Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

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– The impact of cultivar, year of production and region of origin

Analys av folatinnehåll i svenska bruna bönor (*Phaseolus vulgaris* L.) från Öland

– inverkan av sort, produktionsår och ursprungsregion

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Abstract

The term folate refers to all naturally occurring derivates of pteroic acid that are biologically active, except folic acid. Over 150 vitamers are possible, but only around 50 are found in foods. Pulses are beneficial from an environmental aspect due to their nitrogen fixation availability, but also from a nutritional point of view, since they are protein rich and contain high amounts of essential micronutrients such as folate. Swedish brown beans (*Phaseolus vulgaris*) are mainly grown on the island of Öland, but test cultivations have recently been performed successfully on the island of Gotland and in parts of Skåne. The beans demand high (>6.5) pH due to the symbiosis with the rhizobia bacteria, high humidity during germination and flowering and dry conditions during harvest and drying. In this study the folate content was determined by a microbiological assay in the cultivars Karin and Katja, to examine if the content differed between cultivars, years and regions. If such a relation would exist, the aim was to explain the differences by meteorological conditions. Furthermore, the aim was to determine the water content of the beans, to examine if it would be related to the factors cultivar, year or region. The content of folate was determined to be 157±26 µg/100 g in Swedish brown beans from Öland. The factors cultivar and region did not have an impact on the folate content in the beans. The folate content differed between years, plausibly explained by different meteorological conditions between the years. The beans from 2009 contained the highest contents of folate, when the meteorological conditions were optimal. The water content in Katja differed between years, with the highest amounts in the beans from 2009, indicating that water binding substances such as proteins and carbohydrates were present to a higher degree. The water content differed between Katja and Karin, indicating the presence of different amounts of water binding substances in the cultivars.

Keywords: folate, *Phaseolus vulgaris*, Swedish brown beans from Öland, cultivar, meteorological conditions, water content

Sammanfattning

Termen folat refererar till alla naturligt förekommande derivat av pteroinsyra som är biologiskt aktiva, förutom folsyra. Över 150 vitamerer är teoretiskt möjliga, men endast omkring 50 återfinns i livsmedel. Baljväxter är fördelaktiga för miljön på grund av deras kvävefixerande förmåga, men också ur en näringsmässig aspekt då de innehåller höga halter av proteiner och essentiella mikronäringsämnen såsom folat. Svenska bruna bönor (*Phaseolus vulgaris*) produceras främst på Öland, men testodlingar har nyligen utförts framgångsrikt på Gotland och i delar av Skåne. Bönorna kräver jordmånar med högt (>6,5) pH på grund av symbiosen med rhizobia-bakterierna, hög fukthalt under groning och blomning och låg fukthalt under skörd och torkning. I denna studie användes en mikrobiologisk metod för att bestämma folatinnehållet i sorterna Karin och Katja, för att undersöka om halterna skilde mellan år, sorter och regioner. Om en sådan skillnad fanns, var syftet att förklara skillnaderna med hjälp av meteorologiska förhållanden. Vidare var syftet att bestämma vattenhalten i bönorna, för att undersöka om den varierade med faktorerna sort, år eller region. Folatinnehållet i svenska bruna bönor från Öland bestämdes till 157±26 µg/100 g. Faktorerna sort och region påverkade inte folathalten i bönorna. Folatinnehållet skilde mellan år, möjligen på grund av olika meteorologiska förhållanden. Bönorna från år 2009 innehöll högst halter av folat, då de meteorologiska förhållandena var optimala för bönodlingen. Vattenhalten i sorten Katja skilde mellan åren, med de högsta halterna under år 2009. Detta indikerar att bönorna från 2009 innehöll högst andel vattenbindande substanser såsom proteiner och kolhydrater. Vattenhalten skilde även mellan de olika sorterna Katja och Karin, vilket antyder att sorterna innehåller olika andel av vattenhållande substanser såsom proteiner och kolhydrater.

Nyckelord: folat, *Phaseolus vulgaris*, svenska bruna bönor från Öland, sort, meteorologiska förhållanden, vattenhalt

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1 Introduction

The term folate refers to all naturally occurring derivates of pteroic acid that are biologically active, except folic acid (Ball, 2006). Folate deficiency can affect anyone, but is mainly an issue for women in childbearing age, specifically pregnant and lactating women (Livsmedelsverket, 2016a). The biochemical role of the vitamin is to function as a one-carbon carrier, participating in the vital formation and metabolism of RNA and DNA, S-adenosyl methionine and several amino acids. In these reactions a folate coenzyme donates a one-carbon unit to the reaction, resulting in a reduced folate, which can continue to receive other one-carbon units. Folate deficiency subsequently results in defaulting synthesis of RNA and DNA, which can lead to megaloblastic anaemia (Ball, 2006). There are reported risks associated with excessive intake of synthetic folic acid from fortified foods and food supplements. Folate works intimately with vitamin B_{12} in the vital methylation reaction in the human metabolism, and the excess of folate can hide the deficiency of vitamin B_{12} and subsequently cause haematological symptom. Another problem associated with excessive consumption of folate is the decreased efficiency of antagonists. In cancer treatment the antagonist Methotrexate is used, and can consequently be resisted by excessive folate. No adverse health effects are however associated with excessive intake of natural folates, thus ingestion of folates from foods is preferable (EFSA, 2006).

The folate consumption is proven to increase together with increased consumption of dried legumes (Mitchell, 2009; Mudryj, 2012), such as Swedish brown beans. Further, the climate effect of legumes is fairly small and they are well suited in crop rotation systems. They are also easy to store (NCM, 2012). Swedish brown beans are currently grown mainly on the island of Öland, but recent test-cultivations suggest that there are additional suitable regions in Sweden for the production of the beans (Lundgren, 2017- personal communication). In 2016 the Swedish government determined to propose a national food strategy (Proposition 2016/17:104). The strategy includes the ambition to encourage and support the

native food and agriculture industry. Therefore, there is a nutritional value as well as a political value to examine the folate content in Swedish brown beans.

Studies suggest that weather and soil affects the content of folate in crops, whereas cultivars do not seem to have an effect. This includes pulses such as dry beans (Goyer & Navarre, 2007; Han & Tyler, 2003). The extent of the variation in crops is however not well-known, thus more research is needed (Goyer & Navarre, 2007).

1.1 Vitamins

Vitamins are a group of organic nutrients that cannot be synthesized in sufficient amounts by the consuming organism, and are thereby essential. They have various functionalities and are widely distributed among human foods. There are thirteen vitamins essential for humans, of which four are fat-soluble: Vitamin A, D, E and K. Vitamin C, and all the vitamin B are water-soluble. The vitamin B group consists of eight vitamins: thiamine (B_1), riboflavin (B_2), niacin (B_3), panthothenic acid (B_5), pyridoxine, pyridoxal or pyridoxamine (B_6), biotin (B_7), folic acid (B_9) and cobalamins (B_{12}). Some of the vitamins have different vitamers, meaning structurally related derivates. The difference in structure can influence the properties of the vitamin, such as stability, bioavailability and activity. The precursors of vitamins, namely provitamins, can be converted to vitamins in the human body metabolism (Ball, 2006; Zempleni *et al.*, 2014).

Many of the B-group vitamins function as coenzymes in the human catabolism. Thus the deficiency of them can result in accumulation of some substrates in the body or alternative pathways. The deficiency of some of the vitamins can lead to diseases, whereas others do not have distinct deficiency symptoms. Vitamin deficiency can be caused by inadequate ingestion, defective absorption, inadequate utilization, or increased requirement due to increased excretion or catabolism (Ball, 2006; Zempleni *et al.*, 2014).

1.2 Folate

Over 150 folate derivates are theoretically possible, although there are seldom more than around 50 found in foods (Nollet & Toldrá, 2013). The mother compound folic acid consists of a pteroic acid with a mono L-glutamic acid linked to it. When the pteridine ring is reduced, the two biological active forms 7,8-dihydrofolate (DHF) and 5,6,7,8-tetrahydropteroylmonoglutamic acid (THF) are

generated. These in turn can be substituted with a one-carbon adduct linked to the nitrogen on position N^5, N^{10} or both and form various structures of folate. Some intermediates are 5-formyl-, 10-Formyl- , 5-methyl-, 5-formimino-, 5,10-methylene- and 5,10-methenyl-THF (Figure 1). Typically five to seven glutamate residues are linked to all folates through γ-linkages.

