Development of a bean beverage

The effect of different treatments on sensory properties and nutritional content

Master Thesis by

Louise Beckius
Klara Schaar

Lantmännen

LUNDS UNIVERSITET
Lunds Tekniska Högskola

Abstract

This master thesis aimed at exploring different methods of producing a beverage based on Lantmännen's Swedish grown white beans. A flour was milled from beans that had been soaked and boiled in order to inactivate antinutrients such as lectins, trypsin inhibitors and phytic acid, and subsequently dried and milled. A water-based beverage with 8% bean flour was boiled for 5 min. 1.5% of rapeseed oil was added and the beverage was mixed with a high shear mixer for 1 hour. This basic beverage then underwent different treatments and was analysed for protein content, Raffinose Family of Oligosaccharides (RFO) content, pH and viscosity as well as sensorial properties such as taste and sandiness. The treatments included fermentation with *Lactobacillus plantarum* 299v, centrifugation to exclude insoluble components, as well as treating the beans with sodium bicarbonate in order to increase pH and thereby increase solubility of proteins and dietary fibers. The fermentation process produced an acidic (pH 5), probiotic beverage with an unique flavor and a shear-thinning behavior. Cenrifugation gave a milk-like beverage both in color and consistency with a protein content of around 0.3 g/100 g beverage. Increasing pH of the beans could give a centrifuged beverage with a protein content of up to 0.9 g/100 g beverage. The sensory analysis showed that this treatment gave a decreased sensation of sandiness due to higher solubility of dietary fibers, and that there was a greater general liking of the centrifuged beverages compared to the non-centrifuged. It was concluded that there was a strong potential in producing a bean-based beverage with these methods, but for the fermented and non-centrifuged samples it seems necessary to add some form of flavoring in order to increase the consumers' general liking and acceptance for this type of product.

Sammanfattning

Examensarbetets mål var att utforska olika metoder för att tillverka en dryck baserad på Lantmännens svenskodlade vita bönor. Ett mjöl tillverkades på bönor som blötlagts och kokats för att inaktivera antinutrienter så som lektiner, trypsininhibitorer och fytinsyra, varpå de torkades och maldes. En vattenbaserad dryck med 8% bönmjöl kokades i 5 minuter. 1,5% rapsolja tillsattes och drycken mixades med en högsjuvningsmixer i en timme. Denna dryck behandlades sedan på olika sätt och analyserades för proteininnehåll, mängden raffinos oligosackarider (RFO), pH och viskositet samt sensoriska egenskaper. Behandlingarna inkluderade fermentering med *Lactobacillus plantarum* 299v, centrifugering för att avlägsna olösliga komponenter samt behandling av bönorna med bikarbonat för att öka pH och på så vis öka lösligheten av protein och kostfibrer. Fermenteringsprocessen resulterade i en syrlig (pH 5), probiotisk dryck med en unik smak och med ett sjuvtunnande beteende. Centrifugering gav en mjölkliknande dryck till både färg och konsistens med en proteinhalt på ungefär 0,3 g/100 g dryck. Att öka bönornas pH resulterade i en centrifugerad dryck med en proteinhalt på upp till 0,9 g/100 g dryck. Den sensoriska analysen visade att denna behandling också gav en minskad känsla av sandighet i drycken som inte centrifugerats, på grund av kostfibrernas ökade löslighet, och att det var en generellt högre grad av acceptans för de centrifugerade proverna än de ocentrifugerade. Sammanfattningsvis visade studierna att det finns en stor potential i att producera en bönbaserad dryck med någon av dessa metoder, men att de fermenterade och ej centrifugerade dryckerna kan kräva någon form av smaksättning för att öka acceptansen för denna typ av dryck hos konsumenterna.

Foreword

The idea of developing a bean beverage arose partly from a project course in life sciences at the Faculty of Engineering at Lund University in collaboration with Lantmännen, where a smoothie containing beans, fruits and berries was developed. This thesis is a continuation of the idea of producing a bean based beverage but instead, the possibilities of producing a new type of plant based milk with white beans have been explored.

The practical work has been performed at the Department of Food Technology and Nutrition at Lund University. The initial trials have been performed by both authors while Louise Beckius has been responsible for the fermentation part and RFO analysis and Klara Schaar for the pH adjustment part and protein analysis. The sensorial evaluation was conducted together but the statistical analysis of the hedonic evaluation was performed by Louise Beckius and the statistical analysis of the triangle test was performed by Klara Schaar. Both authors have contributed equally to the written report.

We are greatful for getting this oppurtinity of learning more and experimenting with beans, which we both find is an exciting raw material with great potential in development of healthy and sustainable products. We especially want to thank our supervisors at Lantmännen R&D; Emma Nordell and Lovisa Marais Martin, for the opportunity of doing this thesis together and for the support and endless optimism that you spread. We also want to thank Annelie Moldin and Emelie de Craene at Lantmännen Cerealia, for valuable inputs in the early part of the development and for supplying the beans. Finally, we want to thank everyone at the Department of Food Technology and Nutrition at the Faculty of Engineering, Lund University, especially the following persons:

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List of abbreviations

RFO: Raffinose Family of Oligosaccharides

WHC: Water-holding Capacity

RDS: Rapidly Digestible Starch

SDS: Slowly Digestible Starch

RS: Resistant Starch

TSA: Tryptic Soy Agar

MRS: de Man, Rogosa and Sharpe

VRBD: Violet Red Bile Dextrose

DM: Dry Matter

E%: Energy percent

ANOVA: Analysis of Variance

PSD: Particle Size Distribution

n: flow behavior index

k: flow consistency index (Pa s^n)

CFU: Colony Forming Units

1.0 Introduction

The consumption of beans is today quite small in Sweden. Beans could be used for many new types of food applications, while also contributing to a more sustainable agricultural practice and reduce environmental impact from food consumption while enabling healthier eating habits. (Baljväxtakademin 2016) The high protein- and fiber content in beans gives them a well-balanced nutritional profile, and contributes with many vital components for a healthy diet. For those who do not eat animal-based products, beans are an ideal source of protein and energy. The fiber content can help prevent diseases and give beneficial physiological effects. (Rebello, Greenway et al. 2014)

A negative side effect of the high fiber content is however that some consumers experience discomfort in the form of flatulence when ingesting beans. This is caused by the presence of Raffinose Family of Oligosaccharides (RFO) and other soluble fibers. This in addition to the presence of antinutrients can decrease consumer acceptance of beans. Antinutrients and RFO are usually decreased to an acceptable level through soaking and boiling, and can be further decreased through fermentation. (Barampama and Simard 1994)

One type of unexplored food application for beans is in the form of a beverage, e.g. plant milks. The term plant milk refers to beverages that can substitute dairy milk, such as soy – and oat milk.
There is, to the authors' knowledge, no plant milk based on white beans or any other pulse, apart from soy beans, on the market today. According to Boye, Zare et al. (2010) the reason for this is the pulses' high starch and fiber contents, which makes the production of low-viscous products difficult. The main challenges in producing a bean beverage will be achieving a stable emulsion and reducing the sandy/grainy texture of the beans. Also, the antinutritional compunds should be kept at a minimum, as well as the RFO in order to increase consumer acceptance.

During this project, a basic production process has been developed, and methods for optimizing protein content and improving texture and flavor have been studied, such as fermentation, eliminating insoluble particles using centrifugation, and increasing the pH of the beans in order to change the solubility of fibers and proteins. The beverages were evaluated using analyses of nutritional content, rheological measurements, sensory analyses and other chemical analysis methods.

2.0 Objectives

The main goal of this thesis was to develop a new beverage based on Swedish grown white beans from Lantmännen Cerealia. More specifically, the goals were to:

- Develop a basic production process for a bean beverage. This beverage should obtain a protein level which contributes to at least 12 E% so that it can be labeled as "source of protein", and it should not separate within 10 minutes
- Study the effect of fermentation on the taste, viscosity, sandiness and RFO content of the beverage.
- Study centrifugation as a method of eliminating insoluble particles and decreasing the sandy texture of the beverage.
- Study the effect of increasing pH of the beans in regard to protein- and fiber solubility and the subsequent effect on sandiness, and protein content after centrifugation of the beverage.
- Carry out a literature study of the beans' nutritional content, as well as the antinutrients of beans and methods of eliminating them.

3.0 Literature study

3.1 Cultivation of beans

Phaseolus vulgaris is the latin name for the plant on which common beans are grown. Other beans that belong to the same group are Navy bean, Great Northern Bean, California small white bean, Kidney bean and Pinto bean. There are both twining and bush varieties of *P. vulgaris*. The bush forms can reach 1 m in height and the twining forms that grow supported by poles, can become 4 m high. The beans can grow in linear, round or flattened pods up to 15 cm long. Beans originate from South and Central America but are now cultivated all around the world. India is the leading cultivator with 21% of the worlds production (EOL 2012).

The cultivation of beans can increase biodiversity due to their ability of fixing nitrogen in the atmosphere and with the help of microorganisms converting it to bound nitrogen in the ground which improves fertility of the soil and can be used by plants. Their ability to fix nitrogen also means that they need less fertilizers, thereby reducing their environmental impact from agriculture compared to many other crops (FAO 2016).

3.2 Chemical composition and nutritional content of beans

Since there is little research available the beans from Lantmännen Cerealia, studies where other types of common beans have been used will instead be referred to in this theoretical background. The nutritional content of the Swedish grown white beans from Lantmännen Cerealia as well as the nutritional content of white beans according to the National Food Agency Food Database can be found in Table 1 (GoGreen 2013, The National Food Agency Food Database 2016).

Table 1. Nutritional content of white beans from Lantmännen Cerealia and other white beans in a dried state compared to boiled state.

Nutritional value/100g	**White beans (dry), Lantmännen Cerealia**	**White beans (dry), Livsmedelsverket**	**White beans (boiled), Lantmännen Cerealia**	**White beans (boiled), Livsmedelsverket**
Energy (kJ/kcal)	1250 kJ/300 kcal	1337 kJ/319 kcal	550 kJ/140 kcal	452kJ/108 kcal
Fat (g)	2.7	1.6	1.2	0.6
Saturated fats (g)	0.5	0.2	0.2	0.1
Carbohydrates (g)	37	45.7	17	14.2
Sugar (g)	3	3	1.4	1
Fiber (g)	20	15.8	9	7
Protein (g)	21	22	9.5	7.8
Water (g)	-	11	-	69

3.2.1 Carbohydrates

3.2.1.1 Dietary Fibers

Dietary fibers are defined by the American association of cereal chemists (AACC) as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human small intestine with complete or partial fermentation in the large intestine. Hemicelluloces, cellulose, oligosaccharides, pectins, waxes, gums and lignin belong to the group. (Rodríguez, Jiménez et al. 2006) The dietary fiber composition of legumes can vary, the content in navy beans is 3.4-6.6% crude fiber, 0.1% lignin, 3.2% cellulose, 0.5-4.9% hemicellulose. (Oomah, Patras et al. 2016)

Health promoting effects of dietary fibers include protection against cardiovascular disease, constipation, irritable colon, diabetes and colon cancer. (Rodríguez, Jiménez et al. 2006) Dietary fibers can be divided into soluble and insoluble fibers. The dietary fiber content of dry *P. vulgaris* is approximately 23-34%; 20-28% insoluble and 3-6% soluble fiber. (Tosh and Yada 2010) Dietary fibers can also be divided into other groups depending on their function in the intestinal tract; such as fermentable and non-fermentable. The fermentable fibers are not metabolized in the small intestine but will instead be fermented by the bacteria in the large intestine, leading to production of gases, short-chain fatty acids, anti-microbial substances and also improvement of the immune system. The production of gases (carbon dioxide, methane and hydrogen gas) is the reason behind bloating and stomach ache, which are common symptoms after consumption of fiber-rich foods. (Johansson 2014)

The non-fermentable fibers on the other hand will not be metabolized in the small intestine nor in the large intestine, but will still have other functions in the large intestine that have positive effects on the health. They have the capacity of binding water, increasing the volume of the feces and giving it a softer consistency, thereby increasing the rate by which the food passes through the large intestine. This means that substances that potentially can cause cancer in the intestinal tract will be hindered to attach to the intestinal mucosa. Improving the movements of the large intestine also prevents constipation. An important difference between fermentable and non-fermentable dietary fibers is that the consumption of non-fermentable fibers does not contribute to the production of gases. (Johansson 2014) An example of what type of fibers that belong to the different groups can be seen in Table 2.

Table 2 Two classifications of fibers with examples of fibers belonging to the different groups. (Rodríguez, Jiménez et al. 2006, Johansson 2014))

Fermentable	Beta-glucans, pectin, plant gums, mucin, inulin, oligosaccharides,resistant starch and some hemi-celluloses
Non fermentable	Cellulose and some hemi-celluloses
Soluble	Beta-glucans, pectins, galactomanan gums, oligosaccharides, inulin
Insoluble	Lignin, cellulose, heimcellulose

The water holding- and water binding capacity of dietary fibers affect their physiological and technological properties. Insoluble fibers have a high water-holding capacity (WHC) and this capacity seems to increase with higher pH, also making the fibers more water soluble. The WHC is defined as the amount of water bound to 1 gram of dry dietary fiber. The fibers interact with water with interactions as well as hydrogen bonds. Particle size of the fiber could also affect the WHC, with a higher capacity at lower particle size due to a larger pore volume and surface area. (Uma Tiwari and Cummins 2016)

3.2.1.2 Raffinose Family of Oligosaccharides (RFO)

RFO are sometimes classified as soluble dietary fibers, and is the type of fiber which is of most interest in this project. RFO, also know as α-galactosides, refers mainly to raffinose, stachyose and verbascose. The RFO content of dry beans, lentils, peas and chickpeas is between 2-10 g/100g dry weight. Raffinose is a trisaccharide consisting of a galactose monomer with an α-1-6 glycosidic link to a sucrose molecule. Adding one or two α-D-linked galactosyl units gives stachyose and verbascose, respectively. (Tosh and Yada 2010) Usually the content of stachyose is larger than raffinose and verbascose in common beans. (Rakshit, Sharma et al. 2015) In a study by Sahasrabudhe, Quinn et al. (1981), where chemical composition of four varieties of white common bean have been analyzed, the contents of stachyose and raffinose in the whole bean flours are 2.74-3.29% and 0.45-0.46% respectively. The structure of sucrose, raffinose and stachyose can be seen in Figure 1.

