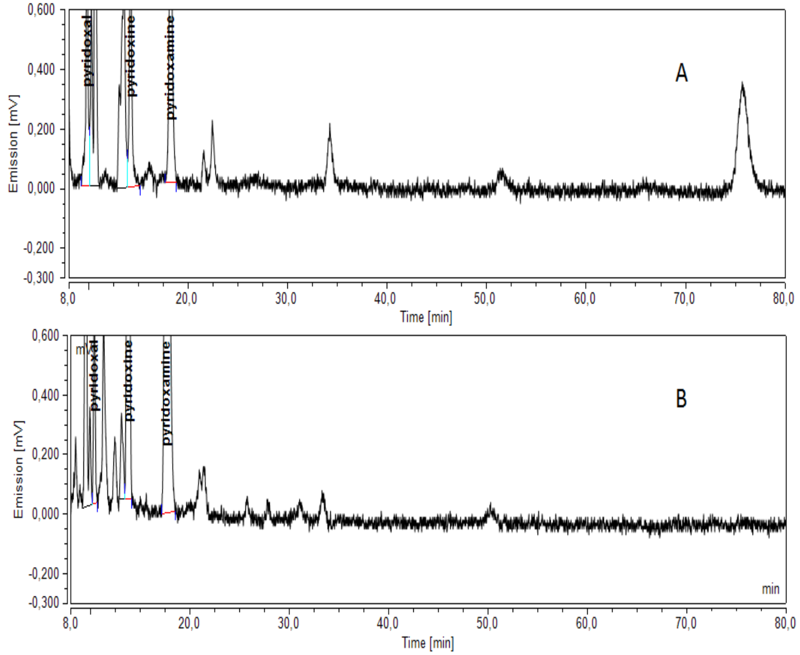


Figure 9. Chromatogram A illustrates a sample filtered with polypropylene material. A peak appears after 76 minutes. Chromatogram B illustrates a sample filtered with cellulose acetate membrane with polycarbonate housing, there is no peak at 76 minutes.

6 Discussion

The content of vitamin B_6 in brown beans from Öland was determined to 0.32 ± 0.02 mg/100g. It is within the range of determination of vitamin B_6 compared to food composition data in Europe (Table 2). It is not possible to say which value is more correct, instead it is a range where the content of vitamin B_6 in brown beans can be found. The sampling could be different. Samples can be of different cultivars, years of cultivation and geographical areas. Preparation is also performed by different analyst and method of analysis is different.

6.1 Vitamin B_6 content in 2008-2010

Total PN was higher 2008 compared to 2009. Glycosylated PN was higher 2008 and 2010 compared to 2009 meanwhile there was no significant difference in free PN between years. This indicates on extra formation of glycosylated PN but no change in formation of other forms of vitamin $B_{6.}$ The non-significant difference for total PN between 2010 and 2008 respectively 2009, may be an indication of too few samples 2010. The fact that plants are believed to form glycosylated PN as a response to stress (Gregory & Ink, 1987; Nishimura *et al.*, 2008) may explain the higher levels 2008. To the authors knowledge there is until now no studies exploring the formation of different pyridoxine forms in legumes due to weather conditions during growth.

Mean precipitation was lower 2008 compared to 2009, highest was in 2010. During the period when the beans need more water (germination and flowering) the precipitation was higher 2009 compared to 2008. During the end of the bean maturation when it approaches autumn, it needs less water and there was less precipitation in this period 2009 compared to 2008. It was generally colder 2010 which may have caused formation of glycosylated PN. This also concerned May when the beans were sawn and they need higher temperatures. The high precipitation in July 2010 may have induced stress and formation of glycosylated PN. It is difficult to predict an eventual effect of the lower temperatures in October 2009 and 2010 because the harvest may already have occurred.

It is apparent that the rain and temperature may differ between years for the same months (Appendix I, Table 14 and 15). Less beneficial weather for the yield have a positive impact on vitamin $B_{6.}$ It could be information interesting for farmers when selling their beans. Future research could include more number of years and more samples each year when analysing the content of vitamin B_6 to get a deeper understanding of the glycosylated formation of pyridoxine in relation to weather.