The vitamin is active when the glutamyl α-carbon is in the L isomeric formation simultaneously as the C^6 of the pteridine ring of the tetrahydrofolates is in the S isomeric form (Ball, 2006; Nollet & Toldrá, 2013; Zempleni *et al.*, 2014). The main vitamers in animal foods are the polyglutamates 5-methyl-THF and 10-formyl-THF, whereas tetrahydrofolate is mostly common in liver (beef and pork) and various species of fishes. In plant foods the polyglutamyl forms of 5-methyl-THF are predominating. Folic acid is a synthetic form that only occurs in fortified foods, where it is used due to its stable form (Nollet & Toldrá, 2013).

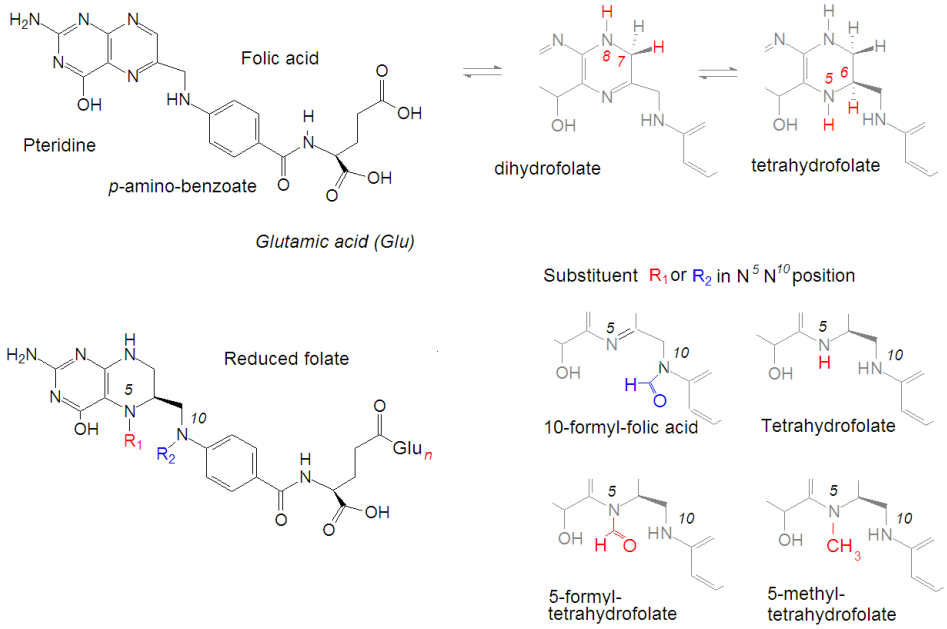

Figure 1. Folic acid and its derivates (Hanna Sara Strandler, 2012).

1.3 Stability of folates

Synthetic folic acid is a yellowish, crystalline powder that have a charring temperature around 250 °C. It is practically insoluble in cold water, somewhat soluble in

methanol, less in ethanol and not at all in acetone, diethyl ether, chloroform or benzene. It is soluble and degradable in hydrochloric acid that have been heated. Folic acid is stable and soluble in alkaline solutions with pH between 6.5-6.8. Furthermore, the vitamers are ionogenic and amphoteric. The N^5-position of the THF (pka = 4.8) and the glutamate carboxyl groups (γ pka = 4.8; α pka = 3.5) are ionic groups of relevance in the pH range of foods. Polyglutamyl folates are more ionogenic than monoglutamyl folates, because of the free α-carboxyl groups on the glutamate residues (Ball, 2006).

The stability of the different vitamers depends on external factors such as pH, UV-light, oxidants, temperature and catalysts, and thus varies considerably. The vitamin activity can be affected by a cleavage of the C^9-N^{10} bond but also by interruptions of the pteridine ring. Less exposed to cleavage are the vitamers with substituted nitrogens, since this will work as a steric hindrance. Dihydrofolate and tetrahydrofolate are considered as the least stable forms, especially the unsubstituted forms (Strandler, 2012; FAO 2001). However, the substituted forms are more susceptible to oxidative degradation (FAO, 2001). Folic acid is the most stable vitamer and can withstand 100 °C for 10 hours (pH 5.0-12.0), when protected from UV-light. Antioxidants, such as ascorbic acid or thiols have a great positive impact on the stability of the vitamers (Ball, 2006).

1.4 Folates in raw and cooked foods

Folates can be found in a broad range of foods, but the highest amounts appears in dark green leafy vegetables, legumes, liver foods, berries and fortified foods such as cereals (Nollet & Toldrá, 2013; Ball 2006).

Since many of these foods are consumed after preparation, it is relevant to determine how much of the vitamins that retain after various types of cooking. The retention factor is used to calculate the percentage of vitamins that retains after preparation (Strandler, 2012).

$$\%\,True\ Retention = \frac{folate\ content\ per\ g\ food \times g\ food\ after\ cooking}{nutrient\ content\ per\ g\ raw\ food\ \times g\ food\ before\ cooking} \times 100$$

When blanching or cooking foods like vegetables, folates leach into the water due to their water-soluble properties. The amount of losses increases with the amount of used water. The losses are mainly due to leaching of folates into the surround-

ing water, and not to thermal degradation (Ball, 2006; McKillop *et al.*, 2002). Several legumes have to be soaked before cooking, such as dried beans and peas. After 16 hours of soaking, studies have shown losses around 20 %. When boiling the legumes the losses reach around 30 %.

Steam blanching and microwaving decreases the losses of folate. The exposed surface area of the foods is also important for the amounts that get lost, with greater exposed areas resulting in greater losses (Ball, 2006).

1.5 Bioavailability and nutritional requirements

The synthetic form of folic acid is more bioavailable than the other vitamers (~85% compared to ~50%). Due to this the dietary folate equivalents are the mass of the naturally occurring folates in micrograms plus 1.7 (85/50) multiplied with the mass of the synthetic form (Ball, 2006; Nollet & Toldrá, 2013; Strandler, 2012).

The requirement of folate varies dependent on individual factors such as sex, age and physiological state, to mention some. The Nordic Nutrition Recommendations (NCM, 2012) have set four levels. LI is defined as the lower intake level, AR the average requirement for normal levels of folate in the blood, RI recommended intake and UL the upper limit, above which there could be a risk of an adverse health effect (Figure 2).

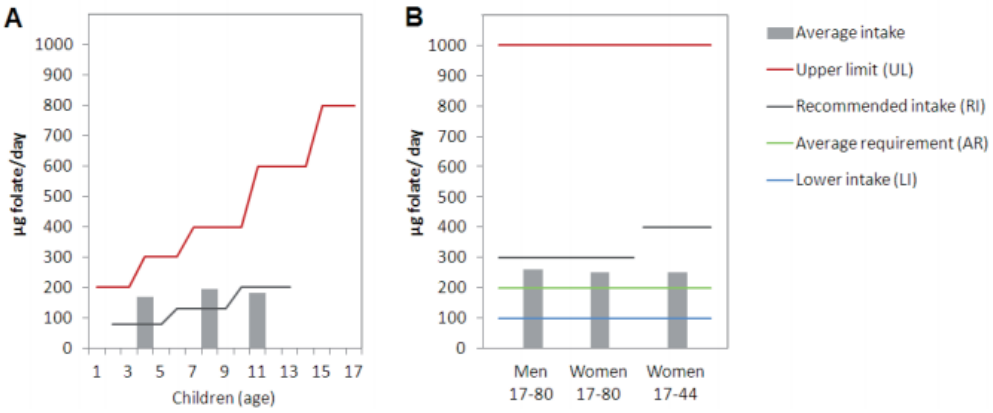

Figure 2. LI, AR, RI and UL for children (A) and adults (B). (Hanna Sara Strandler, 2012). Figure is based on data from NCM 2005, Riksmaten barn 2003 (Enghardt *et al*., 2003) and Riksmaten 97-98 (Becker & Pearson, 2002).

