



# How antinutrients found in legumes and legume-based products affect the bioavailability of nutrients

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Anna Johansson



Independent project in Food Science • 15 credits  
Swedish University of Agricultural Sciences, SLU  
Department of molecular Science  
Agricultural Programme – Food Science  
Molecular Sciences, 2022:15  
Uppsala, 2022



# How antinutrients found in legumes and legume-based products affect the bioavailability of nutrients

*Hur antinutritionella faktorer i baljväxter och baljväxtbaserade produkter påverkar biotillgängligheten av näringsämnen*

Anna Johansson

**Supervisor:** Jaqueline Auer, Swedish University of Agricultural Science,  
Department of molecular Sciences

**Examiner:** Galia Zamartaskaia, Swedish University of Agricultural Science,  
Department of molecular Sciences

**Credits:** 15 credits  
**Level:** First cycle, G2E  
**Course title:** Independent project in Food Science  
**Course code:** EX0876  
**Programme/education:** Agricultural Programme – Food Science  
**Course coordinating dept:** Department of Molecular Sciences

**Place of publication:** Uppsala  
**Year of publication:** 2022  
**Title of series:** Molecular Sciences  
**Part number:** 2022:15  
**Cover picture:** Almost ripe pea field – 2021, Copyright: Anna Johansson

**Keywords:** *Legumes, antinutrients, nutrients, antinutritional factors, bioavailability, absorption and uptake*

## Abstract

As a plant-based diet has been more advocated for various reasons, legumes have become an important pillar in such a diet. Brown beans, yellow peas, green peas and fava beans are the dominating grain legumes grown in Sweden are a natural source of proteins, vitamins and minerals. These nutrients possess an essential role in the function of the human body and a lack of those can cause malnutrition and other health depleting effects. However, the use of legumes is somewhat limited by the presence of other bioactive compounds, referred to as antinutritional factors (ANF). Lectins, tannins, oxalate and protease inhibitors are a few examples that can create problems in the absorption of proteins, iron, potassium, calcium and other nutrients. The level of both nutrients and antinutrients varies between cultivars and within the species, the stage of harvest and other factors that need further investigation. Recent studies have been indicating that ANF prove positive effects on the health like anti-carcinogenic and cholesterol lowering effects. Therefore, the purpose of the report is to inform about potential problems and possibilities that follow legumes, and a legume-based diet, in terms of bioavailability of nutrients.

*Keywords: legumes, antinutrients, nutrients, antinutritional factors, grain legumes, bioavailability, absorption, uptake, nutritional uptake.*

## Sammanfattning

En mer växtbaserad kost har förespråkats av många olika anledningar. I och med detta har baljväxter blivit en viktig pelare i denna typ av kost. Brunna bönor, gula ärtor, gröna ärtor och favabönor är de dominerande baljväxterna som odlas i Sverige och är en naturlig källa till både proteiner, vitaminer och mineraler. Dessa näringsämnen har en väsentlig roll vid uppehållandet av människokroppens funktioner. Brist på dessa kan orsaka undernäring och andra hälsoutarmande effekter. Användningen av baljväxter är dock något begränsad, främst på grund av förekomsten av antinutritionella faktorer (ANF). Lektiner, tanniner, oxalat och proteashämmare är några exempel på ANF och kan, om de intas kontinuerligt, skapa problem vid näringsupptaget av proteiner, järn, kalium, kalcium och andra näringsämnen. Det har dock gjorts studier som tyder på att ANF kan visa på positiva effekter för hälsan också såsom att de kan agera cancerhämmande eller sänka nivåerna av kolesterol i blodet. Därför är syftet med rapporten att informera om potentiella problem och möjligheter som följer med baljväxter, och en baljväxtbaserad kost, när det gäller biotillgänglighet av näringsämnen.

*Nyckelord: baljväxter, antinutrientier, näringsämnen, antinutritionella faktorer, spannmåls baljväxter, biotillgänglighet, absorbering, upptag, näringsupptag.*

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## Abbreviations

ANF	Antinutritional factors
RI	Recommended Intake is a that describes the average amount of a nutrient, sufficient to meet the requirement for 97% of all healthy individuals
PRI	Population Reference Intake, a term used by EFSA to denote the level of nutrient intake, enough for all healthy people in a group
RDA	Recommended Dietary Allowance is a US term that describes the average intake level of a nutrient, sufficient to meet the requirement for 97-98% of all healthy individuals
AI	Adequate intake, a reference value used when there is insufficient data to develop an RDA or RI value
DMT1	Divalent metal transporter 1, a transport protein
IP <sub>1-6</sub>	Myo-inositol-(1,2,3,4,5,6)-hexakis-phosphate are different forms of phytate



# 1. Introduction

## 1.1 Background

Over the past years, there has been an upcoming trend to create new alternative sources of proteins other than meat. Aspects like animal welfare and environmental factors have played a key role in the expansion of plant-based options on the market (Kyriakopoulou *et al.*, 2018). There are also several health arguments that have been raised for reducing meat consumption, particularly an association between a high red meat intake and the risk of some disease (McAfee *et al.*, 2010). When adapting to, or maintaining, a plant-based diet that contains legumes, it is important to have some prior knowledge regarding nutritional factors since the bioavailability of minerals is considered to be lower in legumes. This is mainly due to the presence of absorption inhibitors such as polyphenols and phytates (Mayer Labba, Frøkiær and Sandberg, 2021) or so called antinutritional factors (ANF) (Sharan *et al.*, 2021). Furthermore, legumes have an unbalanced content of essential amino acids (Sandstrom *et al.*, 2012) which increases the risk of nutritional deficiency (Livsmedelsverket, 2021a). By complementing the legumes with other sources of amino acids, either animal originated products or cereal based goods, a satisfactory combination of amino acids can be achieved (Sandstrom *et al.*, 2012).

According to Platel and Srinivasan (2016), both developed and developing countries have a widespread problem with micronutrients deficiencies, mainly caused by poor bioavailability of nutrients from plant-based foods. Thereof, micronutrients such as iron, vitamin A, zinc and iodine (Platel & Srinivasan, 2016) are most likely to be missing if a vegetarian diet is not varied enough (Sanders, 1999). Thus, a vegan diet has to be supplemented with vitamin B<sub>12</sub> through enriched products or supplements, despite variation (Craig, 2009). Certain groups of the population including women of childbearing age, infants, children and adolescents have a higher requirement for some micronutrients such as iron (Sandstrom *et al.*, 2012).