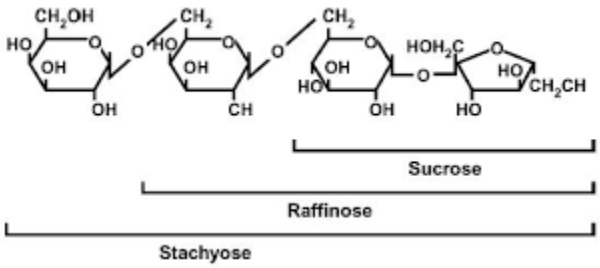

Figure 1. Schematic figure of stachyose, raffinose and sucrose. (Hitachi 2009)

RFO resist digestion in the human intestine due to our lack of the α-galactosidase enzyme. Instead, microorganisms ferment the RFO, producing carbon dioxide, hydrogen and short chain fatty acids. The gas production causes discomfort among many consumers in the form of flatulence, although some studies suggest that one can grow more tolerant to flatulence caused by oligosaccharides, by consuming more pulses and beans. Despite this discomfort, the RFO do have a beneficial effect on the gut microbiota, and SCFA such as acetate, propionate and butyrate have beneficial physiological effects. (Rebello, Greenway et al. 2014, Topping 1996) It is generally assumed that RFO are the main flatulence-causing components in pulses. However, Granito demonstrated in an in vitro study that other soluble fibers could give rise to as much or more flatulence as RFO. Complete or partial elimination of RFO does not remove flatulence entirely. (Granito, Champ et al. 2001)

Soaking legumes helps to reduce the content of RFO in legumes, since the RFO leach out into the soaking water. Also, α-galactosidase enzymes can be activated when in water, starting to decompose the RFO. The higher water to legume ratio, the more RFO are eliminated. Additionally, pH, soaking time and temperature has an effect. (Rakshit, Sharma et al. 2015) For example in red kidney beans that have been soaked for 12 h, the RFO content decreases the most in the initial 4 hours of soaking (65% reduction in raffinose), but continues to decrease during further soaking and cooking, giving a total raffinose loss of 96%. (Nyombaire, Siddiq et al. 2007)

3.2.1.3 Starch

Starch is made up of two types of glucose polymers; amylose and amylopectin. Amylose is a linear molecule, made up of long chains of α-D-glucopyranosyl units linked by 1→4 glycosidic links. Amylopectin is similar, but does also contain glucose units attached by 1→6 glycosidic links, creating branches (Coultate 2009). Figure 2 shows the chemical structure of amylose and amylopectin

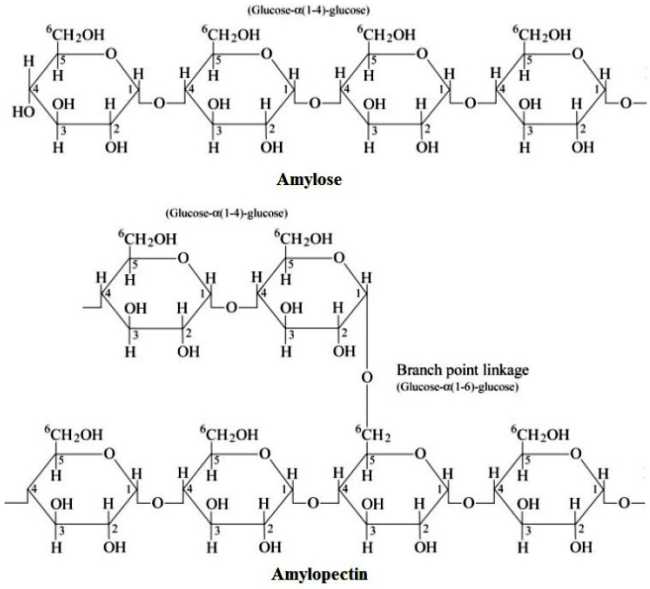

Figure 2. Chemical composition of amylose and amylopectin (Ghanbarzadeh and Almasi 2013)

The average starch content of raw common bean is approximately 48% of the dry weight. The concentration of starch changes during soaking and cooking of beans. 16-22% of solids in *P. vulgaris* are lost during soaking and cooking, leaving an average of 25.5% starch in the beans. The concentration of amylose remains constant, while amylopectin is solubilized and the concentration drops, which decreases the overall starch concentration. Resistant starch (RS) is not changed during soaking but is converted to digestible starch during heating. (Pujóla, Farreras et al. 2007)

Raw navy bean starch consists of 9% rapidly digestible starch (RDS), 13% slowly digestible starch (SDS) and 77 % restistant starch (RS). After cooking, the starch content changes to 88% RDS, 2% SDS and 10% RS. This relatively high proportion of SDS and RS contribute to the low glycemic index response of beans. The amylose content of navy bean starch is approximately 43%. Amylose has

a significant effect on the pasting, swelling and retrogradation. The pasting temperature of Navy Bean starch is 77.8°C, and the peak gelatinization temperature is 71.7°C. (Du, Jian et al. 2014)

3.2.2 Protein

Pulses (beans, peas and lentils) contain a high amount of proteins which are of high protein quality, generally around 18-32% of proteins on a dry basis,. The quality of a protein usually refers to the essential amino acid composition and the digestibility. (Boye, Zare et al. 2010) The protein content of white beans can be seen in Table 1.

Albumins and globulins constitute the main part of the proteins in pulses. There are also smaller amounts of glutenins and prolamins. Both types of proteins are globular, and the albumins are water soluble whereas the globulins are salt soluble and the albumins entail proteins with different functions such as enzymatic proteins, lectins, amylase inhibitors and protease inhibitors. The ratios of albumin to globulin can vary between different pulses. Dry common bean proteins consists of 10-30% albumins and 45-70% globulins. The pulse proteins are low in sulfur-containing amino acids such as methionine and cysteine but quite high in acidic amino acids such as lysine, aspartic acid, glutamic acid and arginine. (Boye, Zare et al. 2010) The limiting amino acids in Great Northern bean are reported by (McWatters and Cherry 1981) to be the sulfur-containing amino acids methionine+cysteine with a chemical score of 29.7% and secondly leucine with a chemical score of 39.4%. The amounts of which they exist in the bean flour as mg amino acid per g protein are 10.4 mg (methionine+cysteine) and 27.6 mg (leucine). The acidic amino acids exist in higher amounts; glutamic acid (224.4 mg/g protein), aspartic acid (164.5 mg/g protein), lysine (67.9 mg/g protein) and arginine (67.5 mg/g protein). Many cereals contain more of the sulfur-containing amino acids and less of lysine, why the combination of pulses and cereal food stuff is a good combination (Boye, Zare et al. 2010).

Pulse proteins have important functional properties such as emulsifying properties, fat binding, water binding and foaming. The properties depend on the amino acid composition, interactions with other components in food systems, processing conditions and the structure of the proteins. (Boye, Zare et al. 2010)

3.2.2.1 Solubility

The degree of solubility of a protein reflects the distribution of polarity throughout the surface of the protein, depending on the amino acids. Solubility greatly influences other functional properties such as emulsification, foaming and gelation. (Kiosseoglou and Paraskevopoulou 2016) The solubility of pulse proteins is generally highest at high and low pH values and lowest at pH 4-6, which is close to the isoelectric point. (Boye, Zare et al. 2010) Apart from pH, solubility is also affected by temperature, concentration of salt and the polarity of the solvent. Heating of food usually causes denaturation of

proteins, often followed by aggregation, and thereby also loss of solubility. The change in functionality of the proteins can affect both consistency and color of the food product. (Culbertson 2005)

3.2.2.2 Emulsifying properties

Proteins can help to stabilize oil droplets in an emulsion by adsorbing to the surface of the droplets, leading to prevention of coalescence into larger oil droplets. Factors that affect the emulsifying capacity of proteins are for example solubility, size, surface hydrophobicity and pH. At a pH close to the isoelectric point of the protein the emulsifying activity is reduced. (Kiosseoglou and Paraskevopoulou 2016) McWatters and Cherry (1981) reports that the proteins in Northern Bean, have high oil emulsification capacity (98% oil emulsified) compared to other legumes.

3.2.3 Antinutrients

Usually it is necessary to both soak and boil pulses before consumption. This is due to the antinutrients present in the pulses that usually can be eliminated through heat treatment and soaking. The antinutrients that are present are for example trypsin inhibitors, lectins, phytic acid and tannins. These compounds can have both harmful and beneficial effects in humans.

3.2.3.1 Lectins and trypsin inhibitors

Lectins are carbohydrate binding glycoproteins which are present in common beans. The glycoproteins are not digested in the gastrointestinal tract, instead they bind to glycosyl groups of the cell membrane which line the digestive tract. This causes several harmful reactions such as the loss of gut epithelial cells, disruption of the digestion, less absorption of nutrients and changes in bacterial flora. This can in turn cause enlargement and deterioration of internal organs and alter the hormone- and immune system. (Vasconcelos and Oliviera 2004) Trypsin inhibitors are proteins that can bind to and inactivate the digestive enzyme trypsin. Sufficient soaking and heating of beans will inactivate lectins and trypsin inhibitors to an acceptable level. (Vasconcelos and Oliviera 2004, Wang, Hatcher et al. 2010)

3.2.3.2 Phytic acid and tannins

Phytic acid binds strongly to cations such as zinc and iron, making them less available for absorption in the intestines. Excessive consumption of improperly treated pulses could therefore lead to deficiency of some minerals, especially for those already at risk for these deficiencies, for example in developing countries where malnutrition is an issue. (Doria, Campion et al. 2012)

The tannin content of beans can range from 0-2% and are present in the seed coat of the bean. Tannins in beans decrease protein digestibility by inactivating digestive enzymes or forming complexes with substrate proteins along with ionizable iron. Similar to phytic acid, this can decrease the amount of

absorbable iron from the diet (Reddy, Pierson et al. 1985). White beans contain little amounts of tannins compared to other types of *P vulgaris*. (Wang, Hatcher et al. 2010)

However, both phytic acids and tannins can present anticarcinogenic effects through their antioxidative capacity. Their binding to metal ions can prevent formation of free radicals. Phytic acid also presents anticalciferation effects (preventing kidney stone) (Doria, Campion et al. 2012) and can regulate the cell cycle, avoiding uncontrolled cell division and forcing malignant cells to apoptosis. (Rebello, Greenway et al. 2014)

Tannins are water soluble, therefore they are eliminated through soaking and/or cooking. Phytic acid is also affected by soaking, during which the membrane permeability of the beans changes, increasing water absorption. This activates phytases, causing hydrolysis and release of phosphate groups of phytic acid, and the molecule loses its ability to bind minerals. The concentration gradient due to the soaking water also causes the phytate to spread to the surrounding water. It is therefore important to rinse the beans and change the boiling water in order to eliminate the phytate and tannins. However, this also reduces the antioxidative capacity of the beans. (Valdés, Coelho et al. 2011)

3.2.4 Lipids

The lipid content in beans is generally around 2-21% and consist to a high degree of the unsaturated fatty acids linoleic (4-22%) and linolenic acids (21-53%). More specifically in common beans, 61.1% of the total fatty acids are palmitic acid, oleic acid and linoleic acid. (Oomah, Patras et al. 2016)

3.2.5 Vitamins and minerals

Cooked beans are a good source of B-vitamins, e.g. thiamin, niacin, riboflavin, pyrodixine and folate. These vitamins have functions associated with fatty acid metabolism and energy metabolism. The folate content is especially high; one can satisfy the daily recommended dose with just two portions of beans (1 portion = approx. 125 g boiled beans). Cooked beans also contain the essential micronutrients zinc, iron and calcium. However, the bioavailabitlity of zinc is quite low, but approximately 20% of the iron and calcium is bioavailable. Beans are low in sodium and high in potassium. This combination helps retain low blood pressure. (Rebello, Greenway et al. 2014)

3.3 Fermentation

Fermentation of legumes such as soy beans, chickpeas and common beans is common in many countries, especially outside of Europe. (Coda, Melama et al. 2015) Barampama and Simard (1994) stated that fermentation of pulses can have a reducing effect on antinutritional factors such as phytic acid, tannins, trypsin inhibitors as well as RFO and is a way of increasing protein digestibility in raw and cooked beans. The authors fermented a slurry made out of common bean flour and water with *Lactobacillus fermentum* ATCC 14931. A concentration of 10^9 cells/ml in the inoculum was used, giving a

concentration of approximately 10^6 cells/ml in the slurry. (Barampama and Simard 1994) Coda, Melama et al. (2015) fermented faba bean flour with *L. plantarum*. The results were a decrease in trypsin inhibitor activity and tannin content, as well as an increase in protein digestibility and free amino acids. *L. plantarum* also has the ability of producing α-galactosidase enzymes which break down the RFO. (Adeyemo and Onilude 2013) *L. plantarum* transforms carbohydrates into lactic acid. Some strains, such as *L. plantarum* A6 are amylolytic, that is they increase α-amylase activity and have the ability of breaking down starch. This has a decreasing effect on viscosity in starch-containing beverages fermented by *L. plantarum* A6. (Espirito-Santo, Mouquet-Rivier et al. 2014) Common bean fermentation can be a way of creating new applications and functional foods based on pulses.