Most nutrients can be provided by consuming sufficient amounts of energy. Folate is however an exception, since some subgroups are risking to suffer from deficiencies, despite a normal diet (NCM, 2012). The National Food Agency of Sweden recommends food supplements of folic acid when planning and during a pregnancy. Pregnant women are recommended to ingest 400 µg in supplements each day until the 12th week. During the rest of the pregnancy it is highly recommended to eat foods that are rich in folates (Livsmedelsverket, 2016a). EFSA (2006) have identified the upper limits for children and young adults based on the lower body weight. The upper limits for adults are based on that there are no studies that indicate adverse health effects below 1 mg of folate per day. The most sensitive groups show an adverse health effect above dosages of 5 mg per day (EFSA, 2006).

1.6 Methods of analysis

The analysis of folates is complicated due to several factors. Firstly, there is a great number of compounds that can occur in foods. The variety and diversity of the folates, the low concentrations that occur in foods and their instability aggravates the determination of them. Additionally, there are enzymes in the foods that can, when associated with the substrate, degrade and modify the folates (Ball, 2006).

A broad variety of techniques are presently used for the determination of folate, including chemical, biological and microbiological methods. The chemical methods include HPLC and UPLC and can be combined with various detectors such as electrochemical, UV and fluorescence. Since all folates absorb light, it is practically possible to detect them with UV methods. However, the only form that is detectable is the synthetic folic acid, since the method is not sensitive enough to detect the natural occurring folates. The fluorescence ability of the reduced folates varies with pH and buffer composition, with fluorescent activity increasing with acidity. Due to this and that the fluorescence varies among vitamers and pH, it is not possible to determine all forms in one analysis. However, this method is preferable to UV detection, since it is more specific. Mass spectrometry (MS) is the most specific and selective detector as of today. Electrospray ionization (ESI) is most often used, since atmospheric pressure chemical ionization (ACPI) risks to degrade the folates due to the high temperatures. Another suitable method would be matrix assisted laser desortion/inonization (MALDI) since it is a relatively mild

method. Positive ion mode is preferable in the analysis of folates, since negative ions would cause the acidic buffer to inhibit the generation of deprotonated molecules. The high cost of the MS limits the use of it worldwide as of today (Nollet & Toldrá, 2013).

Hitherto, the most common method is the microbiological assay. The method is most often preceded by a di- or tri-enzymatic extraction. Briefly, the extraction is performed by the use of heat extraction and amylase addition, to liberate the folate from the food matrix, combined with the use of γ-glutamyl hydrolase, to deconjugate the glutamate residues in order to limit the forms for determination. In protein rich foods, protease can be used to improve the release of protein-bound folates. The extraction step is proceeded by the microbiological assay, in which the bacteria *L. casei* subspecies *rhamnosus* is grown, with the restrictive factor being folate (Nollet & Toldrá, 2013; Strandler, 2012; Zempleni *et al.*, 2013). The microbiological method is cheap and sensitive, but is limited by the fact that the bacteria can be stimulated or inhibited by substances other than folates in the test material (Arcot & Shrestha, 2005).

1.7 Pulses

The term pulses is often defined as the edible seeds of grain legumes. FAO (2016a) defines pulses as crop plants from the *Leguminosae* family that produce edible seeds consumed by humans or animals. In addition to that, only legumes harvested with dry grains are included in the definition. Thus, legumes used for oil extraction, such as the soybean, or those used as vegetables, such as green peas, are not considered as pulses. Accordingly, the common bean, *Phaseolus vulgaris L.* is regarded as a pulse when harvested for dry grain, but not when harvested unripe.

Table 1. *Classification and production of pulses (FAO, 2016a)*

	Production 1994*
Beans, dry	25 093 616
Chickpeas	14 239 010
Peas, dry	11 332 772
Cowpeas, dry	5 588 947
Lentils	4 885 271
Pigeon peas	4 858 102
Broad beans	4 297 465
Lupins	981 480
Vetches	883 238
Bambara beans	287 793
Pulses, others	5 151 560

*In tons per year.

The classification of pulses can be done accordingly to Table 1. The common bean (*Phaseolus vulgaris*) belongs to the category dry beans, so does the lima bean (*Phaseolus lunatus*), scarlet runner bean (*Phaseolus coccineus*), tepary bean (*Phaseolus acutifolius*), adzuki bean (*Vigna angularis*), mung bean (*Vigna radiata*), rice bean (*Vigna umbellata*), and moth bean (*Vigna aconitifolia*).

The production of pulses have been somewhat stable since 1994, and is around 80 000 000 tons per year (Table 1). The consumption is steadily declining all over the world, and is caused by a change in dietary preferences but also in some countries by an insufficient domestic production to cover the request (FAO, 2016b).

1.8 The role of legumes in the ecosystem of the soil

A very important property of pulses is their ability to fix nitrogen. This process is a symbiosis between the plant and the soil bacteria rhizobia that enables the production of pulses on nutrient poor soils, and subsequently mitigates the climate changes that mankind is dealing with. The nitrogen fixation is however somewhat dependent on the climate, since the bacteria rhizobia will be killed by temperatures above 50 °C. This aspect needs to be considered when introducing the crop to new areas.

Pulses are involved in additional biological and chemical processes that maintain and improve the soil biodiversity and structure. They are involved in releasing soil-bound phosphorus, which in turn can be used by other contemporary or future

crops. They also have a role in improving soil aeration and water holding capacity. When organic matters are decomposed, an organic matter called humus is formed. When humus is further degraded it increases and improves the soil aggregation and aggregate stability, the cation exchange capacity and conduces nutrients such as nitrogen and phosphorus to the soil. The humus also increases the water holding capacity as well as storage of carbon from the atmosphere. The aggregates also improve aeration, water infiltration and resistance to erosion and crusting. Legume production contributes with stable soil aggregates which in turn increase pore space. They achieve this by binding and compressing soil particles, which lead to stable soil aggregates. They also affect the soil structure through microbiological activity on their roots, which generate polysaccharides and proteins. These nutrients stimulate mycorrhizal hyphae growth, which spread and expand the root system and thereby the aggregate stabilization.

Soil organisms include various types of micro-organisms (e.g. bacteria and fungi), meso-fauna (e.g. acari and springtails) and macro-fauna (e.g. earthworms and termites). Plant roots can be regarded as soil organisms, considering their interactions with other components of the soil. The diversion of soil organisms is important to maintain a healthy soil but also to have a sustainable agricultural system. The biodiversity contributes with resistance and resilience against stress and also improves the capacity suppress diseases. The diversity of microorganisms is great and can vary due to the physical, ecological, climatic and structural properties of the soil. The increasing population is causing a change in climate and land usage which is damaging specific species of organisms and subsequently threatening the productive capacity of the soil (FAO, 2016a).

1.9 Swedish brown beans

Swedish brown beans belong to the common beans *Phaseolus vulgaris* L. They are mainly grown on the island of Öland, and have since year 2010 protected designation of origin (PDO). The cultivars that are currently on the market are Stella I, Katja, Karin and Bonita (Föreningen för Bruna Bönor på Öland, 2017; Öland´s touristcenter; 2017). An average of 720 hectares per year in Sweden have been used for bean production during the years 1995-2011 and the mean production in quantity for the same period have been 1140 tons per year. The import of brown beans and garden beans from other countries was in 2011 reported to be 1429 tons. The total consumption of brown beans and garden beans in the Swedish population is about 2900 tons (Jordbruksverket, 2012).

The climate and soils of Öland are beneficial for the production of brown beans (Föreningen för bruna bönor på Öland, 2017; Öland's touristcenter, 2017). The soil consists of moraine, postglacial sand and sedimentary rock. The scenery changes above Färjestaden, where it consists less of sedimentary rock. The pH of the soils are relatively high (>7) which is beneficial for the brown beans (FAO, 2017; Jordbruksverket, 2015). Pulses generally get damaged by acid soils, since the nitrogen fixation gets interrupted (FAO, 2017). The soils of Öland are similar to those of the Swedish island of Gotland (Jordbruksverket, 2017; SGU, 2017). Recently test-productions of brown beans were successively performed on Gotland and in parts of Skåne (Lundberg, 2017- Personal communication).