Legumes are a naturally good source of protein and other nutrients such as minerals and vitamins (Hall *et al.* 2017) which makes them an important pillar in a plant-based diet (Bal, 2007). However, the concentration of nutrients is not necessarily a

reliable indicator of an adequate nutrient intake. This is why the concept of bioavailability was developed, to define the proportion of an ingested nutrient, available for storage deposition in the body or its utilization in metabolic processes (Gregory III, 2017). Legumes contain components, other than the classic nutrients. Substances such as phytic acid and lectins belong to the bioactive compounds and if they are being frequently consumed, they can affect the human metabolism. These effects can be regarded as either positive, negative or both *depending on the form ingested and dosage*. Due to their sometimes negative effects, the bioactive compounds are being referred to as antinutrients or ANF. Negative effects include inhibition of mineral uptake and protein digestion (Campos-Vega, Loarca-Piña & Oomah, 2010) leading to interactions with the protein functions and functional properties of legumes-based food to some extent (Sharan *et al.*, 2021). ANF can also disrupt the nutritional adequacy of a legume-based product by causing an improper uptake of nutrients (Campos-Vega, Loarca-Piña and Oomah, 2010).

Therefore, it is crucial to know how different antinutrients affect the availability of nutrients to the human body.

## 1.2 Legume cultivation in Sweden

Legumes require early springs and dry autumns to flourish. The soils need to have proper drainage and preferably a pH between 6.5-4.5. The climate in the south and southwest parts of Sweden is therefore ideal for cultivation. Öland is one of the most dominant regions for brown bean cultivation while other types of legumes are also grown in other regions, such as Gotland and the south east parts of Skåne (Fogelberg, 2008).

The biggest group of legumes cultivated in Sweden includes yellow peas, fava beans, sweet lupines, chickpeas, vetch and all other beans besides brown ones, while the other groups are green peas and brown beans (Jordbruksverket, 2021a).

According to the Swedish Board of Agriculture (Jordbruksverket), 49 900 acreages of legumes was cultivated in 2021. This means that only 2% of the total arable land in Sweden was used for legume cultivation (Jordbruksverket, 2021a). The majority of these pulses were used as animal feed. Thus, a large proportion of legumes are still being imported from other countries for food purposes. Pulses can be harvested at an unripe state and sold fresh, frozen or canned like the green peas or, if harvested at a mature stage like the yellow peas, sold pre-cooked or dried and packaged for further processing (Henriksson, 2017).

### 1.3 Purpose and demarcations

The purpose of the report is to summarize potential problems and highlight possibilities of legumes as a food source, in terms of bioavailability of nutrients. The report will be focused on naturally occurring nutrients and antinutrients in legumes and their impact on nutritional uptake.

As to demarcate the work, the report is focused on products and legumes deriving from four main grain legumes commonly cultivated in Sweden; yellow peas (*Pisum Sativum*), fava bean (*Vicia faba*), green peas (*Pisum sativum var. Hortense*) and brown beans (*Phaseolus Vulgaris*) (Jordbruksverket, 2021; Lindeberg-Lindvet, 2020).

Fava bean occurs in the literature with different spellings and under different names, *faba bean*, *fava bean*, *field bean* and *horse bean* to name a few examples (Sharan *et al.*, 2021). Also, in Swedish the fava beans have many names such as åkerböna, bondböna and favaböna (WWF, 2022). When comparing nutritional value between the legumes on “Livsmedelsdatabasen”, the Swedish translation “*bondböna*” has been used. However, in this report, the beans are referred to as fava bean. As for the yellow and green peas, both groups are referred to as peas unless otherwise is stated.

Recommended intake (RI) is used for minerals and vitamins in this report. RI is used as a replacement for dietary reference values (DRVs) in the fifth edition of Nordic Nutrition: Recommendations 2012 as it was decided to put emphasis on the diet as a whole. Also, RI corresponds to EFSA's term Population Recommended Intake (PRI) and the US version, Recommended Dietary Allowance (RDA) (Sandström *et al.*, 2002). Lastly, vegan and vegetarian diets, with no inclusion of fish or mammals, are defined as “plant-based diets”.

### 1.4 Method

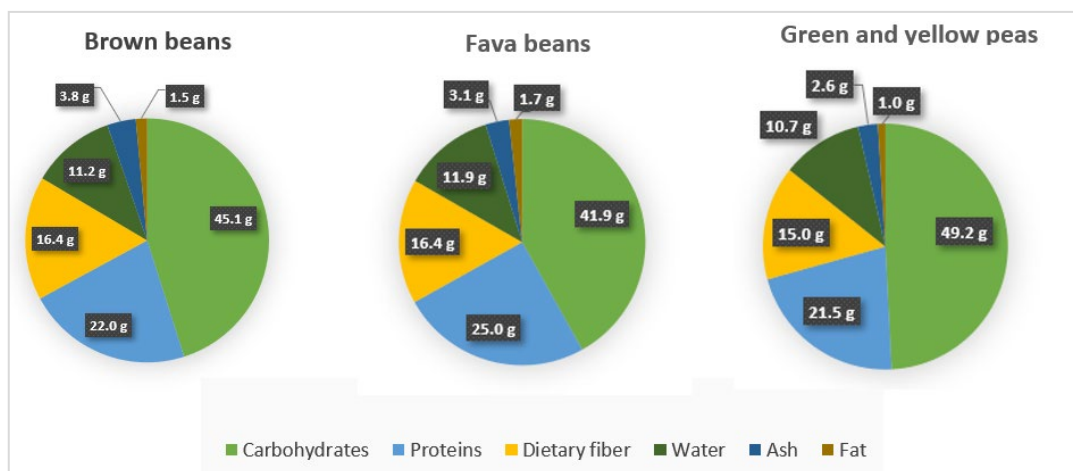
In order to gain an overview of the topic, information was collected through a literature study. The majority of articles used for the report were found using the search engines *Google Scholar* and *PubMed* as these sites were deemed to possess high credibility and present published material that has been reviewed. Scientifically reviewed publications and original works have primarily been used, but some reports from organizations or authorities were also included.

As analytical methods evolve and the composition of crops changes due to breeding, literature and data older than 15 years have been avoided if possible.

Through the work process, the following keywords were used, separately or in combination: *legumes\**, *antinutrients\**, *nutrients\**, *antinutritional factors\**, *grain legumes\**, *absorption\**, *uptake\** and *bioavailability\**. Some articles and certain information have also been collected through searches with Latin names or Swedish translations.