L. plantarum has been used for centuries in order to preserve food. It is a probiotic bacteria which produces aromatic compounds such as lactic acid and acetic acid. Probiotics are bacteria which, when ingested live, can have beneficial health effects. *L. plantarum* 299v is a patented strain and used in the fermented fruit beverage ProViva. It has an anti-microbial effect against many pathogenic bacteria such as *Escherichia coli* and *Listeria monocytogenes* and can have a positive effect on the bacterial flora in the gastrointestinal tract of humans. (Molin 2015)

3.3.1 Cultivation and enumeration media

The agars that were used for detecting growth of *Lactobacilli* in the fermented beverages was Tryptic Soy Agar (TSA) and Rogosa Agar. TSA is a non-selective agar media that supports growth of a wide variety of microorganisms and is therefore used for total bacterial count in this project. (Scientific/Remel 2016)

Rogosa Agar on the other hand is a selective agar for *Lactobacilli*. Acetic acid is added to the media to lower the pH to 6.2 and thereby increasing the specificity for only *Lactobacilli* and not all Lactic Acid Bacteria. (Scientific/Oxoid A 2016)

MRS-broth was also used for pre-cultivation of *L. plantarum* 299v. MRS is a non-selective media for enumeration of *Lactobacilli*, containing polysorbate, acetate, magnesium and manganese that are needed for growth of *Lactobacilli.* (Millipore 2016)

In order to confirm that no *Enterobacteriaceae* were present in the beverage, enumeration on Violet Red Bile Dextrose **(**VRBD) Agar was performed, since this agar is selective against *Enterobacteriaceae*. (Oomah, Patras et al. 2016) Some pathogenic bacteria belong to this family such as *Escherichia coli*, *Salmonella*, *Shigella* and *Yersinia enterocolitica*, that can cause different forms of food poisoning. In production of milk, low pasteurization is enough to inactivate these bacteria. In this project this would mean that detection of these bacteria in the fermented beverages would indicate that contamination have occurred after autoclaving. (Walstra and Wouters 2006) In this study, enumeration of yeast and moulds is also performed on Malt Extract Agar in order to to detect potential spoilage in the beverage by yeast or moulds. (Scientific/Oxoid B 2016)

3.4 Plant milks

Plant milks are often fortified with vitamins and minerals in order to be more nutritionally similar to dairy milk. There are many different types of plant milks, popular ones are soy milk and oat milk, other types available on the market are almond-, cashew-, rice- and coconut milk (Alen 2014). In Table 3, some of the plant milks in Lantmännen Cerealia's (GoGreen) of products are presented together with their nutritional value. (GoGreen 2013)

Table 3 Nutritional content of GoGreen's unsweetened almond beverage, soy beverage, oat beverage and cashew beverage

Nutritional value/100 ml	Almond milk	Soy milk	Oat milk	Cashew milk
Energy (kJ/kcal)	70 kJ /16 kcal	160 kJ/40 kcal	160 kJ/40 kcal	90 kJ/20kcal
Fat (g)	1.4	2.1	0.5	1.5
Saturated fats (g)	0.1	0.3	0.1	0.3
Carbohydrates (g)	0.1	0.8	7.2	0.9
Sugar (g)	0.1	0.7	1.1	0.1
Fiber (g)	0.4	6	0.3	0.3
Protein (g)	0.5	3.7	0.8	0.6
Salt (g)	0.1	0.03	0.05	0.1

3.4.1 Soymilk process

There are many different processes for the production of soy milk. The soy milk can be made of whole soybeans, dehulled soybeans or whole soybeans where the insoluble fraction is removed later in the process. A general soymilk process for whole bean soymilk is described by Shurtleff and Aoyagi (1979) shown in Figure 3.

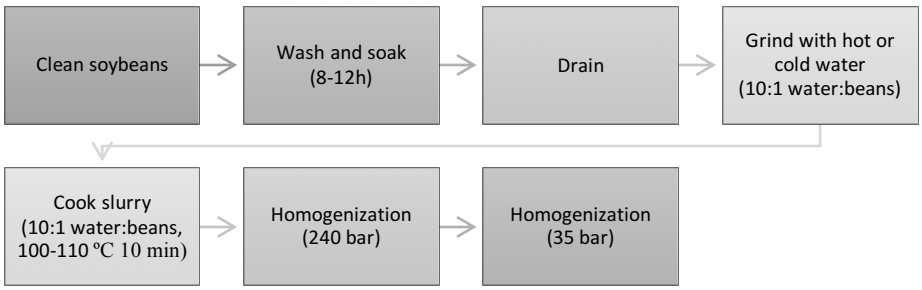

Figure 3 General soymilk process for whole bean soy milk (Shurtleff and Aoyagi 1979)

The minimum time for soaking the soybeans is 3 hours but it depends on the water temperature. The slurry is brought to boil in order to inactivate trypsin inhibitors and sterilize the product. In the soy milk

where whole beans are used the milk is homogenized at high pressure at 90ºC in at least two steps (first at 240 bar, second at 35 bar). In the more common production of soymilk the insoluble residues are removed after the cooking step by filtration, and only one homogenization step with lower pressure is required. 1 kg dry soybeans yields around 6.9 L of soymilk.

Shurtleff and Aoyagi (1979) also mentioned that dehulling, or removal of the seed coat, may reduce soaking time, reduce oligosaccharides, increase shelf-life due to reduction of bacterial count and create a whiter milk with reduced bitter and "green" flavors.

Filtration is a common step to remove insoluble particles, which are the substances causing the grainy/sandy/chalky textural characteristics. Rosenthal, Deliza et al. (2003) compared the effect of homogenization with filtration and enzymatic treatment on the sandy/grainy texture. The enzymes Celluclast, Pectinex and Rohalase were used to hydrolyse insoluble fibers. The size of the insoluble particles is the important factor affecting the grainy or chalky sensation. The conclusions of the study was that both enzymatic treatment and filtration reduces particle sizes and gives lower chalkiness in the soy milk according to the sensory evaluation, compared to the homogenized products (Rosenthal, Deliza et al. 2003). Another way to reduce chalkiness is to pre-blanch the soybeans with alkali such as baking soda (sodium bicarbonate), and adjusting the final pH to 7.5 (Shurtleff and Aoyagi 1979).

3.5 Homogenization & Emulsion stability

Homogenization can induce changes in the microstructure and rheological properties of a dispersion or emulsion by reducing the size of the oil droplets and of the fiber particles in the emulsion. (Castro 2013) This is for example done by decreasing the rate of sedimentation, since this rate increases with increased particle size according to Stoke's law;

$$U_{stokes} = 2gr^2(p_1 - p_2)/9n$$

g = acceleration due to gravity (m/s^2), p_1 = density of oil phase (kg/m^3), p_2 = density of water phase (kg/m^3), n = dynamic viscosity (kg/m*s) and r = particle radius (m), U = the rate of sedimentation (m/s). (Taherian, Fustier et al. 2006)

Fat is often added to plant milks before homogenization. A stable emulsion would require that the majority of oil droplets are below 1 micrometer in size. The addition of oil does not only affect the stability of the beverage, but can also be used to create a turbid/cloudy emulsion in order to imitate the appearance of milk. The microscopic oil droplets suspended in water create an optical effect of "milkiness" (Shachman 2004).

According to Bengtsson (2009) a higher perception of crispiness or graininess is present in fibre suspensions (from potatoes and carrots, in this case) containing larger clusters or aggregates. Homogenization changes the microstructure of the fibres to smaller clusters and single cells or cell fragments, giving a more "slippery and melting" texture. Homogenization of a bean based beverage

could improve both emulsion stability, visual appearance and sensory properties by reducing the perceived sandiness/graininess.

4.0 Materials and methods

4.1 Raw materials and chemicals

For the production of the bean flour and bean beverages Swedish grown small white beans were supplied by Lantmännen Cerealia (see Table 1). Swedish rapeseed oil from the local market was used. Chemicals used were sodium bicarbonate (Santa Maria, Sweden) and citric acid (Santa Maria, Sweden).

4.2 Process formulation

4.2.1 Bean flour

Two different types of flours were produced according to the process scheme in Figure 3. The beans were soaked at a ratio of 1:1 water:beans (v/v) for 9 hours at room temperature and boiled in a pot in the same ratio for 45 minutes. The beans were dried after soaking and boiling at 75°C for 12 hours in a drying oven (Termaks Drying Oven, Termaks, Norway). The dried beans were milled into a flour with a coffee bean miller equipped with knives (Coffee Grinder Prestige, OBH Nordica, Sweden). The flour was milled in batches of 50 g and for about 1 minute per batch. The moisture content of 0.4 g bean flour (soaked and boiled beans) was measured in a moisture meter (MA 30, Sartorius, Germany) twice. It was calculated according to equation 1:

$$Moisture\ content = \frac{m_{before} - m_{after}}{m_{before}} \cdot 100\ (g/100\ g) \qquad (1)$$

The bean flour was also sieved in sieves with different pore sizes in order to determine the approximate particle size distribution of the flour. The sieves that were used had the pore sizes of 63, 175, 420, 555, 800 and 1000 µm. The weight was measured before sieving and after (the fraction that passed through the sieve) for each sieve. The trial was repeated twice. A particle size distribution was constructed and the average particle size could be calculated.

4.2.2 Heat treatment

The flour was mixed with water to different ratios. For the first trials, a ratio of 1:10 (bean flour:water) was used. The slurry was boiled in a pot on a stove with constant manual stirring with a spoon. For the initial trials, the slurry was boiled for 10 minutes. The amount of flour to water was adjusted to the right

ratio after the boiling by weighing the sample and adding water until the same weight as before boiling was reached, since the amount of water decreased due to evaporation.

An experiment for finding the optimal boiling time was performed in order to see the effect of the boiling time on the viscosity of the beverage. The samples were "bringing to boil" and them boild for 5, 10 and 15 minutes. One sample was not heat treated at all, in order to see if heat treatment was necessary in regards to rheological behaviour of the sample. The viscosity and shear stress as a function of shear rate was then measured with a rheometer (Kinexus Pro, Malvern, United Kingdom) by performing a shear-ramp at rates from 20 to 1000 s^{-1}. The used geometry was C25 Splined A0009 SS:PC25 Splined A0008 Al, which measures circular flow in the annular gap between two concentric cylinders. The shape of the viscosity curves were compared as well as the flow consistency indeces (k) and flow behavior indeces (n) from the interpolated power law equations. The power law can be described with equation 2:

$$\sigma = k * -\gamma^n \quad (2)$$
$$\Leftrightarrow \log(\sigma) = \log(k) - nlog(\gamma)$$

where σ = shear stress and γ = shear rate. (Singh and Heldman 2009)

4.2.3 Mixing

In order to obtain a homogenous and emulsified bean beverage, mixing was performed with a disperser tool (Ultra-turrax, IKA Homogenizers, Germany) with 300 ml sample (8% bean flour, 1.5% rapeseed oil) at 13 500 rpm. Due to lack of resources, no homogenization with higher pressure could be performed. The effect of the homogenization time on the sedimentation of the insoluble solids in the beverages was studied by homogenizing the beverage for 10-60 minutes, taking samples every 10 minutes and measuring the height of the upper layer relative to the height of the whole sample. The height of the upper layer was measured every 5 minutes during 60 minutes.This trial was repeated twice, so there are 2 replicates of each measurement.

4.2.4 Color measurements and oil droplet size

The size of the oil droplets after mixing was studied in a light microscope (BX50, Olympus, Japan). Additionally, the change in color after mixing was measured with a colorimeter (Chroma Meters CR-400, Konica Minolta, United Kingdom) obtaining L*a*b values. Each measurement was repeated twice. L represents the level of black/white, L=100 being a perfect reflecting diffuser and L=0 is black. Positive a is towards red, negative a is towards green. Positive b is towards yellow, negative is towards blue. Color was measured both before and after mixing in centrifuged and non-centrifuged samples.

4.2.5 Final process scheme

After these initial trials explained in 6.2.1-6.2.3, a final process was chosen in order to continue the different treatments on the beverage. The times for heating the slurry and mixing the beverage were decided using the results from the initial trials. The process scheme can be seen in Figure 4.

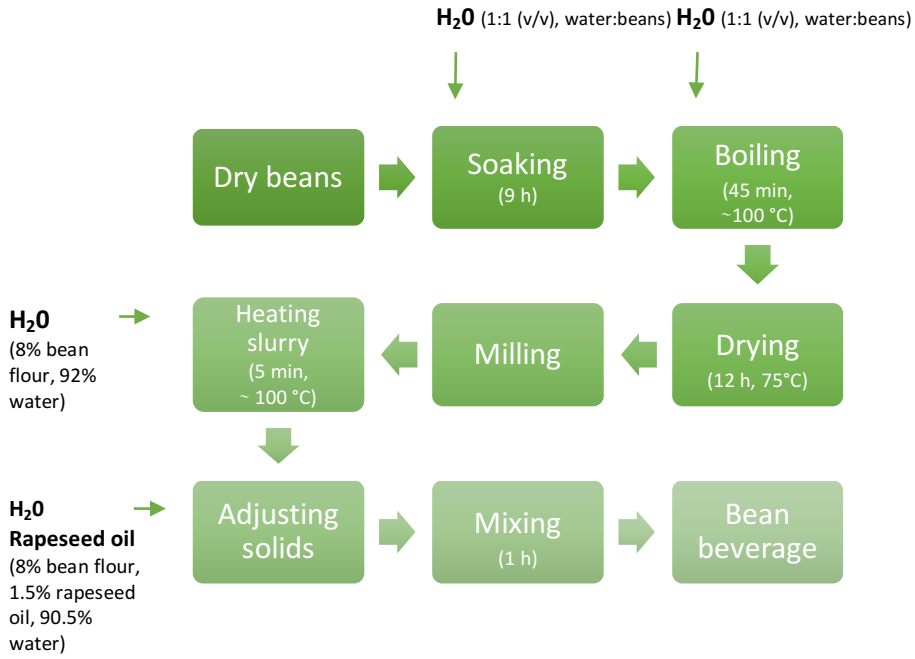

Figure 4. General process scheme for the final basic formulation of the bean beverage.