The beans are normally planted in May and harvested in September and the flowering occurs in July. A warm spring is preferable for the plants in order for the leaves to spread and suppress evaporation and weed growth. The height of the plants only reaches around 35-45 cm, which demands a special harvesting method. Before packing, the beans are dried to around 18 % of moisture (Föreningen för bruna bönor på Öland, 2017; Lundberg, 2017- personal communication; Öland's touristcenter, 2017).

The estimated water need of beans in general is greatly affected by the climate. Beans grown in a hot and sunny climate naturally demand more water. Generally, moderate or high humidity is required during growth, whereas dry weather is necessary when harvesting, drying and threshing of the beans. Too high or low humidity during growth will result in diseased plants (FAO, 2017). FAO has identified the water need for beans in general to be 300-500 mm during total growing period (FAO, 1991). The absolute minimum temperature for beans in general is estimated to be 7°C, the optimal minimum 16°C, the absolute maximum 32 °C and the optimal maximum 25 °C (FAO, 2017).

1.10 Nutritional contents of beans and other pulses

A high intake of dried legumes increases the folate intake, together with dietary fiber and other carbohydrates. The amino acid content can be full worthy when combining legumes with cereals or meats (NCM, 2012). The nutritional content in pulses vary, but there are some common traits. The contents of protein and carbohydrates, including dietary fiber are relatively high (NCM, 2012; Table 2 & 3). The folate content varies considerably among pulses, but it is uncertain if there are real differences or if they are due to variation in analytical methods (Han & Tyler, 2003). The folate content in brown beans was determined to be 140 µg/100 g (edi-

ble weight) by the Danish food composition databank (1991), whilst the Swedish and Norwegian authorities have set it to 394 µg/100 g (1989) (edible weight) (European Food Information Resource, 2017). The method used in these databases is the microbiological method.

Table 2. *Some nutrients in raw mature seeds (Dietary folate equivalents per 100 g of edible weight)*

	Water (g)	Protein (g)	Carbo-hydrates (g)	Dietary fiber (g)	Zinc (mg)	Iron (mg)	Folate (µg)
Adzuki beans	13.44	19.87	62.90	12.7	5.04	4.98	622
Black beans	11.02	21.60	62.36	15.5	3.65	5.02	444
Chickpeas	7.68	20.47	62.95	12.2	2.76	4.31	557
Great northern beans	10.70	21.86	62.37	20.2	2.31	5.47	482
Kidney beans	11.75	23.58	60.01	24.9	2.79	8.20	394
Lentils (raw)	8.26	24.63	63.35	10.7	3.27	6.51	479
Lima beans	10.17	21.46	63.38	19.0	2.83	7.51	395
Navy beans	12.10	22.33	60.75	15.3	3.65	5.49	364
Pinto beans	11.33	21.42	62.55	15.5	2.28	5.07	525

Data from the food composition databases of U.S. Department of Agriculture (USDA, 2017).

Table 3. *Some nutrients in dried and boiled mature Swedish brown beans (per 100 g of edible weight)*

Swedish brown beans	Water (g)	Protein (g)	Carbohy-drates (g)	Dietary fiber (g)	Zinc (mg)	Iron (mg)	Folate (µg)
Dried	11.20	22	45.10	16.40	2.000	5.000	394.0
Cooked	58.50	8.75	16.80	13.20	1.100	2.200	59.3
Commercially cooked	72.20	4.38	15.00	6.40	0.580	1.550	36.5

Data from the food composition database of the National Food Agency of Sweden (Livsmedelsverket, 2017).

2 Aim

The aim of this thesis was to determine the folate content in Swedish brown beans from Öland, to examine if the content differed between cultivars (Karin, Katja), years of production (2008-2010) and regions of origin (Borgholm, Färjestaden, Mörbylånga, Kastlösa, Degerhamn). If such a relation would exist, the aim was further to explain the differences by meteorological conditions. Another aim was to determine the water content of the beans, to examine if it would be related to the factors cultivar, year or region.

3 Materials and Methods

3.1 Sampling

The brown beans were obtained from the producers and were kept in plastic vacuum bags in -70 °C until the analysis. In this study, 26 samples from different years, cultivars and regions were collected (Table 4 and 5; Figure 3).

Table 4. *Cultivars and production year of the beans*

Cultivar	Year	Samples
Karin	2008	1
	2009	7
	2010	1
Katja	2008	7
	2009	7
	2010	2
Bonita	2010	1
Total	2008	8
	2009	14
	2010	4

Figure 3. Island of Öland. The regions of origin of the samples are marked. From top: Borgholm, Färjestaden, Mörbylånga, Kastlösa and Degerhamn.

Table 5. *Cultivars and regions of origin of the beans*

Cultivar	Region	Samples
Karin	Borgholm	2
	Färjestaden	3
	Mörbylånga	1
	Kastlösa	1
	Degerhamn	2
Katja	Färjestaden	1
	Mörbylånga	2
	Kastlösa	4
	Degerhamn	9
Bonita	Degerhamn	1
Total	Borgholm	2
	Färjestaden	4
	Mörbylånga	3
	Kastlösa	5
	Degerhamn	12

3.2 Milling and packaging

The frozen beans were milled by using a RETSCH ZM100. Two sieves of the sizes 4.0 and 0.5 mm were used consecutively to obtain sufficiently fine milled particles. Caution was kept not to overheat the sieves, by putting the milling cutters on cold when necessary. The milled beans were kept in a vacuum bag in -70°C until use (at most 4 weeks).

3.3 Extraction and purification

The analysis was conducted according to SLV-m059-f3.3 (Livsmedelsverket 2016b). Each sample (1.0 ± 0.1 g) was mixed with 100 ml extraction buffer, containing 40 ml 0.1 M phosphate buffer pH 7.8 (di sodium hydrogen phosphate (1.78 %), sodium ascorbate (2 %), thiobarburic acid (0.4 %)) and 60 ml of deionized (Milli-Q®) water. Octanol (1 ml) was added to the samples and aluminum foil was used as cover, prior to autoclaving (121 °C, 15 minutes). The samples were gently mixed and put in water bath to reach room temperature rapidly. Additionally 20 ml of the Phosphate buffer pH 7.8 (0.1 M) was added to the samples. Conjugase solution (3 ml) (Appendix 1) was added to the samples and 1 ml of an amylase solution (Sigma-Aldrich A-6211, 1500 units/ml) was added to the samples and the amylase enzyme blanks. The samples were covered with aluminum foil and incubated in 37 °C for 16 hours. The de-conjugation was stopped by steaming the samples in 100 °C for three minutes. The samples were rapidly cooled to room temperature in a water bath, followed by addition of ascorbic acid (7.5 %). The pH was measured and if required set to 4.5 by adding hydrochloric acid or sodium hydroxide. The samples were put into volumetric flasks and made up to the mark with deionized Milli-Q® water. Subsequently they were filtered through a pleated filter (Munktell V120H) into an Erlenmeyer flask. A volume of the solution calculated to contain approximately 0.3 ng/ml folate was taken to further analysis.

3.5 Calibration solutions

A standard series was prepared by firstly preparing a folic acid stock solution (Sigma 010M1567) (0.100 mg/ml) according to SLV-m059-f.3.3 (Livsmedelsverket, 2016b). Folic acid (52 mg) was weighed and dissolved in 0.1 M phosphate buffer pH 7.0 and made up to 500 ml with deionized Milli-Q® water in a volumetric flask. A concentration series was prepared by adding 1.0 ml of the stock solution to a 100 ml volumetric flask and made up to the mark with deionized Milli-Q® water; where by 1.0 ml from that flask was added to a 200 ml volumetric flask and

made up to the mark with deionized Milli-Q® water. Finally 3.0 ml was transferred to a 50 ml volumetric flask. Buffer (3 ml) was added to the flask, and made up to the mark with deionized Milli-Q® water.