## 2. Nutrients

Peas, brown beans and fava beans display a favorable composition of nutrients in terms of low fat content [1-4%] and high levels of proteins [20-41%], dietary fibers [20-25%], minerals [1.4-1.7%] and vitamins [0.004 -0.01%] (Mayer Labba, Frøkiær & Sandberg, 2021; Sharan *et al.*, 2021; Livsmedelsverket, 2021b). Different cultivars of the same legume can have varying levels of nutrients and antinutrients within (Mayer Labba, Frøkiær & Sandberg, 2021). Also, genetic characteristics, stage of maturity at the harvest, climate and site of growth influences the total content of nutrients within the spices (Gregory III, 2017). This is why a display of the major components in dried peas, beans and fava beans has been made based on calculations from The Swedish Food Agency in Figure 1 (Livsmedelsverket, 2021b).



**Figure 1.** Content of nutrients per 100 grams of dried brown beans, fava beans, green and yellow peas respectively (Livsmedelsverket, 2021b).

According to Nordic nutrition recommendations (2012) the recommended intake of different nutrients depends on sex, age and levels of physical activity. For example: A child of 1-3 years old needs a daily dose of 150 µg of the vitamin folate, while 4-8-year-olds dose is set to 200 µg. Males and females of 19-50 years, is recommended around 400 µg of folate per day while a pregnant or lactating women need 500-600 µg (Institute of Medicine, 2011).

## 2.1 Proteins

Proteins represent about 20% of the dry weight in beans and peas (Duranti, 2006) while the amount of proteins can be as high as up to 41% in fava beans (Sharan *et al.*, 2021). Globulin, albumin, prolamin and glutelin are the dominating classes of proteins in legumes, with globulins being the most abundant protein (Lu *et al.*, 2020). It constitutes 35-72% of the total protein fraction (Singh, 2017) and is referred to as storage proteins (Lu *et al.*, 2020). Globulins contain high amounts of arginine, lysine and glutamine (Singh, 2017).

Albumins represent the second largest group of proteins in legumes and are known to possess enzymatic and metabolic effects (Lu *et al.*, 2020). In comparison to globulins, albumins contain a higher amount of methionine, lysine and cysteine (Singh, 2017). The two remaining groups of proteins in legumes are prolamins and glutelins, both present in small quantities (Lu *et al.*, 2020). Prolamins contain amino acids such as leucine, glutamic acid and proline. While glutelins have a similar amino acid composition to prolamins, their sequences include higher levels of histidine, glycine and methionine (Sharan *et al.*, 2021).

Dietary proteins have two main roles. First, they act as a source of nitrogen and amino acids and second, as a source of energy (Sandstrom *et al.*, 2012). Their nutritive value varies due to the number of essential amino acids and their digestibility. Thus, a “high-quality protein” could be defined as one with all essential amino acids and a high digestibility. Generally, in staple foods, plant proteins contain more than adequate amounts of amino acids in terms of: histidine, isoleucine, leucine, phenylalanine, tyrosine and valine. What limits their use are the lack or lower levels of lysine, threonine, tryptophan (Damodaran, 2017). In terms of essential amino acids, globulins and albumins have relatively low levels of sulfur-containing amino acids such as tryptophan, cysteine and methionine (Duranti, 2006).

## 2.2 Dietary fiber

Per 100 grams of dried legumes, dietary fibers make up 20-25 % (Livsmedelsverket, 2021b). There are several ways to classify dietary fibers and the two most accepted variants are either based on their solubility or their fermentability. If classified based on solubility, there are two categories of dietary fibers: insoluble (poorly/non-fermentable) or soluble (fermentable) dietary fibers. The dietary fibers in legumes consist of galactomannan, glucomannan, pectin, xyloglucan, lignin, cellulose and non-starch polysaccharides. The first four types



are classified as soluble and the remaining are referred to as insoluble (Mudgil, 2017).

Soluble dietary fibers facilitate the water adsorption in the intestine (Bal, 2007) which generally increases the transit time through the gastrointestinal tract. Slowing down the digestion results in a prolonged emptying of the stomach and leads to a more evenly distributed absorption of glucose and other nutrients. There is also a reduction in the glycemic response and plasma cholesterol as a result of the prolonged absorption (Mudgil, 2017) which is beneficial as it reduces the risk of several diseases (Huber & BeMiller, 2017). Dietary fibers have also positive effects on the intestinal microflora of humans (Mudgil, 2017). The activity of dietary fiber has been stated as prebiotic as they pass the small intestine undigested. Instead, are the soluble fibers fermented by the colon's intestinal flora. This has beneficial effect for the gastrointestinal microorganisms as well as on the human host (Huber & BeMiller, 2017). Insoluble fibers decrease the intestinal transit time while increasing the fecal bulk and excretion of bile acids. Hence, a mixture of soluble and insoluble dietary fibers is preferred. Insoluble fibers are also more abundant in foods as most fiber-containing products often contain around two-thirds of insoluble fibers and one-third of soluble (Mudgil, 2017).

## 2.3 Minerals

The proportion of minerals in the different legumes is declared under "ash" in Figure 1, whereas a more specific overview of each mineral can be seen in Table 1. Amounts of minerals range from 2 – 4% per 100 grams dried peas, brown beans and fava beans (Livsmedelsverket, 2021b). Ash is the residue that remains after a product has been incinerated, leaving only inorganic material. Thus, ash content determination can be used to evaluate mineral content in foods (Park, *et al.*, 2004). Factors determining the composition of minerals in plant-based foods are numerous and include the genetics of the plant, weather conditions during growth, the quality of the soil and the plants maturity when it is harvested (Miller, 2017).

The bioavailability of minerals is mainly determined by their absorption rate in the intestinal lumen and the transit time into the bloodstream. Some minerals are relatively well absorbed and others are not. There are homeostatic mechanisms that up- or down-regulate the intake of many minerals. If there has been an excessive absorption of minerals, it can either be stored in the body or be excreted in the feces or urine. Some forms of iron have less than 1% bioavailability while different forms of sodium and potassium can have up to 90% availability. Other factors that can either reduce or enhance the absorption of minerals are: in which chemical form the minerals are consumed, food ligands, redox activity of the food components if there

are any mineral-mineral interactions, the consumers physiological state, gut microflora and anabolic demands (Miller, 2017).