4.3 Protein analysis

Protein content in was determined by measuring the total nitrogen content (FLASH 2000 N/Protein Analyzer, Thermo Fisher Scientific, USA). According to Commons (2016), the conversion factor of 6.25 can be used when calculating the relative protein content from the nitrogen concentration in beans such as jack, lima, navy and mung beans. Since there is no information on the average nitrogen content in the proteins in the white beans from Lantmännen Cerealia, the conversion factor of 6.25 is used in this study.

The value of the protein content obtained from the protein analysis and the the dry matter (DM) of the sample that was measured were used to calculate the protein content in the beverage sample. DM was obtained by drying a small sample of the beverage in a metal cup in a drying oven at 105°C for 12h or until constant weight. DM was calculated according to equation 3, and the protein content in the beverage was calculated according to equation 4:

$$DM = \frac{m_{after\ drying}}{m_{before\ drying}} \cdot 100\ (g/100\ g) \qquad (3)$$

$$Protein\ content\ in\ drink = \frac{Protein\ content_{measured} \cdot DM}{100}\ (g/100\ g) \quad (4)$$

4.4 Recipe formulation

In order to find out the optimal concentration of bean flour in the beverage, a theoretical calculation of the the amount of protein (as g/100 ml beverage) and the energy percentage (E%) from proteins was performed according to equation 5:

$$E\%_{\ protein} = \frac{E_{from\ 1\ g\ protein} \cdot m_{protein}}{E_{total}} \quad (5)$$

Where $m_{protein}$ is equal to the amount of bean flour/100g beverage (8g) times the bean flours protein content (22g/100g according to a measurement) E_{total} was calculated using the energy content of the beans and oil according to equation 6.

$$E_{total} = \frac{m_{oil} * E_{oil} + m_{beans} * E_{beans}}{m_{beverage}} \quad (6)$$

The E% was calculated for beverages with 6%, 8%, 10% and 12% bean flour. The protein content was also measured twice in each beverage to get a measured value of the E%, in order to compare the two. An E% which fulfils the requirements for labelling the beverage as a "source of protein" was wanted (>12%). (European Comission 2016) The consistency was also evaluated internally. Additionally, different concentrations of the rapeseed oil (1%, 1.5%, 2%, 3%) were dispersed in the beverage where after the taste, consistency, sandiness and lubricating effect was evaluated internally. The basic recipe was thereafter formulated, which can be found in section 5.2.

4.5 Fermentation

Fermentation of the bean beverage was performed in order to investigate the effect on the amount of RFO, sandiness, viscosity and taste. The bean beverage was fermented with the bacterial strain *L. plantarum* 299v (Probi, Sweden), stored at -80°C. There were two replicates. Before starting the fermentation process, growth curves and absorbance curves needed to be assessed, to get a relationship between absorbance and growth, the maximum concentration of the specific bacterial strain, and thereby determine the concentration and volume of the inoculum to use for the fermentation. The standard procedure for microbiological cultivation that was used can be seen in Appendix 1 - Fermentation. A similar set-up as in the study by Barampama and Simard (see section 3.2) was chosen for this study regarding inoculation concentrations.

In order to eliminate microorganisms that potentially exist on the beans naturally, the beverage had to be autoclaved before inoculation. The beverage was autoclaved during different amounts of time (5, 10, 15 minutes) at 121°C to see the effect on growth on TSA. The fermentation procedure was performed according to the following protocol. All measurements and samples were taken from both beverages, and each measurement was performed in duplicates:

1. Pre-cultivation of *L. plantarum* 299v in MRS medium until the maximum concentration was reached (10^9 cells/ml was obtained after 24 h).
2. From the absorbance and growth curves, the volume of the inoculum needed in order to have the wanted starting concentration was calculated. An absorbance of about 0.380 corresponded to about 10^9 cells/ml and 0.4 ml of the inoculum was needed. The cells were washed according to the standard protocol in Appendix 1.
3. Two 450 ml beverages were autoclaved in a 500 ml glass bottle at 121°C for 15 minutes. The beverages were cooled down to room temperature. Samples (50 mL) from both were taken before the autoclaving for measuring of viscosity and pH.
4. Before the inoculation, on day 0, sampling was performed in order to confirm that no microorganisms are present before inoculation. An undiluted sample and a sample diluted to 10^{-1} in the peptone and NaCl solution were spread onto duplicate agar plates (VRBD, Malt, Rogosa and TSA). The pH and viscosity was also measured before the inoculation.
5. The remainder of the beverages (400 ml) were inoculated with 0.4 ml of *L. plantarum* 299v. The beverage was then incubated in an anaerobic clock at 37°C for 7 days.
6. On day 3 and 7 of fermentation, sampling was performed according to the same procedure as on day 0 but with other dilutions. The dilutions used each sampling day and the incubation conditions for the different agars can be seen in Appendix 1 - Fermentation (Table 1). The beverages were incubated again in an anaerobic clock at 37°C between day 3 and day 7. On day 3 and 7, pH was measured and on day 7, pH and viscosity was measured.
7. Samples from day 7 were taken and dried in a convection oven (105°C, overnight) and milled into a flour for further analysis of RFO. A sample of the fermented beverage was also sent for external analyses of nutritional content and of raffinose and stachyose content.

4.6 pH adjustment

Trials were performed where the pH of the soaking- and boiling water was adjusted using sodium bicarbonate in order to increase the solubility of fibers and proteins in the beverage. The concentrations of sodium bicarbonate were chosen based on recommendations by Shurtleff and Aoyagi (1979) for whole bean soymilk production. The beans were soaked or boiled in different concentrations of sodium bicarbonate (water:bean ratio was 1:1 (v/v)); boiled in 0.25% and 0.5%, and soaked in 0.5%, 2.2% and 4% (w/w). This was in order to obtain different pH in the beverage. The beans were then dried and milled according to normal procedure. A beverage (8% flour) was prepared from each type of flour, obtaining beverages with pH of 6.8 (without sodium bicarbonate), 7, 7.4, 7.6, 8.1, 8.8 and 9.8. Samples were mixed with an Ultra-turrax for 10 min (13 500 rpm). All samples were centrifuged (Allegra x-15r,

Beckman Coulter, USA) for 20 min at 1000G. A non-centrifuged sample at each pH was saved for protein analysis.

The same trials were repeated a second time, with the difference that pH was lowered to around 6.8 (around the same pH as the standard beverage) using citric acid before centrifugation. The yield after centrifugation (mass supernatant/total mass) was determined, as well as the pH, dry matter and protein content of both the supernatant and the non-centrifuged samples. All measurements were made twice.

A small internal sensorial analysis was conducted to evaluate the taste, consistency and sandiness of the samples with and without centrifugation.

4.7 RFO analysis

RFO analysis was performed with a Megazyme Raffinose/Sucrose/D-glucose assay kit. The principle of the method is that raffinose, stachyose and verbascose are hydrolyzed enzymatically with α-galactosidase and invertase into D-glucose, D-sucrose and D-galactose. The D-glucose concentration is then measured spectrophotometrically, enabling calculation of the original RFO concentration. The assay procedure was performed according to attached protocol in Appendix 2. (Megazyme 2015) Each sample was measured in duplicates.

4.8 Sensory analysis

A sensory analysis with 14 panelists was performed, including a hedonic test and a triangle test. The panelists were untrained male and females from different countries. All panelists but one were in the ages 20-30 (the last one was in the ages 31-40).

4.8.1 Hedonic evaluation

The hedonic test included a 9-point scale evaluating the panelists general liking and their perceived sandiness/graininess of the sample. Samples evaluated were:

1. Control sample (8% bean flour beverage)
2. Sample treated with sodium bicarbonate (pH was 7.5 before being lowered to 6.9)
3. Centrifuged version of sample 1
4. Centrifuged version of sample 2

The sensory analysis questionnaire can be seen in Appendix 3 – Sensory analysis. Analysis of variance (ANOVA) was performed on the data from the hedonic evaluations in order to find out how the mean value of the different samples vary at a significance level of 95 %. The difference of the samples was significant if $F_{crit}<F$, or if the p-value was lower than 0.05. Although, to find out which mean values that

were significantly different to each other, a Tukey test were performed. The differences in mean values between all samples were calculated and compared with a w-value, which was calculated according to the equation 7:

$$w = q_{\alpha(k,df)} \cdot \sqrt{\frac{SE}{r}} \quad (7)$$

where

$q_\alpha = value\ obtained\ from\ a\ table\ of\ Tukey\ q - values$ (Statistics 2016)

$df = degrees\ of\ freedom\ (within\ groups)$
$k = number\ of\ treatment\ levels$
$SE = standard\ error\ of\ a\ treatment\ mean$
$r = number\ of\ replications$

If the difference in mean values for each pair that is compared is higher than the calculated w-value, there is a significant difference on a 95% significance level.

4.8.2 Triangle test

The purpose of the triangle test was to evaluate if there was a perceived difference in sandiness/graininess in samples 1 and 2. The panelists received two sets of three samples (the order of the samples was randomized amongst the panelists), one set where sample 1 was the different one and the other set where sample 2 was the different one. The panelists were also able to comment on the reason they thought the sample was different.

The results of the triangle test were analyzed according to the χ^2-test as described by Lawless and Heymann (2010) where the observed results can be compared with the expected results by chance. If the value exceeds the χ^2 value expected by chance, the null hypothesis can be rejected. H_0 = there is no significant difference between samples 1 and 2.
χ^2 is calculated according to equation 8:

$$\chi^2 = \sum \frac{(|O-E|-0.5)^2}{E} \quad (8)$$

In a triangle test it can be calculated according to equation 9:

$$\chi^2 = \frac{(|O_1 - E_1| - 0.5)^2}{E_1} + \frac{(|O_2 - E_2| - 0.5)^2}{E_2} \quad (9)$$

where

$O_1 = Observed\ number\ of\ correct\ choices$

$O_2 = Observed\ number\ of\ incorrect\ choices$

$E_1 = Expected\ number\ of\ correct\ choices$

$E_2 = expected\ number\ of\ incorrect\ choices$

5.0 Results & Discussion

5.1 Process formulation

5.1.1 Bean flour

The PSD of the bean flour was determined by sieving. The distribution curve in Figure 5 shows that approximately 45% of the flour can be found in the particle size interval of 63-175 µm, 26% in the interval of 175-420 µm and approximately 9-10% each in respective size intervals of <63 µm, 420-555 µm and 555-800 µm. The calculated average particle size is 248 µm. In a study by Han, Janz et al. (2010), PSD of different commercial pulse flours (ground from whole seeds) are reported. In Navy bean flour, the largest portion (63.4%) of the particles was found in the size range of 177-250 µm, which is comparable with the PSD and the average particle size of the bean flour used in this study. The measured moisture content of the bean flour is 5 ± 0.1 g/100 g. According to Shurtleff and Aoyagi (1979), the dietary fibers' effect on mouthfeel in beverages depends on particle size, viscosity and water absorption. In general, mouthfeel can be improved by reducing particle size of the fibers to less than 20 µm, which is the detection limit in the mouth for particles. Fibers that have higher water absorption tend to give a softer mouthfeel. In order to decrease sandiness in this bean beverage, the average particle size of the flour needs to be reduced more, possibly by milling the beans into a finer flour, or by increasing the water absorption of the fibers.

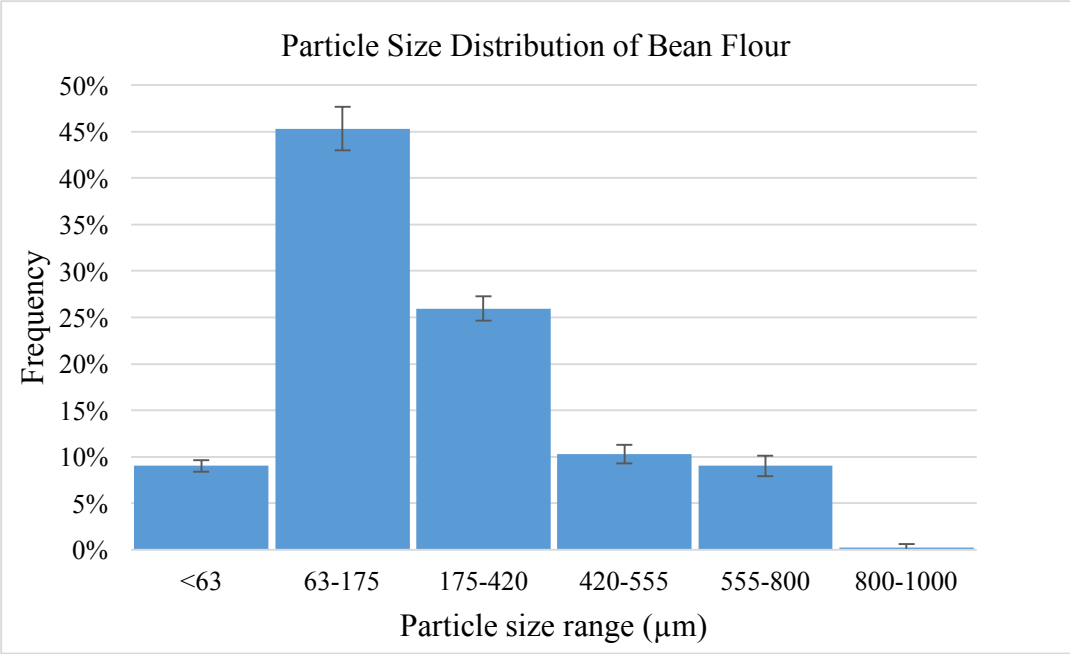

Figure 5 The Particle Size Distribution of the bean flour determined by sieving the flour through sieves with different pore sizes, shown as weight percentage of the flour belonging to the different size ranges. The error bars represent the standard deviations based on two replicates.