3.6 Microbiological assay

The samples and the calibrations samples were diluted by the use of a Gilson ASPEC® liquid handler. The instrument was primed 5 times with Milli-Q® water and additional 5 times with basal medium (Appendix 1). The samples and the calibrations solutions were transferred into 16*100 mm test tubes, and 13*100 mm test tubes were used for the diluted solutions. Four concentrations of each sample were obtained in duplicates. The racks were covered with a silicon cover and lid and sterilized in 121 °C for 5 minutes. The racks were quickly cooled down to room temperature in a water bath. An inoculum was prepared by mixing 4 ml of growth medium with 4 ml of Milli-Q® water and sterilized in 121 °C for 5 minutes. The inoculum was cooled down and 1600 µl cryo-protected *Lactobacillus. casei ssp. Rhamnosus* (Appendix 1) was added. The inoculum solution (25 µl) was added to all test tubes except five blanks. The test tubes were covered with silicon cover and a lid and put in 37 ± 0.5 °C for 22 hours.

3.7 Turbidimetric method

The turbidity of calibration solutions, samples and blanks was measured with a spectrophotometer (Gilson ASPEC Spectrophotometer) at the wavelength 550 nm whereupon the total folate concentration could be determined with three significant digits.

3.8 Samples

In this study, 26 samples from different cultivars, years and regions were randomized and analyzed (Table 4 & 5). In each batch 5-8 samples and amylase enzyme blanks were included in duplicates (A and B) to compensate for interbatch-variation. A-samples were analyzed immediately, whereas B-samples were kept in -20°C until analysis (at most 1 week).

3.8.1 Quality assurance

Certified reference material (CRM) 485 for mixed vegetables were analyzed in duplicates in different batches to measure the precision and accuracy. The obtained average value from each batch was assured to be within the range of 80-110 % of the reported certified value. A quality control chart was generated by analyzing four control samples in duplicates and calculating the mean and standard deviation. One control sample was subsequently included in every batch, and allowed to deviate from the calculated mean with maximum 3 standard deviations. Spike sample analysis was performed on four samples to further verify the validity and accuracy of the method, by calculating the recovery. The recovery was allowed to be between 80-110 % of the expected value. A third tool that was used to assure the quality of the results was the analysis of FAPAS-samples according to Food Chemistry Proficiency test Report 21103.

3.8.2 Testing of protease

A test was performed to investigate if protease had a significant effect on the detected amount of folate in the control sample. Four control samples were analyzed according to the method, with the modification that 1 ml protease (Sigma-Aldrich P-5147,15 units/ml) was added to the samples after adding extractions buffer and octanol and incubated for 3 hours in 37 ±0.5°C, prior to the first autoclaving (121 ± 1°C in 15 minutes). The results were run in a paired sample t-test with the confidence level 95 %, together with the results from four samples with no added protease.

3.8.3 Determination of dry weight

Glass containers with lids (25 mm deep and a diameter of 50 mm) were dried in 102 ± 2 °C and put to cool down before usage. The glass containers were weighed without samples, upon which 1-2 g of the homogenized samples were added. They were subsequently dried in 102 ± 2 °C, and put to cool down in an vacuum desiccator, until the weight decreased ≤ 1 mg or increased in two following weigh-ins. The scales that were used had the capacity to read of 1 mg and the readability of 0.1 mg.

3.8.4 Meteorological conditions

The meteorological conditions (precipitation and temperature) for one location on Öland (southern cape) was obtained from SMHI (Swedish Meteorological and Hydrological Institute) for 2008-2010.

3.8.5 Statistical analysis

A principal component analysis (PCA) was conducted to be able to get a visualization of trends and patterns. A general linear model (GLM) ANOVA was subsequently conducted. Folate concentration and water content were used as response variables on the factors: cultivar, year and region. Minitab version 17 and The Unscrambler version 10.1 (CAMO software A/S, Norway) were used for statistical analyses. $P < 0.05$ was considered statistically significant.

4 Results and Discussion

4.1 Accuracy and reproducibility of method

The inter-batch variability of the control samples was considered adequate (within ± 2 SD) for all batches, except for one batch, which was re-analysed. Results from the analysis of CRM 485 mixed vegetables imply a high precision and accuracy (Table 6). This can be concluded since the results of the A and B sample are not significantly different, and the detected amount is 89 % of the reported certified amount. The recovery of the spiked samples was determined to be 91 % (Table 7), which is in between the acceptable marginal (80-110 %). The analysis of the FAPAS resulted in a z-score of 0.4 (acceptable range ±2). The combined quality control results imply an adequate accuracy, precision and reproducibility of the microbiological method on the analysis of the brown beans.

Table 6. *Results from analysis of CRM 485 Mixed vegetables (N5747)*

Sample	Folate (µg/100g)	Amount detected (%)
A	278	
B	280	
$\bar{x}$	279	89

Table 7. *Recovery of spiked control samples*

	Folate (µg/100g)	Folate spiked (µg/100g)	Folate added to samples (µg/100g)	Recovery (%)
	121	204		
	130	203		
	126	223		
	115	229		
$\bar{x}$	123	215	100.97	91

4.2 Impact of protease

The t-test showed no significant difference between the control samples with added protease compared to the ones with no added protease ($p=0.103$). Due to that, protease was not added to the samples in the study.

4.3 Meteorological conditions

The meteorological conditions of southern cape of Öland for the years 2008-2010 as well as mean values for 2002-2010 and 1944-1990 can be observed in Table 8. The precipitation for the years 2008-2010 appears to vary. The least amount of precipitation occurred in 2008 and the most in 2010. In 2010 the amount of precipitation was 15 % more than in 2008. Simultaneously, the mean temperatures differed between the years. The highest mean temperature was obtained in 2008 and the lowest in 2010.

Table 8. *Precipitation (mm) and temperature (°C) on Öland 2008-2010*

Year	Precipitation	Temperature
2008	427	8.9
2009	439	8.0
2010	490	6.6
$\bar{x}$ (2002-2016)	459	8.1
$\bar{x}$ (1944-1990)	400	7.0

The meteorological conditions for the months May until September 2008-2010 can be observed in Figure 4 and 5.

The precipitation for 2008 was highest in autumn (August-September) and least in June-July. Since the flowering, which occurs in July, is dependent on high humidity, this is not the ideal situation for the growth of the beans. The harvest and drying of the beans, which occurs in autumn (September), is optimally performed in dry weather. Too high humidity can be detrimental for the beans, by the emergence of diseases. Therefore it is likely to assume that the harvest was not optimal in 2008. The Swedish brown beans demands mild temperatures. Too high temperatures would kill the rhizobia bacteria and too low (frost) would kill the beans (FAO, 2017). The temperature is most important during seed germination, which occurs in May. Since the only obtained data is a mean temperature, frost during night-time cannot be excluded.

The meteorological conditions for 2009 differed from 2008 in terms of precipitation. The precipitation in 2009 was highest in spring and summer and lowest in autumn, which is ideal for the beans. The temperatures are similar to those of 2008, but also here frost cannot be excluded.

In 2010, the amounts of precipitation was highest for spring and summer and lowest for autumn. However, in July the amount of precipitation reached 103 mm (compared to 21 mm in 2008 and 51 mm in 2009). The mean amount for 2010 was 490 mm, which implies that 20 % of that participation occurred in July. Although humidity is desirable during flowering, too high humidity can damage the crops. The mean temperature for May 2010 was considerably lower than for the same period in 2008 and 2009, which also could have affected the crops in a negative way.

According to FAO, beans generally need 300-500 mm of precipitation for the total growing period. The total amount reaches around 220 mm per year, for all accounted years.

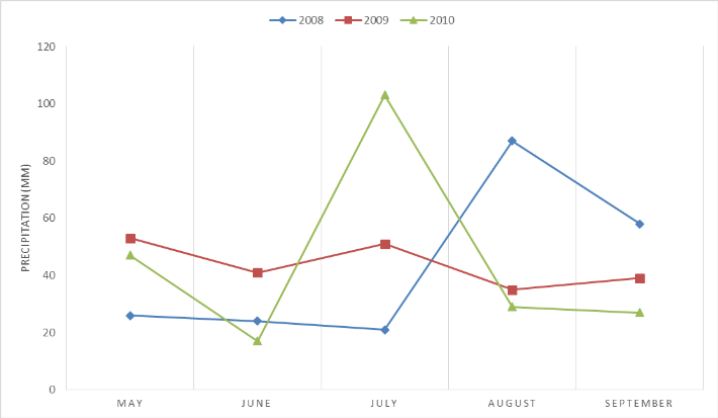

Figure 4. Precipitation (mm) on southern cape (S) of Öland for May-September, 2008-2010.