6.2 Water content in the cultivars

The water content was significantly different between the cultivars Karin and Katja. There are many uncertain factors of how the beans have been treated which can have affected the water content. The beans are dried to at most 18 percent water content after harvest, but it can also be less. The beans have been stored for different times and can have lost water during this time, and they have in this study been milled in different batches. Even though two sieves were used to reduce the friction and heat formation, some bean samples were slightly heated during milling. It was unfortunately not noted which bean samples it concerned. Nevertheless, even if the beans are treated differently the result indicates on an actual difference in the water-holding capacity (WHC) between Karin and Katja. The higher value in Katja is probably due to a higher content of polar molecules as carbohydrates and proteins with hydroscopic abilities. Carbohydrates and proteins are the main molecules absorbing water in beans (Deshpande & Cheryan, 1986). A study by Elhardallou & Walker (1993) concluded that higher content of dietary fibre in legumes was associated with higher WHC. It can also be that Karin has a thicker coating and it takes longer time for water to be released from the bean. Sefa-Dedeh & Stanley (1979) discovered that the thickness of the coating can affect the passage of water. Further research about the chemical composition in Karin and Katja is needed to conclude the nature of the difference in WHC. A consequence can be that Karin keeps better quality for longer time because it loses more water. Although, this depends on how the water is bound in the beans and what the water activity is. This needs to be investigated. Another consequence could be that Katja maybe contain more water per unit also when cooked. This would reduce the nutrient content per 100g. Further research is needed.

The cultivar Katja had higher water content 2009 compared to 2008 and 2010. However, 2010 only comprised of two samples and is thereby difficult to interpret. The beans were stored for different times but in question of water content, it should be negligible due to the long storage time, 7 to 9 years. A possible explanation is that weather conditions have influenced the chemical composition of the beans and thereby the water holding capacity. According to Graham & Patterson (1982) the protein content is often altered in plants when exposed to low temperatures. The carbohydrate content increased in beans exposed to drought (Mafakheri *et al.*, 2011). Though, the weather conditions were more beneficial 2009 when the water content was higher in Katja. Further analysis is needed to elucidate the change in chemical composition. It is likely there would have been a difference between years and water content also for Karin but no trend was found. This may be due to too few sample from the different years.

6.3 Geographical area and fertiliser

There was no difference between geographical areas and this can probably be explained by the similar soil conditions. Another explanation can be the uneven distribution of samples between the geographical areas, more analyses are needed. There was also no difference between fertilizers and this could possibly be explained by the fact that beans do not need much fertilizers since it fixates nitrogen and fertilizers thereby have a low impact on the formation of vitamin B_6.

6.4 Nutritional aspects of brown beans from Öland

Even though it is not common with vitamin B_6 deficiency, the reduced bioavailability of glycosylated PN may have health implications. For groups at risks of deficiency it may be beneficial to be aware of the reduced bioavailability in plants to assure sufficient intake of vitamin $B_{6.}$ The formation of the amount of glycosylated PN in foods is limited and could be extended (Fitzpatrick, 2011). For the healthcare, it could be helpful with more knowledge of the amount of glycosylated PN when formulating food advices.

There is not much research about processing and the effect on vitamin B_6 except for cooking and frying. Kabir, Leklem & Miller (1983) found that the glycosylated PN increase in cauliflower and broccoli after it had been frozen. To understand how different processing techniques of foods affect the content of vitamin B_6, further research is needed.

There is little research on health effects of consuming brown beans from Öland. Though, in Table 1 one can conclude that they have nutrient levels similar to other commonly consumed pulses which are associated with health benefits. Since beans are consumed cooked the proceeding step of this study would be to cook the beans to get knowledge about the retention of vitamin B_6. As seen in Table 1, the National Food Agency of Sweden previously has performed the analysis. The retention was determined to a third of the vitamin B_6 content in brown beans from Öland (100g dried/100g cooked beans). It can be assumed that the levels in the brown beans from Öland in this study would be similar. That would correspond to 0.11 mg/100g from the results and this is in the range of commonly consumed foods (Table 3). This is a contribution of 10 percent of the daily estimated requirement for women and 8.5 percent for men. Vitamin B_6 is widely distributed in foods and brown beans from Öland have the possibility to contribute to the daily intake.

6.5 Quality control

The quality of the control samples was satisfying as well as the recovery test and the FAPAS proficiency test. In the calculations of recovery, only two samples were included instead of four due to human error. It was decided it was enough since there were good repeatability and reproducibility in the analyses.