5.1.2 Heat treatment

The viscosity measurement results from the trials with different heat treatments of the bean beverage (0, bring to boil, 5, 10 and 15 min boiling time) can be seen in Figure 6. The viscosity is plotted as a function of shear rate. It is clear that all samples have a shear thinning behavior, and approximately the same rheological behavior, apart from the sample which has not been heat treated which is less viscous both at a lower and higher shear rate. This is confirmed by the flow behavior index (n); the n-value is similar for all samples except the unheated sample. Its n-value is close to one, meaning it has close to newtonian behavior. The other samples have a $n<1$, meaning they have a shear thinning behavior. The flow consistency index (k) describes the viscosity at a shear rate of 1 s^{-1} and is lower for the unheated sample than the others, which are close to 0.2 Pa s^n (Cunningham 2016). The n- and k- values can be seen in Table 4.

A boiling time of 5 minutes was chosen for the process, since this was assessed to be a sufficient time to obtain the wanted viscosity in order to facilitate mixing, while at the same time minimizing the overall time for heat treatment.

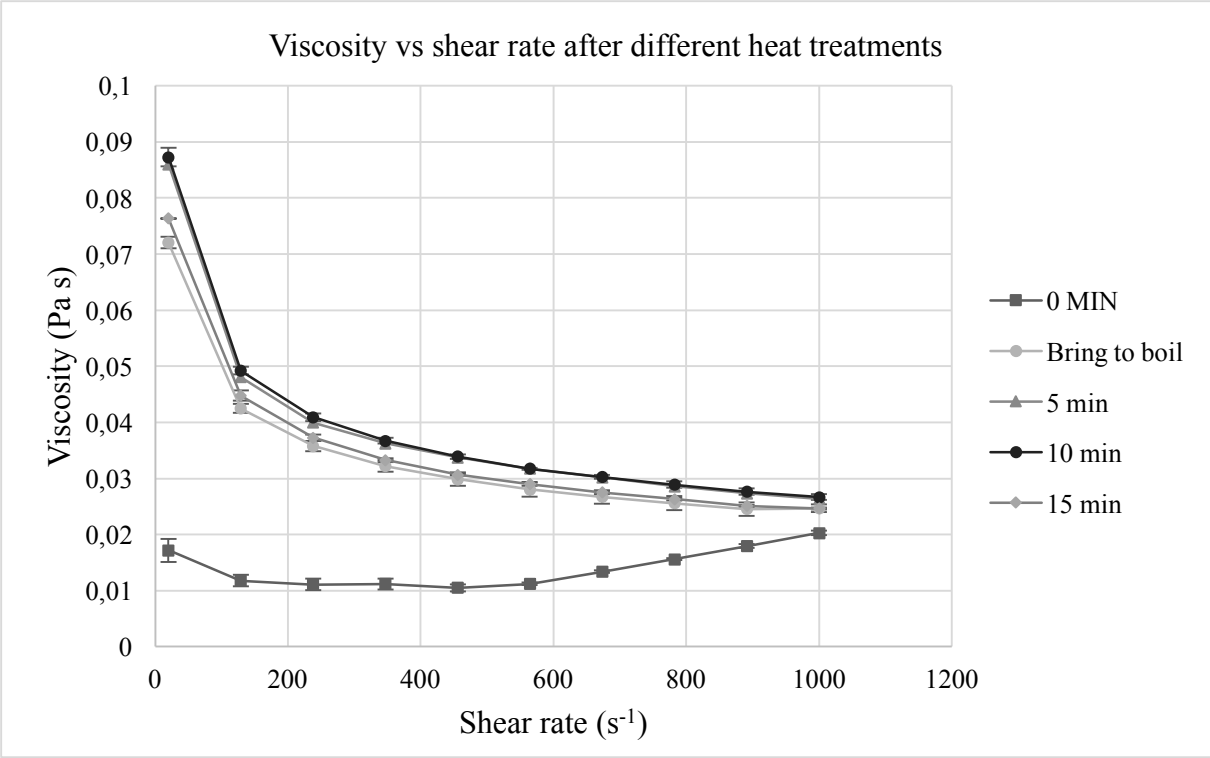

Figure 6 Viscosity as a function of shear rate for samples heat treated for different amounts of time (0 min, bring to boil, 5 min, 10 min and 15 min). The error bars represent the standard deviation based on two measurements

Table 4 . Power law index and flow consistency index from interpolated power law equations

	0 min	**Bring to boil**	**5 min**	**10 min**	**15 min**
Flow behavior index (n)	1.01	0.72	0.70	0.70	0.71
Flow consistency index (k) (Pa s^n)	0.004	0.17	0.21	0.21	0.18

5.1.3 Mixing

The result from the mixing experiment using an Ultra-turrax (13 500 rpm), where the effect of mixing time on the extent of sedimentation was studied, can be seen in Figure 7. A trend of decreased sedimentation rate and sedimentation extent with increased mixing time can be seen. The samples where no sedimentation had occurred when the sample had been standing for 10 minutes after the mixing was performed, was the samples that was mixed for 40, 50 and 60 minutes. Additionally, the sample that had been mixed for 60 minutes did not show sedimentation until 50 minutes after that the mixing was stopped. The goal with the mixing process was to obtain a beverage where no sedimentation occurs within 10 minutes of letting the beverage stand. The mixing time of 60 minutes was chosen, since this sample showed a lower sedimentation rate than the samples that had been mixed for 40 and 50 minutes. An important observation during the mixing process was that there was a high degree of foam formation. After only 10 minutes of mixing, the volume of the foam was larger than the volume of the liquid, which can be seen in Figure 8 showing a picture of a beverage that has been mixed for 10 minutes.

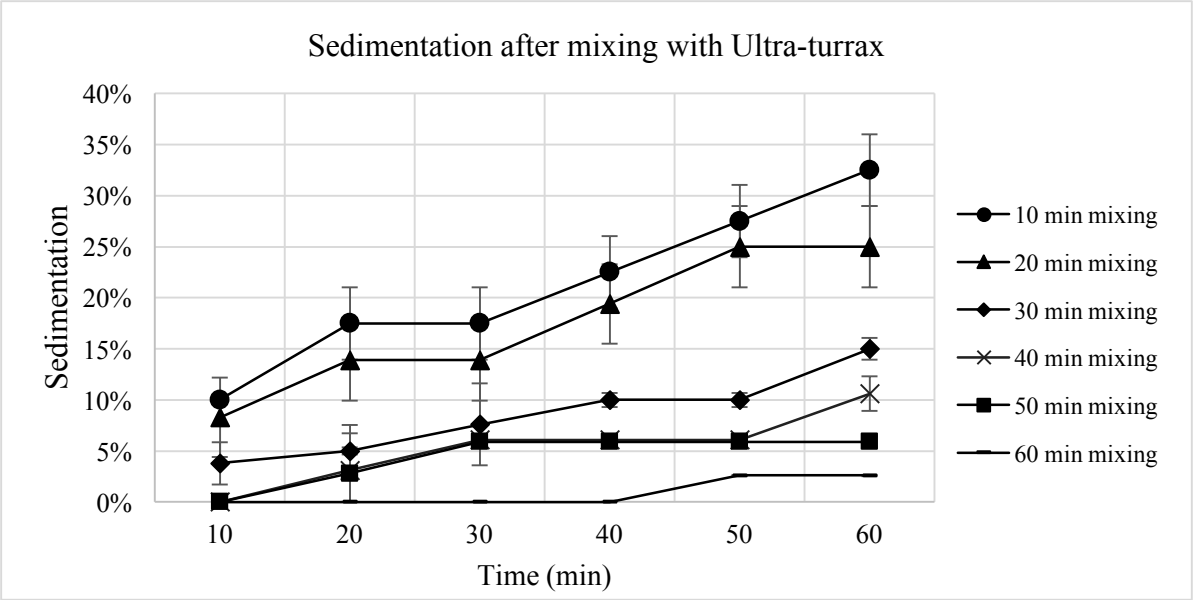

Figure 7. The extent of sedimentation (height of upper layer divided by height of the whole sample) in percentage as a function of time after the total mixing time of 10, 20, 30, 40, 50 and 60 minutes respectively. The extent of sedimentation was measured with 10 20, 30, 40, 50 and 60 minutes respectively. The extent of sedimentation was measured with 10 minutes interval after that the mixing had been performed. The error bars represent the standard deviations based on two replicates.

Figure 8. A picture of a basic bean beverage that has been mixed with an Ultra-turrax for 10 minutes, showing a large extent of foam formation.

The homogenizers available at the laboratory could not be used to homogenize the bean beverage because of the high amount of fibers in the products that could wear out the valve and pumps in the homogenizer. Therefore, mixing was done with a high shear dispersion instrument, (Ultra-turrax) instead. Mixing was performed at the speed of 13500 rpm and since low pressure is applied compared to using a homogenizer, this process cannot be compared to using a homogenizer, especially a high pressure homogenizer which is often used in the production of whole bean soy milk. Under optimal conditions, a high pressure homogenizer would be used in order to obtain a more homogenous and stable product regarding size and structure of both fibers and oil droplets.

The foam formation during mixing indicates that the proteins that are present have the capacity of stabilizing foams. The foam formation could potentially be a problem if the process would be scaled up to an industrial scale. Although, mixing trials were also conducted in another high shear mixer where larger volumes (2-3 L) can be mixed (data not shown in the report). This mixer gave rise to almost no

formation of foam in comparison to mixing with the Ultra-turrax. However, it could be an important consideration to add a defoaming substance as in the soymilk production, in order to avoid loss of material, pumping problems etc.

5.1.3.1 Color measurements and oil droplet size

In figure 9, a microscopic image is shown displaying the oil droplets in a sample after 1h mixing and after centrifugation at 1000G for 20 min. The scale helps to establish the average droplet size, which is approximated to the size of the third largest droplet. The average diameter of the droplets is estimated to 5 µm. This was clearly enough to ensure sufficient emulsion stability (at least 10 min after mixing).

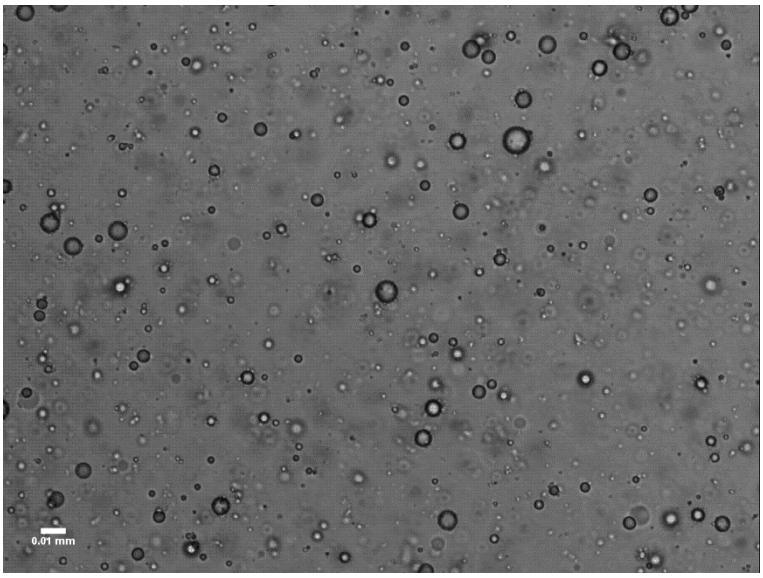

Figure 9. Control sample after centrifugation, after 1h mixing. Zoom = 20x

Color measurements were made before and after mixing, both for centrifuged and non-centrifuged samples. The data can be seen below in Table 5. It is clear that both control- and centrifuged samples increase in L-values after centrifugation, meaning that they increase in whiteness. The centrifuged sample (CC) before mixing seems to be more towards black/green (b=-9.26). This can be due to that the sample was not completely opaque before mixing and the surface underneath in the colorimeter was black. Both a and b values were close to zero, meaning that they had no distinct color, with an exception of C (After), which was more yellow. One can also see the color change in Figure 10.

*Table 5. L*a*b values before and after mixing. C = control sample, CC = centrifuged sample. The results are presented ± standard deviation.*

Sample	L	a	b
C (Before)	56.52 ± 1.71	-1.91 ± 0.03	0.67 ± 0.34
C (After)	74.41 ± 0.06	-0.84 ± 0.07	4.88 ± 0.20
CC (Before)	31.19 ± 0.28	-1.72 ± 0.09	-9.26 ± 0.08
CC (After)	76.14 ± 0.15	-1.83 ± 0.03	-0.24 ± 0.14

Figure 10. Figure showing the color change before and after mixing.
The sample to the left (423) is before mixing and the sample to the right (571) is after mixing.

5.1.4 Final Process

The data obtained from the initial trials, the heat treatment experiment and the mixing experiment combined with results from the literature study, resulted in a final process formulation. The process scheme including all process parameters can be seen in Figure 11. This process was used as a base when applying different treatments to the beverage (addition of oil, centrifugation, pH adjustment and fermentation).