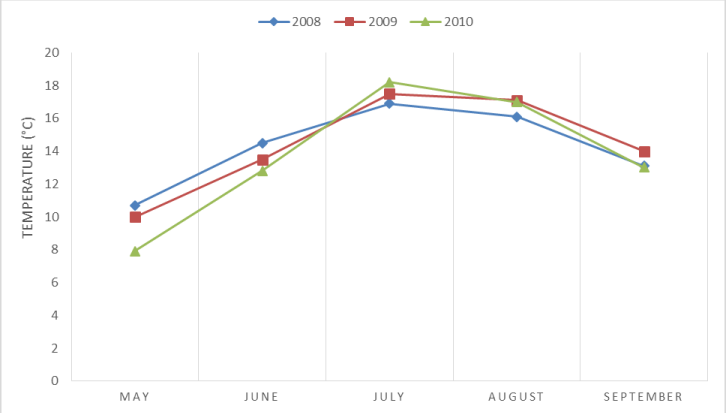

Figure 5. Mean temperature (°C) on southern cape (S) of Öland for May-September, 2008-2010.

4.4 Folate content in Swedish brown beans from Öland

Table 9. *Folate content in dry weight in Swedish brown beans from Öland 2008-2010 and average for the same period in dry and edible weight*

Year	Folate* (µg/ 100g)	Standard deviation (µg/ 100g)	Coefficient of variation (%)
2008	140[a]	±14	10
2009	194[b]	±16	8
2010	190[c]	±18	10
$\bar{x}$ (2008-2010)			
Dry weight	177	±30	17
Edible weight	157	±26	16

*Statistically significant differences are distinguished by different letters.

The average folate content was determined to be 157±26 µg/100 g (minimum 96 µg/100 g and maximum 197 µg/100 g) in edible weight in the brown beans from Öland (Table 9). This content can be compared to the content determined by the National Food Agency of Sweden, which is set to 394 µg/100 g in brown beans. Since these values are around 30 years old, one consideration could be if the content of the beans have differed due to classical plant breeding. This is however not likely, since the Danish National Food Institute reported values of 140 µg/100 g in 1991. The large deviation could instead be explained by the variation that can occur due to different sampling, different laboratories and use of method. Since the content seem to differ between years (Table 9), it is plausible that this factor at least partly could explain the variation. Folate content can differ between different

years, and possibly also due to other factors and variables that have to be considered in the determination of the natural variation of the folate content.

It can however be concluded that the folate content in brown beans is in the range of 140-394 µg/100 g due to the Nordic food databases (Swedish, Norwegian and Danish). This is a fairly high content in a foodstuff (compared to e.g. 202 µg/100 g in raw spinach) and thus Swedish brown beans can be considered as a good source of folate (Livsmedelsverket, 2017). The high content of folate in Swedish brown beans can hence be used in the introduction and marketing of the beans to new and already existing markets.

4.5 Factors influencing the folate content

The factors cultivar and region did not have an impact on the folate content, whereas the year had a significant effect (Table 9 & 10). A previous study have implied that cultivar may not influence folate content in dry beans, which complies with the results (Han & Tyler, 2003). No comparable data is available since no previous studies have been conducted to investigate the folate content between different cultivars of Swedish brown beans.

The factor region did not affect the content of folate significantly. On the contrary, Han & Tyler (2003) identified the location as an affecting factor for the content. One explanation for the results is that the specific regions in this study were located closely to each other. They were all located on the same coast on the same island, which resulted in similar meteorological conditions as well as soils. The soils of Öland are similar in that sense that they have a suitable pH (>7) for the production of Swedish brown beans. Another fact that has to be considered is the uneven distribution among regions in the sampling. Only two samples were analysed from Borgholm, while 12 were analysed from Degerhamn. It is therefore likely that the outcome would have differed with more samples.

Table 10. *Factors influencing the folate content in all beans*

Factor	R-sq (adj) %*	p-value**
Cultivar		0.762
Year	71.88	0.000
Region		0.206

*Percentage in variation explained by the independent variable that affects the dependent variable.
**p-value <0.05 is significant.

The folate content differed between years (Table 10; Figure 6). The lowest amounts were found in beans from 2008, and the highest in 2009. Since only four samples were analysed from 2010, the data from those samples are not considered as sufficient. The difference between 2008 and 2009 is however considerable. The folate concentrations follow the meteorological conditions. As concluded, in 2008 the humidity was relatively low during germination and flowering and high during harvest and drying. The unfavourable meteorological conditions in 2008 seem to have resulted in lower folate contents than for 2009 and 2010. On the contrary, the meteorological conditions seemed optimal for 2009, which seemed to have resulted in higher concentrations of folate. Regardless the small sampling from 2010, the high precipitation in July, which was discussed earlier, may not have affected the crops in a negative way. The trend shows the opposite result.

The impact of weather on the folate content can be useful for producers in order to predict and estimate folate content in Swedish brown beans. It is also useful for producers in dry areas to be aware of the impact of the humidity on the folate content. Further research is needed, since the amount of years and samples was limited in this study.

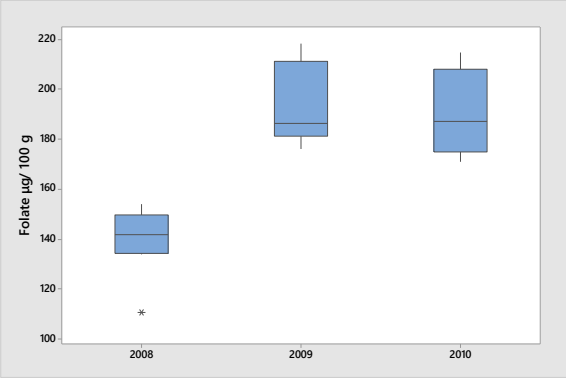

Figure 6. Folate content (µg/100g) in all cultivars for the years 2008-2010. The minimum values, first quartiles, medians, third quartiles and maximum values can be observed in the graph. Outliers are marked as *.

4.6 Factors influencing the water content

One interesting observation was that the water content of the beans seemed to be related to cultivar and not year or region (Table 11; Figure 7 & 8). The water content was initially around 18 % (Föreningen för bruna bönor på Öland, 2017) and all diversities should be due to storage and water holding capacity of the beans. The storage conditions were similar for all beans from collection and until they were analysed. The storage conditions should consequently not have affected the

results considerably. Little is known about the different cultivars and their water holding capacity. Plausibly, the difference could be due to water holding substances, such as some carbohydrates and proteins. Dietary fibers are concluded to contribute considerably to the water holding capacity of legumes (Elhardallou & Walker, 1993). Another variable that is suggested to affect the water holding capacity is the thickness and composition of the coating of the beans (Sefa-Dedeh & Stanley, 1979). A thicker coating naturally lead to less evaporation of water from the beans. Since no data on different coatings and content of water binding substances in the different cultivars are available, no certain explanations can be given for the difference in the water content and water holding capacity.

Surprisingly, the factor year did not affect the water content in all beans. However, the water content in Katja differed between years (Table 11; Figure 9). One explanation could be that the considerable variation between cultivars masked the variation between years together with the fact that the sampling from Karin was insufficient between years to obtain reliable values.

The difference in water content between years in Katja could be due to the difference in presence of water binding substances. The beneficial meteorological conditions in 2009 could also have increased the production of water binding substances, such as proteins and carbohydrates. The results from 2010 are contradicting, but are not as reliable due to insufficient sampling.

The water content in the beans is interesting from several aspects. It could affect the durability of the beans, simultaneously as it could affect the quality and sensory of the finished product. Hence it is of interest for the producers and the merchants in the bean industry that different cultivars may have different water content. More research is however needed to make further statements, since the sampling in this study was limited.

Table 11. *Factors influencing the water content in all beans*

Factor	R-sq (adj) %*	p-value**
Cultivar	47.40	0.000
Year		0.083
Region		0.643

*Percentage in variation explained by the independent variable that affects the dependent variable.
**p-value <0.05 is significant.