The result of the CRM 485 value was different than from the certified value. Analyses earlier performed at the Swedish National Food Agency were in concordance with values in this report and these values were accepted during revision by Swedac. Kall (2003) also got values higher than the certified value. According to the certification report (Finglas *et al.*, 1998), ten laboratories participated in the analyses. Seven of the laboratories used microbiological analyses with PL or PN as external standards and three laboratories used HPLC determining the three vitamers. According to this study, PM approximately constitute 70 percent of the total vitamin B_6 content in CRM 485. The corresponding value for

Kall (2003) was 80 percent. It may be that the vitamin B_6 content is underestimated in the certified value. It indicates on the necessity of all the vitamers to be represented in the analysis as standard.

6.6 Choice of column, mobile phase and filter

The column Phenomenex Kinetex 2.6u C18 (150x4.6 mm) was chosen over Hyperclone 3u ODS (C18) 120A (150x4,6mm) because of better separation of the peaks for pyridoxal, pyridoxine and pyridoxamine. The run time was also significantly shorter. Mobile phases with 5.25 percent and 4 percent acetonitrile (ACN) were tested with both columns and 4 percent ACN gave greater separation of peaks and was thereby chosen.

From this study, it is concluded when analysing brown beans from Öland, it is recommended to use filters with cellulose acetate with polycarbonate housing compared to polypropylene material. They seem to be less selective in the filtration of compounds found in the beans. The filters with polypropene membranes had been used at the Swedish National Food Agency in analyses of other foods where there had not been a peak at 76 minutes. Thereby contamination by the filters is ruled out.

6.7 Future research

Interesting future research for brown beans from Öland could be to germinate them. Studies have found increased vitamin content and mineral bioavailability by germination of beans (Vidal-Valverde *et al.*, 1998; Wang *et al.*, 2015). If vitamin B_6 increase or if the proportion of glycosylated PN changes in brown beans from Öland due to germination, is to the author’s knowledge still unknown.

7 Conclusion

The content of vitamin B_6 in brown beans from Öland (cultivar Karin and Katja) was 0.32 $\pm$ 0.02 mg/100g (minimum 0.28 mg/100g and maximum 0.35 mg/100g). On average, 49 percent was glycosylated. There was no difference in the content of vitamin B_6 between cultivars. There was also no difference for geographical area and type of fertilizer with any variable. There was a difference between year of cultivation, suboptimal weather conditions caused formation of glycosylated PN which resulted in higher total PN. The water content was significantly higher in Katja compared to Karin. For Katja, there was a significant difference between water content and years.

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Acknowledgements

I would like to thank the team of nutrients and heavy metals, at the department of chemistry at the National Food Agency of Sweden, for interesting learnings and an enjoyable time. Especially thanks to my supervisor Hanna Sara Strandler for all discussions and ideas during the working process. I had such a great time in the laboratory and I want to thank Anna von Malmborg for all the help and patience. I would also like to thank my supervisor at SLU, Roger Andersson, for input during the working process. Finally, I would like to thank my classmate Sara Babakirad for all discussions about our bean projects and all the fun we had!

Appendix I

Weather conditions (average precipitation and temperature (°C)) on the southern cape of Öland between the years 2002-2016, collected from SMHI (Table 13). The mean precipitation and temperature in 1944-1990 is also presented (Table 13). The precipitation (mm) and temperature (°C) in May-October between the years 2002-2016 is shown in Table 14 and 15.

Table 13. *Precipitation (mm) and temperature (°C) on Öland*

Year	Precipition (mm)	Temperature (°C)
2002	483	8.3
2003	441	7.7
2004	473	7.5
2005	302	8.0
2006	426	8.7
2007	508	8.6
2008	427	8.9
2009	439	8.0
2010	490	6.6
2011	398	7.8
2012	473	7.6
2013	414	8.1
2014	518	*
2015	452	9.2
2016	541	8.8
Mean 2002-2016	452 ± 58	8.1± 0.7
Mean 1944-1990	400 ± *	7.0± *

*Missing value

Table 14. *Precipitation (mm) in May-October on the southern cape of Öland 2002-2016*

	May	June	July	August	September	October
2002	51.7	26.1	50.1	0.2	17.9	120.9
2003	18.5	46.2	130.7	35	26	47.6
2004	23	25.7	65.7	96.8	41.4	71.8
2005	32.1	15.4	36.8	42.6	10.0	17.1
2006	30	27.0	5.7	110.7	21.7	48.8
2007	44.0	44.2	137.9	68.7	25.7	9.3
2008	26	24	21	87	58	55
2009	53	41	51	35	39	42
2010	47	17	103	29	27	44
2011	21.8	96.9	44.1	23.2	22.8	6.7
2012	23.3	70.0	32.2	41.4	39.9	63.9
2013	47.6	43.4	47.2	16.9	36.3	48.5
2014	21.4	54.7	83.0	90.2	45.4	49.3
2015	40.0	19.8	48.1	17.5	68.8	9.0
2016	30.8	50.0	56.1	44.6	9.2	149.0
Mean	32.0	43.3	61.5	49.0	30.4	53.5
Standard deviation	11.3	23.3	38.8	35.1	16.9	44.4