Minerals are divided into major and trace minerals. The first group includes calcium, phosphorus, potassium, sulfur, sodium, chloride and magnesium and are needed in greater quantities than the trace minerals. The trace group consists of components such as iron, zinc and nine others (Miller, 2017). Essential minerals in both groups are important components crucial for upholding body functions (Silva *et al.*, 2019). Minerals are not affected by extreme pH, heating, light or oxidizing agents during food processing (Miller, 2017). However, leaching of minerals during cooking and physical separation from milling can decrease the levels of minerals during processing (Oghbaei and Prakash, 2016).

*Table 1. Mineral content per 100 grams of dried peas, fava beans and brown beans and the Recommended Intake (RI) per day of the different minerals.*

Minerals needed in (mg/day)	Peas <sup>a</sup> (mg)	Brown beans (mg)	Fava beans (mg)	RI Females (18-60 y)	RI Males (18-60 y)
Potassium	1100	1040	278	3100	3500
Phosphorous	380	420.0	220	600/700 <sup>b</sup>	600/700 <sup>b</sup>
Magnesium	120	131	45	280	350
Calcium	59	135	42	800/900 <sup>b</sup>	800/900 <sup>b</sup>
Iron	6	5	1.5	15	9
Zinc	3.8	2	1.2	7	9
Sodium	2	25	254	- <sup>c</sup>	- <sup>c</sup>
Sodium chloride	0	100	600	- <sup>c</sup>	- <sup>c</sup>

Minerals needed in (µg/day)	Peas <sup>a</sup> (µg)	Brown beans (µg)	Fava beans (µg)	RI Females (18-60 y)	RI Males (18-60 y)
Iodine	45	1.9	25.3	150	150
Selenium	2	2.2	0	50	60

NOTE: The different values were collected from following sources: All RI values were collected from (Sandström *et al.*, 2012) and the declaration of minerals from (Livsmedelsverket, 2021b).

- The mineral content for yellow and green peas are similar and are therefore presented as one group.
- When two values are written: value/value the values represent individuals at the age of 21-60 years/ individuals at the age of 18-20 years.
- The RI values were not established or declared for.

### 2.3.1 Potassium (K)

Potassium is an essential nutrient, predominantly found in the intracellular fluids as a cation. This is why potassium is widely distributed in foods derived from living tissue (Stone, Martyn & Weaver, 2016). Potassium travels into the cells through special proteins on the surface of the cell, creating a concentration gradient that maintain the polarization of membranes. This function enables both muscle contraction, nerve transmission and the vascular tone (Palmer, 2014; Miller, 2017). The risks of potassium deficiency are very rare as the intake of the component is almost always greater than the required need (Miller, 2017). With an insufficient consumption of potassium over long time several problems can occur. It can lead to an increased risk of developing kidney stones, a heightened blood pressure, urinary calcium excretion, bone turnover and an increased sensitivity to changes in the sodium intake which in the long run affect the blood pressure. Hypokalemia is a state that is caused by a severe deficiency of potassium. This state is characterized by fatigue, constipation, malaise and muscle weakness. If more severe, the effect on muscle contraction can also inflict on the rhythm of the heart (National Institute of Health, 2021a).

Currently, no adverse effects from overconsumption of potassium via foods have been detected, therefore there are no upper levels for consumption from food sources (Miller, 2017). The RI value for magnesium is 280 mg per day for females of 18-60 years and 350 mg for males belonging to the same age group (Sandström *et al.*, 2012).

Little is known about factors that inhibit or enhance the bioavailability of potassium from different sources of the diet (Melse-Boonstra, 2020). Although, it was demonstrated that boiling could cause a loss of up to 50% of the potassium content. This can be explained by the fact that potassium often occurs in free form in food (Miller, 2017). Also, it was suggested that salting of foods and reducing the liquid can reduce the potassium levels of the food product (Stone, Martyn & Weaver, 2016). One could also argue that when legume seeds accumulate phytic acid, it forms salts together with certain cations like potassium, which would reduce the bioavailability of the mineral (Rousseau *et al.*, 2020).

### 2.3.2 Phosphorous (P)

Along cereals, milk and meat, legumes contribute to the largest dietary intake of phosphorous in the Northern countries (Sandstrom *et al.*, 2012). For example, 100 grams of boiled peas, brown beans and fava beans contain approximately 123, 162 and 190 mg phosphorus respectively (Livsmedelsverket, 2021b). The biggest proportion of phosphorous in legumes is represented by phytic acid and its different derivatives (Urbano *et al.*, 2000). In these forms, phosphorus is poorly utilized or

unavailable for digestion by humans because of the lack of enzymes required to hydrolyze the phytate and release the phosphorus (Ravindran, Ravindran and Sivalogan, 1994).

In the body phosphorus is often combined with calcium creating a strong mineral in teeth and bones. Phosphorus is also present in the cell structure, in DNA/RNA structures and in cellular metabolism as a building block in ATP. The mineral can also help maintain the acid/base balance. The RI values stretches from 4200 mg per day for an infant to 900 mg per day for women (Sandström *et al.*, 2012). Adults at the age of 18-60 years, have an RI of 600- 700 mg per day (Table 1).

### 2.3.3 Magnesium (Mg)

Magnesium is involved in maintaining many functions in the body such as protein synthesis, bone structure, energy metabolism (ATP) and muscle contraction. Additionally, it is a cofactor to over 300 enzymes (Bohl & Volpe, 2002). Magnesium concentrations in the body are strictly regulated by the kidneys, which daily excrete around 120 mg of magnesium via urine. If the levels of magnesium decrease under the desired level, the excretion is reduced (National Institute of Health, 2021b).

Deficiency and overconsumption of magnesium are not a common problem. However, overconsumption can occur if magnesium supplements are used or in people with kidney failure. Symptoms caused by an exaggerated intake are diarrhea, cramping, nausea and general intestinal stress (Miller, 2017). Habitually low intakes of magnesium can arise due to certain health conditions, causing early symptoms like nausea, loss of appetite, vomiting, tingling, numbness and more (National Institute of Health, 2021b). To meet the sufficient nutrient requirements of almost all healthy individuals the RI values are set between 80 mg for kids from 6-11 months up to 350 mg for males from the age of 14 and up (Sandström *et al.*, 2012). The RI values for females from the age of 10 years are set to 280 (Table 1).