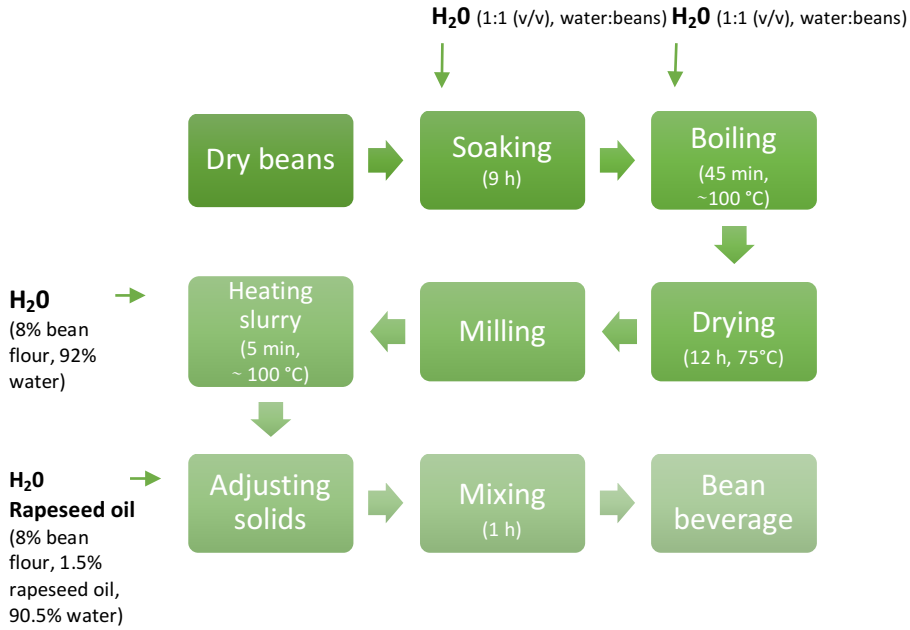

Figure 11 Process scheme for the final basic formulation of the bean beverage.

Although this process is functional for producing the bean beverage, it is rather inefficient regarding both time and energy consumption since there are several heat treatment steps and since a long soaking time is used. This was to ensure inactivation of antinutrients, since these components were not to be analysed. More tests should be performed on the effect of soaking- and boiling time and temperature on all antinutrients in white beans in order to optimize the process.

5.2 Recipe formulation

Based on the results from the calculation of E% from protein, the protein analysis and the small sensorial analysis, a recipe for the basic formulation of a bean beverage was made. Table 6 shows the theoretical values of protein content in the beverage and the E% from protein in the beverage. The measured values of DM, protein content per dry matter and in the beverage are shown in Table 7.

A concentration of 8% bean flour was chosen, based on the high theoretical E% from protein (18.0%) and the measured protein content of 1.7% in the beverage. Addition of 1.5% rapeseed oil was chosen, since this would equal a total fat content of 1.7 g/100 g which is the same as Lantmännen Cerealia's original soy beverage (see Table 3). The recipe can be seen in Table 8. This recipe was used as a base when applying different treatments to the beverage (centrifugation, pH adjustment and fermentation).

Table 6. Theoretical protein content and E% from protein in 6, 8, 10 and 12% bean beverages. The measured protein values are presented ± standard deviation based on two measurements.

	Flour (%)	**6**	**8**	**10**	**12**
Theoretical	Energy/100g (kJ)	130.5	155.5	180.5	205.5
	Protein in beverage (%)	1.3	1.7	2.1	2.5
	E% from protein	16.1	18.0	19.4	20.5

Table 7. Measured DM and protein values (per DM and in beverage) in beverages with 6, 8, 10 and 12% bean flour. The measured protein values are presented± standard deviation based on two measurements

	Flour (%)	**6**	**8**	**10**	**12**
Measured	DM (%)	5.1 ± 0.8	7.7 ± 0.3	8.4 ± 0.8	10.8 ± 0.1
	Protein per DM	22.8 ± 0.2	22.6 ± 0.5	23.0 ± 0.3	22.9 ± 0.3
	Protein in beverage (%)	1.2 ± 0.2	1.7 ± 0.02	1.9 ± 0.2	2.5 ± 0.06

Table 8. Recipe for the basic formulation of a bean beverage.

Ingredient	**Amount (%, weight/weight)**
Bean flour	8
Rapeseed oil	1.5
Water	90.5

The bean flour concentration of 8% was chosen because this would theoretically allow the beverage to be labelled as “source of protein”. Since the theoretical protein content in the beverage with 8% bean flour was the same as the measured protein content (1.7%), the real E% was expected to be similar. A beverage with 6% of bean flour would also allow this labelling, but it was assessed that 8% would give a better overall nutritional profile, even though this gave a more viscous and sandy beverage. Another factor was that it would be better to have a more concentrated bean beverage when applying centrifugation, since this would increase the amount of nutrients in the supernatant.

When the protein content of beverages with 6-12% bean flour was measured, the obtained values were all close to the theoretical values or slightly lower (6 and 10%). The standard deviations for the 6% and 10% samples’ DM measurements were also larger than the other two (0.8% compared to 0.3% and 0.1 %), so a larger number of replicates would likely have given a smaller standard deviation and results closer to the theoretical protein content. The deviations can be due to that the beverages were not totally homogenous when the sample was taken, giving variations in DM. Also, some water or bean flour can be lost during the process, because the samples are transferred between different pots, beakers and containers several times. This would give variations in DM.

5.3 Fermentation

5.3.1 Autoclaving

Some obstacles were met when fermenting, in the form of contamination. During the initial trials, a glass bottle containing the beverage was heated in a boiling water bath for 20 minutes before fermentation to pasteurize the beverage. The beverage was inoculated with *L. plantarum* 299v and incubated anaerobically at 37°C. After three days, the beverage was contaminated with an unknown microorganism. It exerted a foul smell and grey and purple clusters had been formed in the beverage. A similar effect had been observed when the boiled beans were dried; if they were dried at 55°C instead of 75°C, sometimes contamination of an unknown microorganism occurred. This microorganism could possibly be some form of *Bacillus*. *Bacillus* spores could have survived heat treatment prior fermentation and then had become activated at 37°C and 55°C. This has not been confirmed with microbiological tests however but is a likely theory. Because of this, autoclaving of the beverage before fermentation was determined to be necessary. An autoclaving trial was performed in order to find out which temperature and time was needed to inactivate all microorganisms that was found naturally in the beans or that could have contaminated the beverage during the preparation steps. The result from the trial showed that the beverage had to be autoclaved for at least 15 minutes at 121°C for no growth to occur on TSA.

5.3.2 Growth of *L. plantarum* 299v during fermentation

The growth of *L. plantarum* 299v in the bean beverage during fermentation was determined by detection of Colony Forming Units (CFU) of *L. plantarum* 299v on Rogosa Agar and the total bacterial growth on TSA, on day 0 (before inoculation), day 3 and day 7. pH was also measured at the sampling occasions. The growth curves on Rogosa Agar and TSA together with the change in pH can be seen in Figures 12 and 13 respectively. No growth could be seen on day 0 on Rogosa Agar and TSA, as well as on Malt Agar and VRBD Agar (data not shown), meaning that the autoclaved beverages contained less than 1 CFU/ml of living *Lactobacilli*, *Enterobacteriaceae*, mold or yeast before inoculation. In both figure 11 and 12, it can be seen that the maximum concentration of bacteria was reached on day 3, which is on average $1.6 \cdot 10^7$ CFU/ml on Rogosa Agar and $0.9 \cdot 10^7$ CFU/ml on TSA. The pH decreases from 6.5 (±0.11) on day 0 to 4.7 (±0.01) on day 7. This indicates that there are probably-no other bacteria than *L. plantarum* 299v growing in the beverage. The slightly higher maximum concentration on Rogosa Agar appears reasonable since this agar is more selective towards *Lactobacilli*.

The growth seems to be constant or decrease slightly after day 3 and the pH does not change significantly. Therefore, fermentation of the bean beverage with *L. plantarum* 299v for longer than three days does not seem to be necessary in this setting.

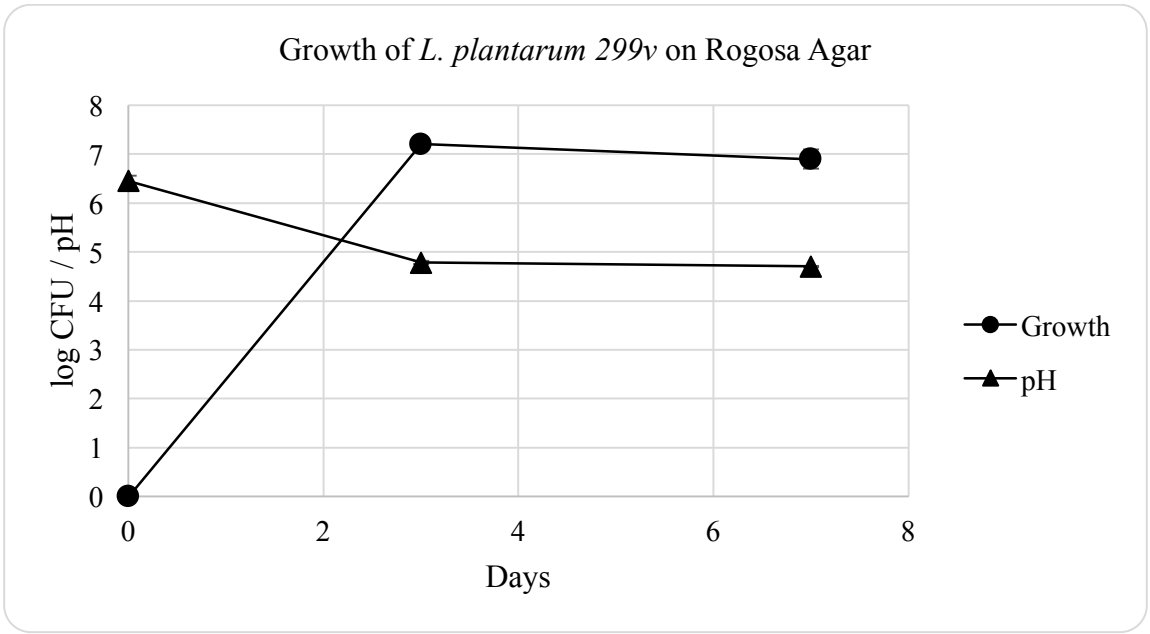

Figure 12. Growth curve of L. plantarum 299v in the bean beverage as log CFU detected on Rogosa Agar on day 0, 3 and 7, marked as line with dots and pH curve marked as line with triangle. Error bars for the growth curve points represent the standard deviations based on two replicates (two separate fermentation occasions)

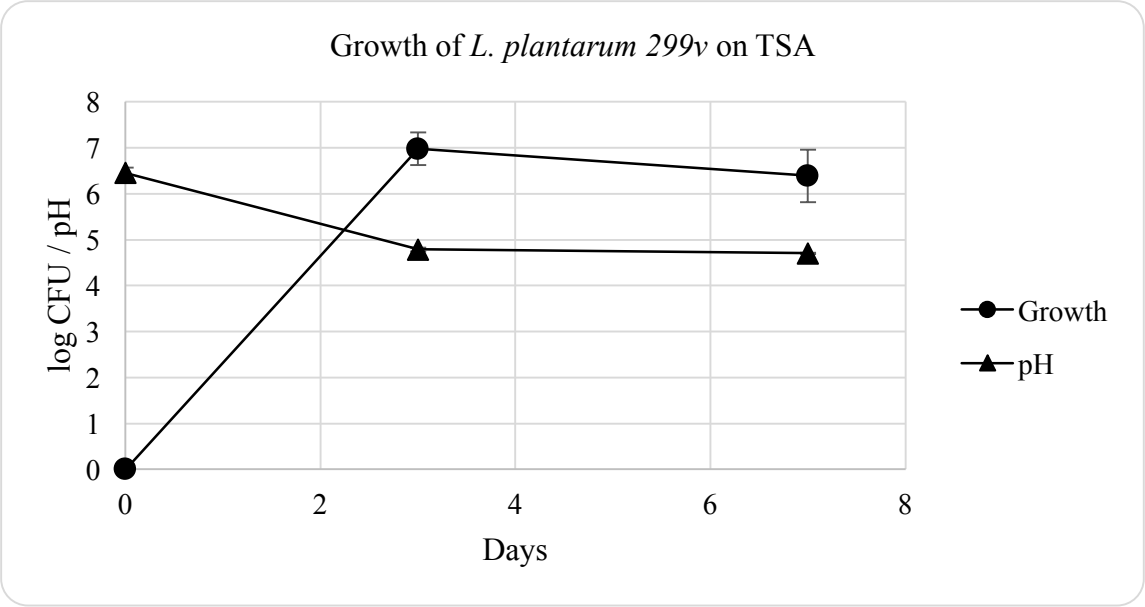

Figure 13. Growth curve of L. plantarum 299v in the bean beverage as log CFU detected on TSA on day 0, 3 and 7, marked as line with dots and pH curve marked as line with triangle. Error bars for the growth curve points represent the standard deviations based on two replicates (two separate fermentation occasions)

5.3.3 Rheology

The results of the rheological measurements made on the fermented (and autoclaved), autoclaved, and non-autoclaved samples can be seen below in Figure 14, presented in the form of viscosity as a function of shear rate. It is visible in the figure that autoclaving has an increasing effect on the viscosity of the sample, while fermentation does not seem to affect the viscosity significantly. The flow behavior index (n) and flow consistency index (k) also confirms this (Table 9), showing that $n<1$ and decreases after fermentation. This means that the samples have an increased shear thinning behavior. Another sign of the increase in viscosity by autoclaving is that the "initial viscosity", represented by the k-value, increases with autoclaving and fermentation.