Table 15. Temperature (°C) in May-October on the southern cape of Öland 2002-2016

	May	June	July	August	September	October
2002	10.2	15.0	15.8	20.2	15.1	7.6
2003	9.1	13.9	17.9	17.6	14.2	7.2
2004	9.4	12.8	13.6	18.0	12.7	9.0
2005	9.1	13.2	18.1	16.1	14.3	10.6
2006	9.1	14.2	19.7	18.3	16.4	12.4
2007	10.5	15.5	15.6	18.3	16.4	12.4
2008	10.7	14.5	16.9	16.1	13.1	10.5
2009	10.0	13.5	17.1	17.1	14.0	7.3
2010	7.9	12.8	17.0	17.0	7.3	8.2
2011	9.3	14.3	17.3	16.9	11.9	8.1
2012	9.7	12.9	15.9	16.7	12.9	8.2
2013	10.0	14.7	17.1	17.6	13.7	11.1
2014	10.3	14.1	19.1	14.8	11.9	7.8
2015	9.2	12.9	15.5	17.7	14.8	10.2
2016	11.3	15.5	17.5	16.1	10.0	9.3
Mean	9.8	14.1	16.9	17.4	13.7	9.5
Standard deviation	0.7	1.0	1.7	1.3	1.9	1.8

Appendix II

Popular scientific summary

Legumes are important foods since they are nutritious and contribute to a sustainable food production. In Sweden, the cultivation of beans is intended to increase. One of the cultivated beans is the brown bean from Öland. This article presents the result from a study where the content of vitamin B_6 is determined in brown beans from Öland. Factors influencing the content of vitamin B_6 is also investigated.

In the developing world legumes are an important source of nutrients. There is an international ambition to increase the production and consumption of legumes. Sweden has formulated a food strategy that aims to increase the native food production with focus on sustainability. Different types of beans are cultivated in Sweden and the area of cultivation is increasing. The brown bean from Öland has got a protected geographical indication (PGI), meaning that some part of the process must be performed on Öland. When growing brown beans, it is important they get enough water during sowing and flowering for good development. During maturation, they need less water. The beans need a warmer climate which is found in the southern part of Sweden. They also prefer the soil with a higher pH-value which is found on Öland.

Vitamin B_6 is one of many important nutrients in brown beans from Öland. It is widely distributed in foods and deficiency in humans is rare but it can occur in vulnerable individuals, such as elderly, alcoholics, people with certain diseases or for people with unilateral food habits. In foods derived from plants (for example legumes and cereals), vitamin B_6 is to a certain extent bound to a sugar molecule. This will lower the ability for humans to digest the vitamin. This binding is not found in foods derived from animals. The study concluded that the content of vitamin B_6 in brown beans from Öland was 0.32 mg/100g. This is in the same range as other commonly consumed foods in Sweden. Vitamin B_6 was in 49 percent bound to a sugar molecule in brown beans from Öland. For healthcare, it is good to gain knowledge about the ability to digest vitamin B_6 from foods to be able to formulate dietary advices.

There is no difference in the content of vitamin B_6 between the different cultivars of brown beans studied. There is neither a difference in content depending on where on Öland the beans are cultivated, or if they have received natural or artificial fertilizer. But the content of vitamin B_6 has been found to differ between years of cultivation. Sub-optimal weather conditions increase the content of vitamin B_6. This knowledge is good for farmers when they sell their beans. Less beneficial weather for growth leads to lower yields but the vitamin B_6 content is higher. It has also been elucidated that different cultivars of brown beans from Öland contain different amounts of water. This could affect the storage time which is important to farmers; foods with less water can be stored for longer time. The water content in the cultivar Katja differed between years. This is probably because weather conditions can affect how much and what type of proteins and carbohydrates are being formed in beans. Proteins and carbohydrates can bind water. To summarize, this study has gained knowledge beneficial for farmers, as well as in healthcare when formulating dietary advice.