Dietary magnesium is available in many foods such as legumes. The absorption of dietary magnesium ranges from 20 to 60% (Sandstrom *et al.*, 2012). Factors such as the ingested dose, food matrix and the presence of inhibitors and enhancers have an impact on the absorbed amount. The consumed dose highly inflicts on absorption. If a higher dose of magnesium has been ingested, the uptake decreases whereas if the intake is divided into lower doses throughout the day, it is heightened. A heightened intake of other minerals, phytate, fermentable fibers and oxalate decreases the bioavailability of magnesium. Components that enhance the

uptake of dietary magnesium are proteins, medium chained triglycerides and low indigestible carbohydrates such as inulin or mannitol (Schuchardt & Hahn, 2017).

#### 2.3.4 Calcium (Ca)

Calcium has several functions within the body. The most prominent one is its structural role in the skeleton. It also plays a major regulatory role in various physiological and biochemical processes. It is involved in cell division, muscle contractions, transmission of nerve impulses, cell membrane functions, hormone secretion, intracellular adhesion and blood clotting etcetera. The most common form of calcium found in foods are bound in complexes to other components like proteins or carbohydrates. Calcium can also work as a cross-linker between polysaccharides and proteins (Miller, 2017).

The RI of calcium, ranges between 540 mg for infants up to 900 mg for pregnant or lactating women and children of 10-17 years (Sandström *et al.*, 2012). The RI values for adults within the age span of 18-60 are listed in Table 1. If the intake of calcium is too low for a longer period of time, it can lead to several chronic diseases including hypertension and osteoporosis. Bioavailability of calcium in foods depends on the concentration and presence of enhancers or inhibitors (Miller, 2017). Two inhibitors that are present in legumes and decrease absorption of calcium, are oxalate and to some extent phytate. Together with calcium, oxalate forms a chelate that is vastly insoluble which thereby inhibits the uptake (Campos-Vega, Loarca-Piña & Oomah, 2010; Miller, 2017).

#### 2.3.5 Iron (Fe)

Of the legumes mentioned in the report, fava beans are the legume with the highest content of iron with 7 mg per 100 gram dried product, closely followed by the peas with 6 mg and lastly the brown beans with 5 mg (Livsmedelsverket, 2021b). Iron is a part of the oxygen-carrying hemoglobin and myoglobin proteins. It is also involved in growth, neurological development, some hormone synthesis and other cellular functions (National Institute of Health, 2021c).

There are two types of dietary iron, namely heme and non-heme iron. Heme iron can be found in foods with an animal origin, whereas non-heme iron can be found in both plant-based and animal-based sources (Fuqua, Vulpe & Anderson, 2012). The bioavailability of iron varies. Between 15-35% of the consumed amount of heme iron becomes absorbed while the percentage for non-heme absorption is lower, 2-20% (Abbaspour, Hurrell & Kelishadi, 2014). Iron in the plant is mainly in the ferric form ( $\text{Fe}^{3+}$ ). Ferric iron has to be reduced to ferrous form ( $\text{Fe}^{2+}$ ) before

it can be absorbed with the help of divalent metal transporter 1 (DMT1) (Fuqua, Vulpe & Anderson, 2012). This transporter is not specific for iron and can transport many other divalent metals including calcium, manganese and toxic metal cadmium. These metals can inhibit iron absorption through competition for DMT1 (Garrick *et al.*, 2003). Other inhibitors of iron adsorption are phytate, calcium, oxalates and polyphenols which can reduce the absorption of both heme and non-heme iron (Samtiya, Aluko & Dhewa, 2020). There are also enhancers of iron absorption. Vitamin C is one component that enhances the absorption of non-heme iron by increasing the solubility of the mineral or acting as a co-factor when the iron is reduced from ferric to ferrous form (Melse-Boonstra, 2020). Some organic acids like citric and lactic acid can also promote the absorption of non-heme iron (Drago, 2017). Phytate, calcium, oxalates and polyphenols reduce the absorption of both heme and non-heme iron (Samtiya, Aluko and Dhewa, 2020). Tannins, present in legumes, can also reduce the bioavailability of non-heme iron (Drago, 2017).

The RI values for iron reach from 8 mg up to 15 mg, where children of 6-11 months are recommended the lower dosage and pregnant women the highest. Infants younger than 6 months have no RI values set (Sandström *et al.*, 2012), instead, their group has an Adequate intake (AI) value of 0.27 mg per day (National Institute of Health, 2021d). As displayed in Table 1, males and females in the age span of 18-60 years have an RI of 9-15 mg per day, respectively (Sandström, 2012). If there is an excess of iron in the body, humans are able to store iron within cytoplasmic ferritin (Fuqua, Vulpe & Anderson, 2012). Depletion of iron mainly decreases the creation of erythrocytes that without iron as a fuel, causes anemia. A deficiency of iron is also a broader condition causing harm to other organs including the heart, which is dependent on iron provided myoglobin and energy in order to sustain its mechanical contractions (Camaschella, 2019).

### 2.3.6 Zinc (Zn)

Zincs belong to the group of trace minerals and act as a cofactor to certain metalloenzymes increasing catalytic activity. Zinc also supports wound healing and plays a role in immune functions, protein synthesis, DNA synthesis and cell division (Miller, 2017). Zinc is also important for pregnant women, adolescents and children as its support's a normal growth (National Institute of Health, 2021d).

Since the body lacks any specialized mechanisms for storing zinc, a steady intake on daily bases is required to maintain sturdy levels in the body. Although, the RI value for zinc is rather low, ranging from 0.3 mg for babies to 12 mg for males of 14-17 years (Sandström *et al.*, 2012). Similar to magnesium, the absorption is connected to the amount of zinc that is consumed. If there are high doses of zinc within the food, the absorption is less effective than if lower levels are present. A

possible reason for this is the oversaturation of the zinc transporting mechanisms (Drago, 2017). The amount of zinc present in 100 gram dried fava beans, brown beans and peas are 1.2, 2.0 and 3.8 mg, respectively (Livsmedelsverket, 2021b). Symptoms connected to deficiency are loss of appetite and impaired immune functions. In a longer term, there is also a risk of growth retardation (Tuerk & Fazel, 2009).

Dietary zinc is usually found in complexes with nucleic acids or proteins. When coming from plants, like legumes, the bioavailability is lower than zinc coming from animal sources (Drago, 2017). Of all the minerals that are prone to form a complex with phytic acid, zinc is the one with the highest affinity. These complexes can be formed either in the pulses or in the gastrointestinal tract (Rousseau *et al.*, 2020). In rare cases and if the iron/zinc ratio becomes 2:1 the uptake of iron can overload the uptake of zinc, as they are being absorbed through the same passages (Drago, 2017).