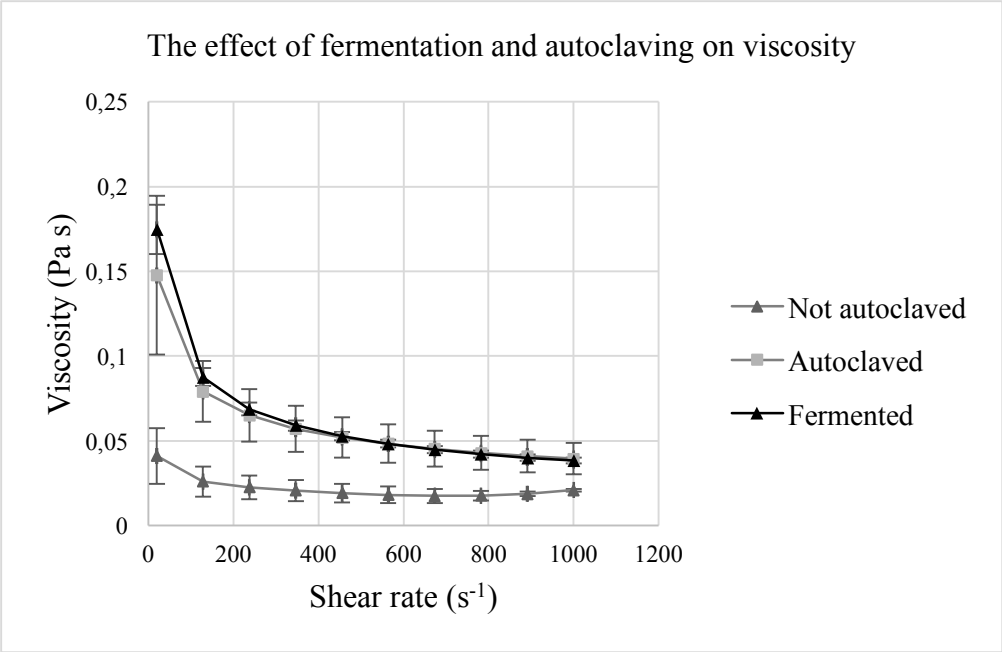

Figure 54. Viscosity curve for samples before and after autoclaving, and after fermentation.

Table 9. Power law index and flow consistency index before and after autoclaving and after fermentation.

	Not autoclaved	**Autoclaved**	**Fermented (and autoclaved)**
Flow behavior index (n)	0.81	0.67	0.60
Flow consistency index (k) (Pa s^n)	0.06	0.38	0.62

According to Du, Jian et al. (2014) the pasting temperature for starch is around 78°C, meaning that the peak viscosity of the starch should be reached at lower temperatures. The increase in viscosity after autoclaving is therefore most likely due to a change in the dietary fibers at 121°C. A theory could be that the dietary fibers take up more water at this temperature and swell causing an increase in viscosity.

Replacing the autoclaving step with pasteurization could potentially be a way of avoiding the increase in viscosity and to prevent potential chemical and flavor changes in the beverage. Ultra high temperature (UHT) pasteurization would be necessary in order to inactivate bacterial spores, which pose a risk for the success of the fermentation and for the safety of the product. UHT treatment is usually around 138°C but for a shorter time than 20 min (2 s), so it is a less harsh heat treatment than autoclaving (Cornell University 2007). Another method could be to dry the boiled beans at a high temperature, enough to inactivate bacterial spores. It would be both energy- and time efficient to eliminate the autoclaving step.

That fact that the fermentation did not have a measurable decreasing effect on viscosity could partially be explained by the inoculation volume being too small, i.e. that there were not enough bacteria to hydrolyze all starch and fibers in order to obtain a lower viscosity. Another probable explanation could be that the 299v strain of *L. plantarum* does not produce amylases that hydrolyze the starch and does not produce the right enzymes to break down fibers, since it seems to be both the starch and the

fibers that contribute to the viscosity of the product. Even if the bacteria produce amylases, the absence of the enzymes breaking down fibers would still mean that the fibers are intact and might contribute more to the viscosity than the starch and therefore no change in viscosity would be seen.

The fact that the sandiness did not decrease can be explained by the decrease in pH. The proteins are least soluble at pH 4-6, and after both day 3 and 7 during the fermentation the pH was below 5. This means that the proteins might form aggregates which can have an effect on the consistency, making it less smooth and containing more distinguishable particles. The low pH may also have decreased the WHC of the fibers. As the sandy effect of the fibers decreases with alkali pH, the opposite may be true for acidic pH.

5.3.4 Sensorial properties

An internal sensorial evaluation of the sandiness, consistency and taste of the fermented bean beverage was performed. The comments were that the taste was distinctly sour, the beverage had a thicker consistency than the control beverage and no difference in sandiness could be distinguished compared to the control beverage. The odor was similar to that of sourdough.

The fermented beverage was unfortunately contaminated prior to the sensory analysis and could not be included. A proper sensory evaluation of the taste, sandiness and general liking on the fermented beverage would be interesting in the future in order to see the consumers' opinion of this new type of fermented beverage.

5.4 pH adjustment

5.4.1 Sensorial properties

The taste, consistency and sandiness of the pH-adjusted samples with and without centrifugation were evaluated in the internal sensorial analysis. Without centrifugation, a disctinct decreased sensation of sandiness was observed after treatment with sodium bicarbonate, compared to the control samples (neutral pH). This effect was tested further in a triangle test described in section 5.6.2. Additionally, the samples with pH 8 and higher had a quite unappealing flavor as a side effect of the added sodium bicarbonate. Some samples tasted bitter and others were soapy. The samples where pH had been adjusted back to 6.8 had a better flavor but were still affected by the added sodium bicarbonate.

The centrifuged samples, when mixed with oil, obtained a more white color than the control beverage as mentioned earlier (section 5.1.3). No sandiness was detected and the beverage had a thin and smooth consistency, somewhat similar to that of cow's milk. It had less of a typical "beany" taste than the control beverage (that had not been centrifuged).

A disadvantage with this treatment was that the addition of sodium bicarbonate affected the flavor of the beverages negatively. Using other food grade alkali chemicals which affect flavor less, such as potassium bicarbonate and phosphoric acid, would solve this issue.

5.4.2 Processing

After pH adjustment, the foam formation during mixing with Ultra-turrax was much larger in volume and had a stiffer consistency. Another observed effect during processing was that at higher pH, the beans became soft quickly and started to dissolve during boiling compared to the original state, giving a yellow or green color to the boiling water. There was also more foam formation during boiling of the beans compared to the original state of the beans.

An explanation of the increased foam formation at higher pH could be that the polysaccharides that exists in the water film become more charged and stabilize the air bubbles together with the proteins. The polysaccharides bind more water and increase in volume, increasing the viscosity of the liquid phase, preventing rapid drainage of the foam and increasing the foam stability. (Dickinson 1992)

The observation that the beans started to dissolve while cooking at higher pH, might indicate that there was a greater loss of nutrients into the boiling water compared to boiling at neutral pH. Perhaps using the boiling water instead of new tap water when formulating the beverage would reduce the nutrient loss. However, this could also lead to a higher level of antinutrients and RFO in the beverage, since these will have leached into the water. Another solution could be to shorten the boiling time of the beans after alkali treatment.

5.4.3 Protein analysis

Figure 15 shows the fraction of the protein in the bean beverage which is retained in the supernatant after centrifugation, at different pH. One can see that the percentage of retained proteins increases with increased pH, and seems to decrease slightly after the pH has been adjusted back to 6.8, before centrifugation. A pH of 9.8 gave the maximum increase in retained proteins, from around 20% (at pH 7) to around 69%. This could be because the proteins are least soluble at pH 4-6 and increase in solubility at higher pH. Adjusting pH back to 6.8 did not seem to affect the amount of protein being retained in the supernatant, although the percentage decreased from 69% to 50%. In the final centrifuged bean beverage, 50% retained proteins correlates to a protein content of around 0.9g/100g beverage.

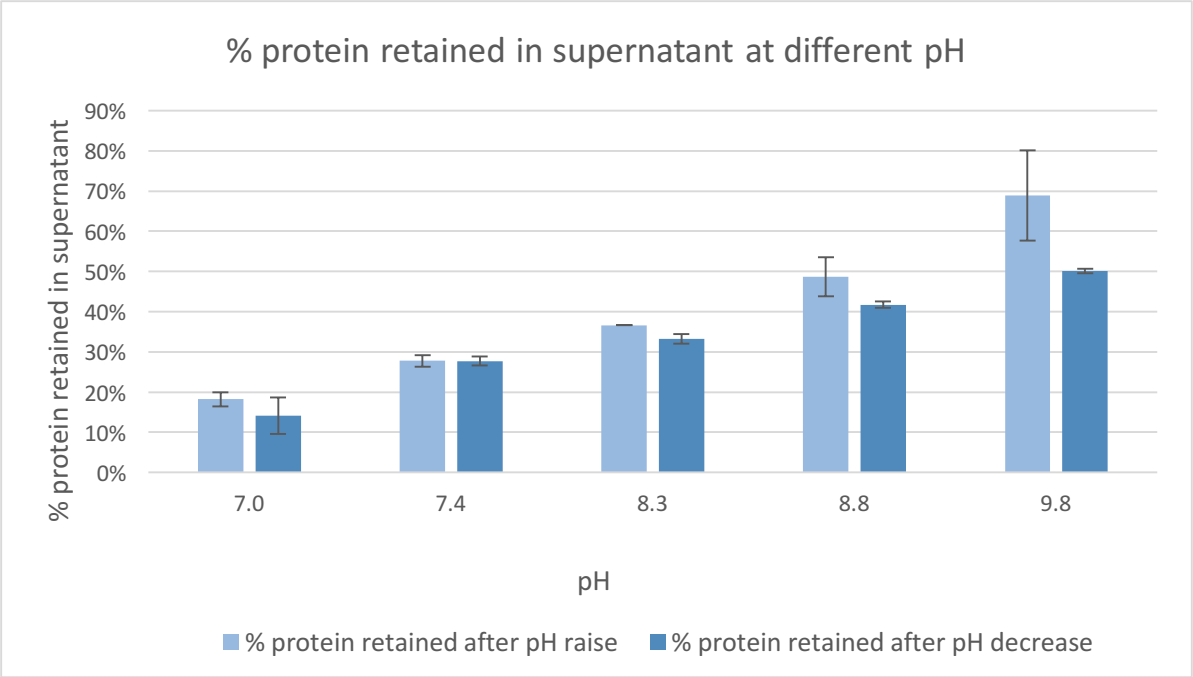

Figure 15. % protein retained in the supernatant (after centrifugation) at different pH. The left bars respresent the % protein retained in the beverage with increased pH, the right bars represent the % protein retained after pH has been adjusted back to 6.8-6.9. The error bars represent the standard deviation based on two measurements.

5.4.4 Centrifugation yield

Centrifugation of samples at 1000G for 20 min gave an average yield (supernatant mass/total mass) of 51.8 ± 4.9%. The method of centrifugation was efficient in eliminating all insoluble particles in the beverage. However, the yield of supernatant to total sample was quite low. Approximately half of the mass of the beverage was "lost" in the form of insoluble fibers, starch etc. This means that this method brings a large loss of raw material, as well as nutritional components. Other methods of separation should be investigated, such as different forms of filtration. The sediment formed during centrifugation was a smooth mass, probably containing mostly fibers and starch.

5.5 RFO analysis

The results from the RFO analyses performed in this study with the assay kit from Megazyme is not included in the report due to large standard deviations in all analysis occasions, even for the control soy bean flour that came with the kit. There was a systematic increase in the RFO content for each analysis occasion. In other words, the method showed low reproducibilty. The reason for the non-reproducible results could not be found. It is important to mention that the method is not trivial and more time and effort is needed in order to understand and optimize this specific analytical method.

5.6 Sensory analysis

5.6.1 Hedonic evaluation of general liking and sandiness

ANOVA was run on the data from the hedonic evaluation of the following 4 samples in general liking and in sandiness respectively:

1. Control sample (8% bean flour beverage)
2. Sample treated with sodium bicarbonate (pH was 7.5 before being lowered to 6.8)
3. Centrifuged version of sample 1
4. Centrifuged version of sample 2

The results from the ANOVA in the evaluation of general liking showed that there was no significant difference between any of the four samples on a 0.05% significance level. Even when the raw data was centered using the mean values of the evaluators and further normalized based on the standard deviation of the evaluators, no significance could be found.
However, when the panelists had to pick one of the samples that they preferred the most, 46.2% of the panelists preferred sample 3, 23.1% preferred sample 4, 15.4% preferred sample 1 and 15.4% preferred sample 2. From these numbers it is hard to draw any conclusions about any sample being preferred over the others since, 46.2% is not a convincing number. Still, a trend towards higher degree of liking of the centrifuged samples than the non-centrifuged samples could be seen.

The scores in general liking varied a lot between the panelists, which could be explained by individual experiences and preferences generally for plant milks and that many participants were automatically comparing it too much with cow's milk. Since the intention was not to imitate cow's milk but rather to develop a new type of beverage, this comparison could make the panelists give lower scores to the beverage. The non-centrifuged samples could probably be more appreciated if used as a base for example smoothies, where the thickness would be more suitable and the beany flavor more masked.

The results from the ANOVA of the hedonic evaluation of the sandiness on the same four samples, showed a significant difference between the samples. In order to find out which samples that were significantly different to each other, a Tukey test was performed. The differences in mean values between all samples were calculated and compared with the calculated w-value of 2.0066. The difference in mean values for the compared pair is significant if the difference is higher than the calculated w-value. The summarized result is shown in Table 10, and the data from the ANOVA and Tukey test can be seen in Appendix 2 - Sensory analysis. It can be seen that there were significant differences in the sandiness between all samples except between sample 1 and 2 and between sample 3 and 4. In other words, the panelists perceived a higher degree of sandiness in the non-centrifuged samples compared to the centrifuged samples but they were not able to distinguish any difference in the

sandiness between the two non-centrifuged samples (control and pH adjusted) and between the two centrifuged samples (control and pH adjusted).

Table 10. Result from Tukey test, where the differences in mean values between all sample pairs is shown. The a indicates a non-significant difference and b indicates a significant difference.