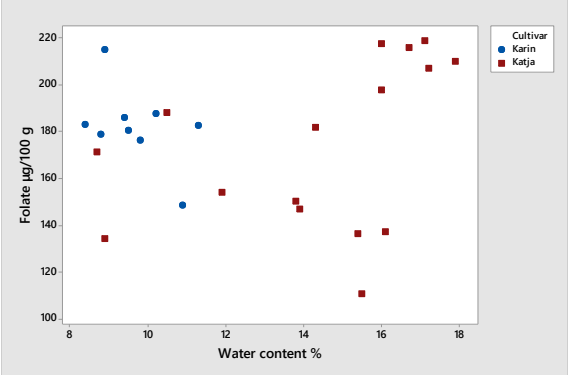

Figure 7. Folate content and water content (%) in the cultivars Karin and Katja for the years 2008-2010.

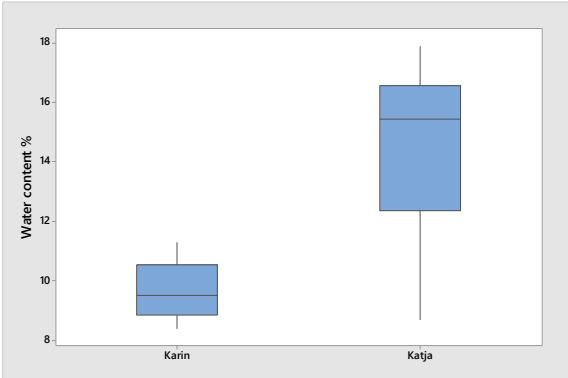

Figure 8. Water content (%) in the cultivars Karin and Katja for the years 2008-2010. The minimum values, first quartiles, medians, third quartiles and maximum values can be observed in the graph.

Table 12. *Factors influencing the water content in Katja*

Factor	R-sq (adj) %*	p-value**
Year	57.15	0.002
Region		0.839

*Percentage in variation explained by the independent variable that affects the dependent variable.
**p-value <0.05 is significant.

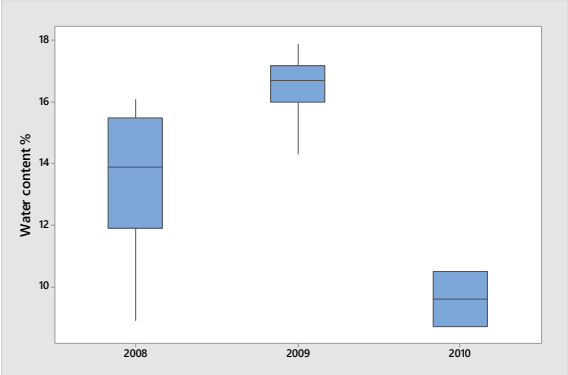

Figure 9. Water content (%) in Katja for the years 2008-2010. The minimum values, first quartiles, medians, third quartiles and maximum values can be observed in the graph.

5 Conclusion

This study concluded that Swedish brown beans from Öland contain 157 ± 26 µg/100 g folate (minimum 96 µg/100 g and maximum 197 µg/100 g). The production year had an impact on the folate content, whereas the cultivar and region did not. The optimal meteorological conditions for a good crop yield also resulted in higher contents of folate in the beans in 2009. The water content of the beans differed between years in the cultivar Katja. The optimal meteorological conditions in 2009 consequently resulted in an increased water holding capacity of the beans. The different cultivars Karin and Katja also contained different amounts of water, which should be due to physiochemical properties of the specific cultivars, that is an effect of the presence of water holding substances.

References

Arcot, J. & Shrestha, A. (2005). Folate: methods of analysis in foods. *Trends in Food Science & Technology*, 1-14. https://www.researchgate.net/profile/Jayashree_Arcot/publication/43452461_Folate_Methods_of_analysis/links/572ed77408ae3736095b1a10.pdf [2017-05-04]

Ball, F.M.G. (2006). *VITAMINS IN FOODS: Analysis, Bioavailability, and Stability*. New York: Taylor & Francis Group.

Becker, W. & Pearson, M. (2002*). Riksmaten 1997-98, Kostvanor och näringsintag i Sverige - Metod och resultatanalys:* National Food Administration, Sweden.

Elhardallou, SB. & Walker, AF. (1993). The water-holding capacity of three starchy legumes in the raw, cooked and fibre-rich fraction forms. *Plant Foods for Human Nutrition*, 44, 171-179. https://www.ncbi.nlm.nih.gov/pubmed/8397397 [2017-05-25]

Enghardt Barbieri, H., Pearson, & Becker, M. (2003). *Riksmaten - barn 2003*: Livsmedelsverket. ISBN 91 7714 177 6.

European Food Information Resource (2017). *FoodEXplorer. Brown beans, dried.* http://www.eurofir.org/foodexplorer/foodgroups.php [2017-05-04]

European Food Safety Authority (EFSA) (2006). *TOLERABLE UPPER INTAKE LEVELS FOR VITAMINS AND MINDERALS.* http://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf [2017-05-02]

Fapas® (2017). *Food Chemistry Proficiency Test*. *Vitamins in Breakfast Cereal.* February-March 2017. Report 21103.

Food and Agriculture Organization of the United Nations (1991). *WATER HARVESTING.* http://www.fao.org/docrep/u3160e/u3160e00.htm#Contents [2017-05-01]

Food and Agriculture Organization of the United Nations (2001). *Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation. Bangkok, Thailand.* http://www.fao.org/docrep/004/Y2809E/y2809e00.htm#Contents [2017-05-01]

Food and Agriculture Organization of the United Nations (2016a). *Soils and pulses: Symbiosis for life.* http://www.fao.org/3/a-i6437e.pdf [2017-05-01]

Food and Agriculture Organization of the United Nations (2016b). *Promoting regional trade in pulses in the Horn of Africa*. . http://www.fao.org/3/a-i6647e.pdf [2017-05-01]

Food and Agriculture Organization of the United Nations (2017). *ECOCROP, search: Phaseolus vulgaris.* http://ecocrop.fao.org/ecocrop/srv/en/cropView?id=1668 [2017-05-01]

Föreningen för Bruna Bönor på Öland (2017). *Föreningen för Bruna Bönor på Öland*. http://www.brunabonor.se/ [2017-04-27]

Han, J-Y. & Tyler, R. (2003). Determination of Folate Concentrations in Pulses by a Microbiological Method Employing Trienzyme Extraction. *JOURNAL OF AGRICULTURAL AND FOOD CHEMSTRY*. 51, 5315-5318. https://www.ncbi.nlm.nih.gov/pubmed/12926876 [2017-05-20]

Goyer, A. & Navarre, R. (2007). Determination of Folate Concentrations in Diverse Potato Germplasm Using a Trienzyme Extraction and a Microbiological Assay. *Journal of Agricultural and Food Chemistry*. 55, 3523-3528. https://www.ncbi.nlm.nih.gov/pubmed/17419642 [2017-05-03]

Jordbruksverket (2012). *Marknadsöversikt-vegetabilier. Report 2012:26.* http://www2.jordbruksverket.se/webdav/files/SJV/trycksaker/Pdf_rapporter/ra12_26.pdf [2017-05-01]

Jordbruksverket (2015). *Nationell jordartskartering. Matjordens egenskaper i åkermarken.* http://www2.jordbruksverket.se/download/18.4288f19214fb7ec78849af18/1441973777932/ra15_19.pdf [2017-05-03]

Jordbruksverket (2017). *Jordartskarta för Sverige.* http://www.jordbruksverket.se/download/18.37e9ac46144f41921cd2127a/1402997444991/Jordartskarta+f%C3%B6r+Sverige.pdf [2017-05-03]

Livsmedelsverket (2016a). *Råd om folsyra för kvinnor.* https://www.livsmedelsverket.se/matvanor-halsa--miljo/kostrad-och-matvanor/folsyra-for-kvinnor [2017-05-01]

Livsmedelsverket (2016b). *Analys av Folat i Livsmedel med mikrobiologisk metodik.* SLV-m059-f3.3.

Livsmedelsverket (2017). *Livsmedelsdatabasen.* http://www7.slv.se/SokNaringsinnehall [2017-05-01]

McKillop, J.D., Pentieva, K., Daly, D., McPartlin, M.J., Hughes, J., Strain, J.J., Scott, M.J. & McNulty, H. (2002). The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. *British Journal of Nutrition*, 88, 681–688.