## 2.4 Vitamins

Vitamins are organic compounds necessary to maintain metabolic body functions and growth. Generally, vitamins are not synthesized in sufficient amounts with the exception of vitamin D and must therefore be supplied through the diet (Gironés-Vilaplana *et al.*, 2017). Vitamins are needed in smaller quantities, placing them along with the micronutrients (Gregory III, 2017). At present, 13 compounds are universally recognized as vitamins, all classified after their chemical and biological activities. One vitamin can appear in different forms. For example, vitamin A is a group of compounds including carotenoids, retinol and retinal (Combs *et al.*, 2017a). There are two groups of vitamins, fat- and water-soluble vitamins. The fat-soluble include; vitamin A, D, E, and K. Water-soluble include: vitamin C and vitamin B complexes (Combs *et al.*, 2017b).

Most vitamins cannot be used in the form they are absorbed and have to be transformed into their active forms, before being used in the body (Gironés-Vilaplana *et al.*, 2017). The knowledge of how vitamins behave in the food matrix is rather limited, mainly because the challenges to create an analysis model, close to the complexity of a food system, have proven hard. Mainly in terms of imitating physical and compositional variables. Vitamins can have an impact on other foods properties in terms of causing browning reactions, acting as flavor precursors, becoming radical scavengers, or reducing agents. Undoubtedly, environmental and agricultural conditions also influence the content of plant-derived foods although only few studies have been made on the subject (Gregory III, 2017).

Multiple vitamins are present in legumes. Table 2 illustrates the vitamin content per 100 grams of the dried product alongside their RI. The maximum cooking loss of the vitamins is also included in Table 2, as it is a factor that affects the final amount of vitamins available in a legume-based product. Post-harvest losses of vitamins occur naturally; however, the extent depends on temperature conditions, the length of time between harvest and processing and physical damages to the raw products during handling. These circumstances might not have an impact on the net concentration of vitamins but might influence their bioavailability. Prolonged shipments or hold-ups contribute to a decrease in the levels of labile vitamins as ongoing metabolism in the plant's tissue can be responsible for changes in the total vitamin concentration. Further processing of the legume products such as washing, milling and the presence of other food components in the final product can also affect the forms and bioavailability of the vitamins (Gregory III, 2017).

Table 2. Vitamin content per 100 grams of dried peas, fava beans and brown beans and the Recommended Intake (RI) per day of the different vitamins.

Fat-soluble vitamins	Peas <sup>a</sup> (µg)	Brown beans (µg)	Fava beans (µg)	RI <sup>b</sup> Females (18-60 y)	RI <sup>b</sup> Males (18-60 y)	Max. loss <sup>c</sup> (%)
Vitamin A	1.2	1.5	35.0	<b>700</b>	<b>900</b>	40
β-carotene	15	18	420	- <sup>g</sup>	- <sup>g</sup>	- <sup>c</sup>
Vitamin D	0	0	0	<b>10</b>	<b>10</b>	40
Vitamin E	100	300	0.3	8	8	55
Vitamin K	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>	5
Water-soluble vitamins	Peas <sup>a</sup> (mg)	Brown beans (mg)	Fava beans (mg)	RI <sup>b</sup> Females (18-60 y)	RI <sup>b</sup> Males (18-60 y)	Max. loss <sup>c</sup> (%)
Thiamin	0.82	0.55	0.50	1.1	1.3/1.4 <sup>f</sup>	80
Riboflavin	0.18	0.20	0.30	1.2/1.3 <sup>f</sup>	1.5/1.6 <sup>f</sup>	75
Niacin	2.50	2.20	2.50	14/15 <sup>f</sup>	18/19 <sup>f</sup>	75
Niacin equivalents	6.08	5.87	6.67	- <sup>g</sup>	- <sup>g</sup>	- <sup>c</sup>
Vitamin B <sub>6</sub>	0.08	0.30	0.56	1.2	1.5	40
Folate	33 <sup>d</sup>	39 <sup>d</sup>	423 <sup>d</sup>	<b>400</b>	<b>400</b>	100
Vitamin B <sub>12</sub>	0	0	0	<b>2.0</b>	<b>2.0</b>	10
Vitamin C	1	0	0	75	90	100

NOTE: The different values are collected from following sources: RI-values (Sandström *et al.*, 2012), the declaration of vitamins (Livsmedelsverket, 2021b) and the max loss in % (Gregory III, 2017).

- The vitamin content for yellow and green peas are similar and are therefore presented as one group.
- RI values marked in **bold** are given in **µg/day** while regular typed RI values are given in mg/day.
- The content of the vitamin was not established or declared for.
- Values are given in micrograms (µg) as the content was deemed too low for milligrams.



- e. Max. loss = Maximum cooking loss (%).
- f. When two values are written: value/value, the values represent individuals at the age of 31-60/ individuals at the age of 18-30.
- g. There is no RI that is specific for the vitamin equivalent. The RI for  $\beta$ -carotene is declared for under vitamin A, and niacin equivalents under niacin.

The common minerals in peas, brown beans and fava beans and their bioavailability will be explained further in the sections to come.

### 3. Antinutrients

Antinutrients or antinutritional factors (ANF) are components within legumes that lower the nutritional value of a food product by affecting the bioavailability and digestibility of nutrients (Sandberg, 2002). ANF mainly possesses negative effects when being continuously ingested for a longer period of time, as it is the case when a plant-based diet is consumed (Doria *et al.*, 2012; Popova & Mihaylova, 2019). Lectins, phytate, tannins, polyphenols and oxalates are examples of antinutrients found in plants and plants-based foods (Emire, Jha & Mekam, 2016; Popova & Mihaylova, 2019). Many of the antinutrients are an important part of plants' defenses against environmental conditions and predators while others are used to store energy needed when the seed is to germinate (Muzquiz *et al.*, 2012).

Common symptoms exhibited by a high intake of ANF are for example bloating, nutritional deficiencies and headaches. Even though sensitivity to antinutrients varies widely between individuals, mechanical, biochemical and thermal methods have been developed to reduce the levels of ANF. These methods include extrusion, soaking, cooking and germination (Popova & Mihaylova, 2019). Another option to reduce, or fully remove, ANF would be the use of traditional plant breeding or genetical modification of legumes. However, not all ANF have negative effects. Some polyphenols are known to reduce the risk of cancer, heart diseases and diabetes which means that lowering the levels of ANF will make the product more nutritious but not necessarily healthier (Emire, Jha & Mekam, 2016). Saponins is another ANF used as a flavoring agent and foaming agent by the food industry. Alas, by completely removing the ANF from the legumes, some functional properties would be affected (Soetan, 2008) and some health benefits lost.

ANF are usually classified into three larger groups; proteins, glycosides and other substances (Muzquiz *et al.*, 2012).

## 3.1 Proteins

### 3.1.1 Lectins

These types of glycoproteins exhibit reversible and specific activities for binding simple or more complex carbohydrates (Duranti, 2006) with some agglutinating effects on red blood cells (Lagarda-Diaz, Guzman-Partida and Vazquez-Moreno, 2017). If the carbohydrates are attached to the epithelial cells on the gut wall when the lectins bind, it interferes with the utilization of the carbohydrates and thereby the absorption. Some lectins are heat-labile (Muzquiz *et al.*, 2012), meaning that their levels can be reduced by heat treatments (Duranti, 2006). Soaking, germination and dehulling did not remove lectins from the pulses (Sharan *et al.*, 2021).

The toxicity of lectins varies depending on the type. Aside from agglutination properties, some lectins affect mitogenesis and digestion (Muzquiz *et al.*, 2012), and can cause symptoms like diarrhea, nausea and vomiting (Duranti, 2006). Lectins can also bind calcium, manganese and iron and thereby reduce their bioavailability. Not all lectins are toxic. Non-toxic variants can be found in peas and fava beans (Campos-Vega, Loarca-Piña & Oomah, 2010). Some studies indicated beneficial effects of lectins such as their ability to stimulate gut functions. Lectins can also limit the growth of cancer tumors (Muzquiz *et al.*, 2012).

### 3.1.2 Protease inhibitors

Protease inhibitors reach the intestine intact as they are resistant to both pepsin and the low pH of the digestive tract (Roy, Boye & Simpson, 2010). This affects the nutritional value of legumes, as the protease inhibitors bind competitively to digestive enzymes such as chymotrypsin and trypsin and inhibit the digestive effects of proteins which also reduces the levels of free amino acids (Muzquiz *et al.*, 2012). This makes trypsin and chymotrypsin inhibitors the most important proteases from a nutritional aspect (Campos-Vega, Loarca-Piña & Oomah, 2010). The inactivation of chymotrypsin and trypsin can also induce an overproduction and secretion of pancreas enzymes which can lead to an enlarged pancreas (Damodaran, 2017). In order to reduce the levels of inhibitors, cooking prior to consumption is preferred as many protease inhibitors are heat labile (Muzquiz *et al.*, 2012). This is why these ANF do not pose huge problems in industrial and home cooked products as long as the temperature reaches adequate levels to deactivate the protease inhibitors (Damodaran, 2017). Similarly, to other bioactive compounds in legumes, the levels of protease inhibitors differ between cultivars. Some protease

inhibitors have also been suggested to have health-promoting properties, acting as anti-cancerogenic agents reducing tumor sizes (Muzquiz *et al.*, 2012).

## 3.2 Glycosides

### 3.2.1 Alpha-galactosides

There are many different alpha-galactosides inter alia, raffinose, verbascose and stachyose. These compounds are derived from sucrose and stored in the peas, beans and fava beans pulses. Several negative antinutritional effects by alpha-galactosides have been reported including flatulence or its interference with the utilization of nutrients. Alpha-galactosides can also have a decreased effect on the dietary net energy as a high proportion of fermentation will take place in the hindgut. The microflora in the large intestine ferments the alpha-galactosides creating carbon dioxide and thereby causing flatulence. If the levels of alpha-galactosides are lowered, they can have a prebiotic effect as the microbes will maintain fermentation but with a lower gas production (Martínez-Villaluenga, Frias and Vidal-Valverde, 2008).

### 3.2.2 Saponins

Triterpene-typed saponins are present in all legumes and their occurrence in plant-based foods can be associated with both beneficial and deleterious effects (Muzquiz *et al.*, 2012). For example, saponins exert foaming properties in aqueous solutions that can be used in the food industry whereas the downside is that saponins can provide metallic or bitter tastes to products (Sharan *et al.*, 2021). Saponins also have the ability to act as surface-active agents together with other components in the digesta creating interaction with the mucosal cells which in turn, can reduce the uptake of nutrients (Muzquiz *et al.*, 2012).

Similar to other ANF, there is data suggesting that saponins possess anti-cancer activities by suppressing the metastatic potential of melanoma cells. They have also proved beneficial effects in preventing hyperlipidemia (Campos-Vega, Loarca-Piña & Oomah, 2010). Some clinical studies suggested that saponins might reduce the levels of cholesterol in plasma but the mechanism behind it is still not well understood (Muzquiz *et al.*, 2012).

Saponins are heat stable but their levels can be reduced via processing such as germination, fermentation, dehulling and soaking. Duration of the processing, soaking time and salinity of cooking water, determine the magnitude of this reduction (Muzquiz *et al.*, 2012).

### 3.2.3 Vicine and convicine

Vicine and convicine is a category of ANF only found in fava beans and have not been detected in peas or brown beans (Muzquiz *et al.*, 2012). When digested in the large intestine or broken-down during seed development, these compounds are transformed into their aglycones. These aglycones are able to cause favism, a fatal disease for sensitive individuals with a low level of a certain enzyme, glucose-6-phosphate dehydrogenase. This enzyme protects the cells from oxidative damages, and if a person suffers from deficiency, the aglycones of vicine and convicine can create oxidative damages to red blood cells (Sharan *et al.*, 2021; Vårdguiden, 2022).

The levels of vicine and convicine vary depending on the time of the ripening and the cultivar. Younger and older seeds have lower activity of the enzyme that mediate aglycone formation, while mature seeds have a higher enzymatic activity. Vicine and convicine levels can be reduced using hydrogen peroxide on the seeds or via soaking in combination with germination (Sharan *et al.*, 2021).

## 3.3 Other substances

### 3.3.1 Phytic acid

Phytic acid, myo-inositol-(1,2,3,4,5,6) hexakis-phosphates and phytate are widely distributed in the kingdom of plants. The latter is a salt of phytic acid and is the dominating form found in legume seeds (Schlemmer *et al.*, 2009; Muzquiz *et al.*, 2012). Phytate and phytic acid serve as storage compounds for phosphorus and minerals in plants and contain around 75% of the plant's total phosphorus reserves. As the plants germinate, the minerals and phosphate bound in these compounds become more accessible to the plant which promotes the seeds growth (Schlemmer *et al.*, 2009).