Mitchell, C.D., Lawrence, R.F., Hartman, J.T. & Curran, M.J. (2009). Consumption of Dry Beans, Peas, and Lentils Could Improve Diet Quality in the US Population. *Journal of the AMERICAN DIETETIC ASSOCIATION*, 109, 909-913.

Mudryj, N.A., Yu, N., Hartman, J.T., Mitchell, C.D., Lawrence, R.F. & Aukema, M.H. (2012). Pulse consumption in Canadian adults influences nutrient intakes. *British Journal of Nutrition*, 108, 27-36.

Nollet, M.L.L. & Toldrá, F. (2013). *Food Analysis by HPLC*. 3 Edition. New York: Taylor & Francis Group.

Nordic Council of Ministers (2005). *Nordic Nutrition Recommendations 2004. Integrating nutrition and physical activity*. 4th. ed. Copenhagen; Nord 2004:13.

Nordic Council of Ministers (2012). *Nordic Nutrition Recommendations 2012.* https://www.livsmedelsverket.se/globalassets/matvanor-halsa-miljo/naringsrekommendationer/nordiska-naringsrekommendationer-2012-svenska.pdf [2017-05-01]

Sefa-Dedeh, S. & Stanley, D.W. (1979). The relationship of Microstructure of Cowpeas to Water Absorption and Dehulling Properties. *Cereal Chemistry®*, 56, 379-386. http://www.aaccnet.org/publications/cc/backissues/1979/Documents/Chem56_379.pdf [2017-06-01]

Strandler, H.S. (2012). Determination of Folate for Food Composition Data. Development and Improvement of Analytical Methods. Lic. Uppsala: Swedish University of Agricultural Sciences.

Sveriges geologiska undersökning (2017). *Kartor. Kartvisaren. Jordkartvisare. 1:25 000- 1:100 000.* Uppsala: Sveriges geologiska undersökning. http://www.sgu.se/produkter/kartor/kartvisaren/ [2017-05-02]

Sveriges meteorologiska och hydrologiska institut (2017). *Års- och månadsstatistik.* https://www.smhi.se/klimatdata/meteorologi/temperatur/2.1240 [2017-05-04]

United States Department of Agriculture: *Agricultural Research Service: USDA Food Composition Databases*. https://ndb.nal.usda.gov/ndb/search/list [2017-04-13]

Zempleni, J., Suttie, W.J., Gregory III, F.J. & Stover, J.P. (2014). *HANDBOOK OF VITAMINS.* New York: Taylor & Francis Group.

Öland´s touristcenter (2017). *The Swedish Brown Bean.* https://www.oland.se/sites/oland/files/the_swedish_brown_bean.pdf [2017-05-09]

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Appendix 1

1.1 Extraction of gamma-glutamyl hydrolase from chicken pancreas

Chicken pancreas from Guldfågeln was freeze-dried and kept in -20 °C until use. A buffer (pH 6.0) was prepared by dissolving 4.53 ± 0.02 g potassium-di-phosphate, 1.17 ± 0.02 g di-potassium-hydrogen phosphate and 4.0 ± 0.02 g of sodium ascorbate in Milli-Q® water and adjusting pH to 6.0 ± 0.02. The solution was transferred to a volumetric flask and made up to 400 ml. The freeze-dried chicken pancreas (20 g) was grinded and 19.2 ± 0.05 g was added to a beaker. The buffer (200 ml) was added to the beaker while stirring. The mixture was incubated for 1 hour in 37 ± 0.5 °C. The mixture was homogenized in a cold blender (Waring®) for 2*15 s with 30 s pause. The suspension was transferred to an Erlenmeyer flask with 200 ml of the buffer pH 6.0. The suspension was incubated for 1 hour in 37 ± 0.5 °C, distributed in 50 ml conical tubes and centrifuged 10 minutes in 3000 rpm. A carbon dextran solution was prepared by mixing 2.4 ± 0.02 g dextran and 24 ± 0.02 g active carbon in 240 ml Milli-Q® water. The supernatant was transferred to a beaker and 80 ml carbon dextran was added. PH was set to 4.6 ± 0.02. The solution was slightly stirred in room temperature for 30 minutes. The suspension was once again distributed into 50 ml conical tubes and centrifuged for 10 minutes in 3000 rpm. The supernatant was transferred to a beaker and 80 ml carbon dextran solution was added. The solution was slightly stirred for 30 minutes. The supernatant was transferred to a beaker whereupon 80 ml carbon dextran solution was added. The solution was slightly stirred for 30 minutes and then distributed into 50 ml conical tubes. The tubes were centrifuged for 30 minutes in 9000 rpm in 4 °C. The supernatant was filtered through a pleated filter (Munktell V120H) and through a 0.2 µm-filter (Whatman FP30/0.2 CA-S). PH was set to 6.1 ± 0.02. The solution was distributed into 15 ml conical tubes and kept in -20°C until use (maximum 2 years). The folate content was determined by the use of the microbiological assay (SLV-m059-f3.3) (Livsmedelsverket, 2016b).

1.2 Basal medium

Folic acid casei medium (Difco nr 28 22 10) (9.4 ± 0.02 g) and ascorbic acid (0.05 ± 0.002 g) were dissolved in 100 ml Milli-Q® water. The solution was boiled for

1-2 minutes and cooled down to room temperature in a water bath. PH was set to 6.1 ± 0.2.

1.3 Folate CRYO

Glycerol (80 ml, 87%) was mixed with Milli-Q® water (20 ml). The solution was autoclaved in 121 °C for 15 minutes and cooled down to room temperature in a water bath. A growth medium was prepared by dissolving basal medium (4.7 g) Folic Acid Case Medium (Fifco nr 28 22 10) and of L (+) - ascorbic acid (0.025 g) in Milli-Q® water (100 ml). The solution was boiled for 1-2 minutes and cooled down to room temperature. PH was set to 6.1. A folic acid calibration solution was prepared by adding Folic acid stock solution to Milli-Q® water, to a concentration of 100.0 ng/ml. The calibration solution (0.5 ± 0.02 ml) was added to the growth medium. The solution was autoclaved in 121 °C for 15 minutes and then cooled down to room temperature in a water bath. A file was used to scratch the ampoule with the freeze-dried *Lactobacillus rhamnosus* CCUG 21452 (ATCC 7469). Ethanol (70 %) was used to sterilize the scribe. The ampoule was gently burned, put in a sterile gauze and broken open. The growth medium (1.0 ± 0.02 ml) was added to the ampoule and mixed. A portion (0.15 ± 0.02 ml) of the ampoule was re-transferred to the growth medium. The medium was incubated in 37 °C for 18 hours. The glycerol solution was added to the growth medium while stirring gently. Portions of 1.6-2 ml were transferred into sterile plastic vials and kept until use in -70 °C (maximum 6 months).

Appendix 2

Popular scientific summary

The B vitamin folate is vital for the human metabolism, since it is involved in the formation of RNA, DNA and some amino acids. Deficiency is mainly a problem in women in the childbearing age, but it can affect anyone. Folates can mainly be found in dark green vegetables, liver foods and legumes, but also in fortified foods such as cereals. Since fortified foods and food supplements can lead to excessive intake of folate, it is preferable to meet the requirements with natural occurring folate in foods.

Pulses are the edible dried grains of legumes. They are beneficial from an environmental point of view, since their impact on the climate is fairly low, together with the fact that they´re protein rich, full of fibers and essential nutrients such as folate.

Since legumes contain high amounts of folates, simultaneously as the production of Swedish brown beans is increasing, it is highly relevant to examine the contents of folate in Swedish brown beans. This study discovered that Swedish brown beans from Öland contained 157 µg/100 g of folate. The amount can be compared to other folate rich foodstuff, such as spinach, which contains 202 µg/100 g of folate. The folate content in the brown beans is consequently rather high, thus the consumption of Swedish brown beans can be beneficial for people in general and specifically for those who are risking to develop a deficiency.

The study also discovered that different cultivars of Swedish brown beans contain different amounts of water. This is interesting for the industry and merchants of beans, since it excessively affects the quality and sensorial properties of the finished products.