The inhibition effect of phytate on other nutrients varies depending on the form and ratio present in the pulses. It has been suggested that a reasonable absorption of minerals occurs if the mineral: phytate ratio, ranges between 5:1 to 15:1, with an exception for zinc, where the ratio should be around 18:1 (Zhang *et al.*, 2022). The larger phytate molecules called myo-inositol-(5,6) hexakis-phosphates, IP<sub>6</sub> and IP<sub>5</sub>, have larger antinutritional effect than the smaller molecules, IP<sub>4</sub>-IP<sub>1</sub>(myo-inositol-1,2,3,4, hexakis-phosphates). This is because IP<sub>6</sub> and IP<sub>5</sub> have a higher ability to form complexes with minerals than the small ones (Muzquiz *et al.*, 2012). Raw brown beans and fava beans contain IP<sub>6</sub> and IP<sub>5</sub> (Schlemmer *et al.*, 2009; Luo and

Xie, 2012) whereas peas have the same content as the two types of beans, but also include levels of IP<sub>4</sub> phytates (Campos-Vega, Loarca-Piña and Oomah, 2010).

Phytic acid has the ability to form complexes with minerals such as zinc, copper, iron and calcium, insoluble at the physiological pH of the intestine (Campos-Vega, Loarca-Piña & Oomah, 2010; Popova & Mihaylova, 2019) which makes them inaccessible to absorption. In addition, phytases inhibit the enzymes amylase, trypsin and pepsin necessary to digest proteins (Popova & Mihaylova, 2019; Samtiya, Aluko & Dhewa, 2020) and can even form complexes with proteins. Hence, affect the bioavailability of nutrients (Muzquiz *et al.*, 2012).

In Sweden, with a mixed diet, the average intake of phytate per person was calculated to be around 180 mg per day. While a vegetarian in the age span of 35-67, had an average intake of 1146 mg per day. Nevertheless, beneficial properties of phytate have also been observed including antioxidant activity, anticancer properties and prevention of kidney stones. To gain an optimal physiological benefit from phytate, the upper gut has to have a higher phytate content (Schlemmer *et al.*, 2009).

Phytate is rather heat-stable and therefore endures home cooking such as roasting and pressure cooking, while industrial processing facilitated with an enzyme called phytase can result in strong hydrolysis of the phytate compounds (Schlemmer *et al.*, 2009). Another option is soaking, a step often used when handling legumes. First, soaking activates the phytase enzyme present in legumes, which decreases the levels of phytates. Secondly, though some minerals leach out during the process, the remaining minerals and proteins become more accessible for absorption due to the reduced levels of phytates. Fermentation, germination, autoclaving and cooking can also be used to reduce the levels of phytates (Samtiya, Aluko & Dhewa, 2020).

### 3.3.2 Tannins

Tannins are concentrated in the bran fraction of legumes (Popova & Mihaylova, 2019) and are known to add a bitter taste to foods. The astringent mouthfeel created by tannins is due to their ability to bind and precipitate saliva proteins (Ulla *et al.*, 2016). Other tannin-protein complexes may cause the inactivation of digestive enzymes, preventing the body from absorbing proteins and other nutrients properly (Popova & Mihaylova, 2019).

Tannins are heat-stable compounds but can be reduced using fermentation and/or germination, soaking and dehulling. Soaking is an efficient method depending on the soaking time and the used media (salted water, plain water or bicarbonate solution) (Muzquiz *et al.*, 2012).

### 3.3.3 Oxalate

Oxalic acid has the ability to form salts or esters with different minerals such as potassium, sodium, calcium, magnesium and iron. The first two minerals form soluble compounds while the remaining three, combined with oxalic acid become insoluble complexes. Some of these insoluble salts like calcium oxalate cannot be excreted if they have reached the urinary tract, instead they accumulate and cause kidney stones (Popova & Mihaylova, 2019). By forming these calcium oxalate complexes, oxalate also decreases calcium uptake. In general, these salts of oxalate possess poor solubility at an intestinal pH (Campos-Vega, Loarca-Piña & Oomah, 2010). For most people oxalate is not a problem, although, if suffering from conditions like primary and enteric hyperoxaluria, the intake of oxalate has to be reduced. To reduce levels of oxalate, fermentation and cooking are effective (Popova & Mihaylova, 2019).

## 4. Discussion and conclusion

To summarize, the literature review revealed that yellow peas, brown beans, green peas and fava beans are a good source of nutrients, as long as the content of antinutrients are regulated. Regulation can be obtained through processing like germination, dehulling, soaking, autoclavation or fermentation. When removing lectins however, germination, dehulling and soaking did not prove effective. In the case of phytate, aiming to maintain an accepted mineral: phytate ratio within the legume-based product could be an option.

The levels of both nutrients and ANF varied between beans, peas and fava beans as well as within the cultivars. Likewise, the stage at which the legumes were harvested and how the legumes were further processed into new products affected the bioavailability of antinutrients, nutrients and their levels (Miller, 2017; Gregory III, 2017). Choosing cultivars with the most favorable composition of nutrients and lower levels of ANF could offer control already from the stage of growth. This method creates a need for further breeding on low-leveled cultivars and more studies on the impact of environmental and agricultural conditions on the ANF content.

Furthermore, additional studies about the behavior of ANF in the body and in the context of a food matrix are needed to better understand their effect on nutrient-bioavailability. However, investigation of bioavailability and nutrient uptake are challenging. This is mainly because it is difficult to mimic the complicated food matrix in a laboratory environment (Gregory III, 2017). Knowledge concerning the behavior of ANF, nutrients and their bioavailability and their context in a food matrix could be used to further increase the nutritious value of legume-based products.

In contrast to the negative effects of ANF, recent studies also indicated some positive effects. Properties like the ability to lower cholesterol in the blood plasma and anticarcinogenic effects were reported (Campos-Vega, Loarca-Piña & Oomah, 2010; Muzquiz *et al.*, 2012). Thus, to identify optimum levels of ANF in food might be an attractive strategy in the future. This, in order to prevent the risks of developing non-communicable diseases.



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## Acknowledgements

I would like to thank my supervisor Jaqueline for her support and motivation throughout the work, as it is easy to lose perspective working with your own text. Also, a thank you to Nellie Nyd, Signe Magnussen and Henrik Johansson who contributed with new viewpoints and thoughts to improve the report further. Lastly, I would like to thank my family and friends who suffered endless lectures connected to the subject of my report.

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