Sample pair compared	Difference in mean values
1-2	1.214 (a)
1-3	3.929 (b)
1-4	5.857 (b)
2-3	2.714 (b)
2-4	2.786 (b)
3-4	0.071 (a)

By increasing the pH of the beverages it was hypothesized that the dietary fibers' solubility and WHC would increase. This due to a change in hydrophobicity of the functional groups of the fibers, meaning that more of the fibers would be soluble and thereby giving a reduced sensation of sandiness or chalkiness. However, the results from the hedonic evaluation of the sandiness showed that there was no significant difference in perceived sandiness between samples treated and not treated with sodium bicarbonate. This could be due to that the pH was raised only from 6.8 to ca 7.5. Perhaps this raise was not enough for all panelists to distinguish the change in sandiness. Although, there was a significant difference in sandiness between the non-centrifuged samples and the centrifuged samples, which are in accordance with the hypothesis about the sandiness being lower when the insoluble fraction is removed, meaning that it is the insoluble dietary fibers and starch that gives the sandy texture.

5.6.2 Triangle test

5.6.2.1 Statistical analysis

There were 18 correct answers out of 28 in the triangle test, i.e. 64% of the 28 observations were correct. The probability of each independent observation being correct due to chance in a triangle test is $p = \frac{1}{3}$. Therefore, the expected number of correct choices is in this case $E_1 = N * p = \frac{28}{3}$, N being the number of participants. The probability of each independant observation being incorrect is $p = \frac{2}{3}$, so $E_1 = N * p = \frac{28*2}{3}$. Using these values, and $O_1 = 18,\ O_2 = 10$ gives:

$$x^2 = \frac{(|O_1 - E_1| - 0.5)^2}{E_1} + \frac{(|O_1 - E_1| - 0.5)^2}{E_1} = \frac{(\left|18 - \frac{28}{3}\right| - 0.5)^2}{\frac{28}{3}} + \frac{(\left|10 - \frac{28*2}{3}\right| - 0.5)^2}{\frac{28*2}{3}} = 10.71$$

where df=1 and α=0.05, the critical x^2= 3.84 according to a x^2-table.

Since 10.7 > 3.84, the null hypothesis can be rejected, meaning that there is a significant difference between the samples with a 95% degree of confidence. This result is an indication that a raise in pH decreases the consumers' perception of the beverage as sandy. A further increase in pH might have a clearer effect. However, since a greater increase in pH would also affect the flavor of the beverages, the panelists might have distinguished a difference in flavor instead of in texture.

5.6.2.2 Comments from panelists

The comments obtained from panelists during the triangle test can be seen in Appendix 2 - Sensory analysis. A majority of panelists who answered correctly commented on the sandiness/mouthfeel/texture of the sample. Some panelists however commented on the flavor (more/less bitter) and viscosity. It seems as if there was an actual difference in bitterness between the samples with and without adjusted pH as a side effect of the use of sodium bicarbonate. Those who did not answer correctly commented on things such as viscosity, flavor, oiliness and temperature. Others commented on the graininess/texture but did not answer correctly despite this. Some samples may have been slightly different in temperature. This could also have affected the results and could thereby be a source of error in this method. Additionally, a higher number of panelists would have given a more statistically secure result in both the hedonic and triangle test, but this was not possible in this case due to time constraints and lack of participants. This, as well as a more specific question in the triangle test, would probably have given a higher level of significance.

5.7 External analyses of nutritional content

Samples were sent to Eurofins for external laboratory analysis of nutritional content, see Appendix 4 - External analysis for detailed content data. The samples sent for analysis were the same four samples that were used in the sensory analysis and also one sample of the fermented beverage. In Table 11, selected data of the nutritional content can be seen for the five samples that were analyzed. Raffinose and stachyose was measured only in the fermented, centrifuged and control sample, in order to se the change in RFO in fermented samples.

It can be seen that the amount of energy is lower in the centrifuged samples which is logical since less carbohydrates remain in the liquid supernatant after centrifugation.The amount of fat is close to the theoretical value of 1.7 g/100 g in all samples except sample 3 and 5. The lower value in sample 3 is probably due to a mistake in weighing the amount of rapeseed oil. The same reason could explain the low value in sample 5 but it could also be explained by degradation of fat molecules in the fermentation process.

The amount of dietary fiber is the same for sample 1 and 2 and lower (not detectable) in sample 3, 4 and 5 as expected. In sample 3 and 4, most of the dietary fibers were expected to be lost in the insoluble bottom phase, which coincides with the results.

The decrease in dietary fiber content however, does not coincide with the results in section 5.3.3, where it seemed as though the dietary fibers in the beverage increased viscosity after autoclaving and there was no change in sandiness. According to Rosenthal, Deliza et al. (2003), it is the insoluble particles that contribute to the sandiness. Boye, Zare et al. (2010) also confirms that it is the starch and fibers that contribute to the high viscosity. It is possible that some dietary fibers were degraded by *L. plantarum* 299v, and the remaining dietary fibers had a great impact on viscosity. It is also possible that the sample sent for analysis was not homogenous and not representable for the fermented beverage, so the results presented was not the real dietary fiber content.

As mentioned in the literature study, Espirito-Santo, Mouquet-Rivier et al. (2014) means that some strains of *L. plantarum* are amylolytic. Therefore, it seems as though the 299v strain used in this type of fermentation is not amylolytic and the starch remains intact in the beverage which is confirmed by the results from this analysis, and which is also suggested in section 5.3.3.

The raffinose seem to be undetectable in all samples (<0.04 g/ 100 g). The amount of stachyose is 0.07 g/100 g in sample 1 and 2 and 0.05 g/100 g in sample 5. The stachyose values indicate that there is less stachyose in the fermented sample (sample 5) than in the control sample (sample 1), which could be either because of a degradation of stachyose by bacteria, because of natural variation of the stachyose concentration in different parts of the samples or because of measurement error. Although, the difference of 0.02 g is too low to be able to draw any conclusion about the effect of fermentation on RFO content.

0.07 g/100 g stachyose in sample 1 is equal to 0.88 g/100 g bean flour and 0.05 g/100 g stachyose in sample 2 is equal to 0.63 g/100 g bean flour. The values of stachyose and raffinose from this analysis are lower than the contents in whole common beans found in the literature (stachyose: 2.74-3.29%, raffinose: 0.45-0.46% (Sahasrabudhe, Quinn et al. 1981). This is an indication of a decreased RFO content after treatments such as soaking and boiling, but cannot be confirmed since analaysis of RFO content in the dry beans from Lantmännen Cerealia used in this study has not been done.

The protein content of sample 1 and 2 were expected to be similar since no part of the beverage has been removed and solubilization of protein does not affect the total amount of protein analyzed. In the internal analysis of protein, samples 1 and 2 contained approximately 1.8 and 1.7 g/100 g protein, respectively. The external analysis of samples 1 and 2 gave 0.2 g/100 g higher and 0.2 g/100 g lower results, respectively. The differences between samples 1 and 2 in both internal and external analysis could be explained by the samples not being optimally homogenized before the analysis. The difference in the amounts between analysis methods for each sample is probably due to the different analysis methods used (although conversion factor of 6.25 is used in both analysis). However, there should have been a systematic difference in the values, meaning that the values should have been either higher or lower to the same extent. For sample 3 and 4 it is obvious that treatment of sodium bicarbonate increases the solubility of the protein and thereby also the protein content.

In the last row of Table 10, the calculated E% from protein is presented, where it can be seen that sample 1, 4 and 5 has values over 12 E% and can therefore be labeled as “source of protein”. In the

internal analysis of protein, sample 2 does also have a E% over 12, which is expected. It can be seen that E% from protein in sample 4 is enough to reach the desired level of E% compared to sample 3, that has not been treated with sodium bicarbonate. This indicates that alkali treatment before centirfugation is needed in order to reach the desired protein content in the final beverage.

The higher values of salt in sample 2 and 3 are expected since it is the sodium that is measured, which is added in the form of sodium bicarbonate in these samples to raise the pH.

*Table 11. Selected data from the external analysis of nutritional content of 5 samples (1=control, 2=pH-adjusted, 3=centrifuged control, 4=pH-adjusted centrifuged, 5=fermented). *values are calculated and not measured. NA=not analyzed. E% in the last row is calculated afterhand.*

Nutritional value/100 g	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
Energy*	166 kJ/40 kcal	172 kJ/41 kcal	58 kJ/14 kcal	85 kJ/20 kcal	135 kJ/32 kcal
Fat (g)	1.66	1.63	1.23	1.67	1.02
Carbohydrates* (g)	3.37	4.25	0.75	0.59	4.22
Dietary fiber (g)	1.8	1.8	<1.0	<1.0	<1.0
Raffinose (g)	<0.04	NA	<0.04	NA	<0.04
Stachyose (g)	0.07	NA	0.07	NA	0.05
Protein (g)	1.96	1.46	<0.3	0.77	1.48
Salt (g)	<<0.02	0.024	0.011	0.031	<<0.02
E%, protein	19.7	14.2	<8.6	15.1	18.3

6.0 Conclusions

Based on the results and discussion presented in this report, some conclusions can be drawn considering the beforehand established goals. First of all, it can be concluded that three different types of bean beverages have successfully been developed (with variations in the treatments of each beverage); a bean beverage made out of the flour of whole beans (1), a bean beverage excluding the insoluble fraction (2) and a fermented, probiotic bean beverage (3). The main conclusions regarding the goals in 4.0 Objectives, are the following:

- All three beverages did not separate within 10 minutes when the mixing time was 1 hour. The E% from protein in all three beverages is above 12 and can thereby be labeled as “source of protein”. Although, this is only obtained in beverage 2 when using alkali pre-treatment.

- Fermentation of the bean beverage with the probiotic bacteria *L. plantarum* 299v was successful in terms of the bacteria multiplying in the beverage, using carbohydrates to produce acids that lowers the pH in the beverage and other substances giving a distinct acidic taste and characteristic flavor.

The beverage (3) has a shear thinning behavior and a sandy texture. No difference in sandiness could be perceived before and after fermentation and the viscosity did not decrease after fermentation. The most probable explanation for this is that the specific bacterial strain is not able to produce amylase, which is necessary to hydrolyze starch, and that the strain does not produce the necessary enzymes needed to break down the insoluble fibers. The results from both internal and external RFO analysis of the fermented samples were inconclusive.

- Centrifugation was a successful method of eliminating insoluble particles and decreasing the sandy texture of the beverage, which was confirmed by the sensorial analysis. The method can be used for making a milk-like bean beverage (2). Results from the rheology and sensorial analyses showed that the bean beverage (1) has a shear thinning behavior and a sandy texture, when the insoluble fraction remains in the beverage.

- An indication could be seen in the triangle test that the panelists could perceive a difference in sandiness between the control bean beverage (1) and the pH-adjusted version of this bean beverage, meaning that increasing the pH in the beverage followed by decreasing the pH to the original value, decreases the sandy texture. By increasing the pH to alkaline pH, the WHC of the dietary fibers increases and the charges on the surface of the polysaccharide molecules change, leading to higher solubility and thereby reduced sandy texture. The increase in pH to alkaline pH also increases the solubility of the proteins, leading to a higher protein content in the pH-adjusted bean beverage (2).

- Overall, it was concluded that there is great potential to develop a milk-like or smoothie-type beverage based on white beans, using the studied methods of adjusting pH, fermentation and/or separation of soluble and insoluble solids.

7.0 Future recommendations

7.1 Process formulation

In order to increase time and energy efficiency of the process, one could further study the optimum soaking-, boiling-, and heating time. The effect of soaking time and temperature on the tannins and phytic acid should be studied in order to find out if the soaking time could either be removed completely or reduced. In a similar manner, the effect of heat treatment of the flour and water slurry should be investigated in respect to the inactivation of anti-nutritional enzymes such as lectins. If a short heat treatment of the slurry is enough to inactivate these enzymes, the boiling step of the soaked beans could be eliminated, which would save time and energy.

A hammer mill could be used for grinding the beans to possibly obtain a finer flour, alternatively a colloidal mill for wet milling the beans, as is done in soymilk production. Additionally, the formation of foam during mixing could lead to processing problems, meaning that studies on how to minimize the foam formation is necessary to conduct. This could be done by adding a defoaming agent or running the process under vacuum.

7.2 Challenges with sandiness and viscosity

The sandy texture seems to be a question of particle size of the insoluble fractions, so a more finely milled flour could be one way of improving the texture. Homogenization at different pressures and temperature can be performed or different types of filtration such as vacuum filtration or membrane filtration, in order to study its effect on sandiness and viscosity. Applying enzymatic treatment of the insoluble fibers is also possible, where one can use enzymes that break down cellulose, hemicellulose, pectin and lignin.

In this study, trials with enzymatic treatment of the starch with α- and β-amylases were performed without any conclusive results on how the viscosity, sandiness and taste were affected and was therefore not included in the report. Hydrolyzing the starch with amylases, as is done in the production of oat milk, was hypothesized to result in a less viscous and more sweet beverage, since the starch is hydrolyzed into maltodextrins, glucose and maltose. Another way to reduce the viscosity more is obviously by decreasing the concentration of bean flour to 6% or even lower, which would still allow the ”source of protein” labelling.

7.3 More variations and other products

During this project, more ideas of how to vary the basic bean beverage have arisen, as well as ways to utilise the by-products formed during processing.

The bean beverage has a distinct flavor which seem to be either much accepted or not accepted by the consumers. Elaborating with masking the beany flavor by adding for example fruits, berries, chocolate or vanilla, preferably in their natural form to keep the healthy profile of the product, or as artificial flavors, could improve flavor and consumer acceptance of the beverage. An example of a product is a smoothie made out of the pH-adjusted bean beverage, banana, raspberries, blueberries and lemon. According to an internal sensorial evaluation, this type of smoothie had a barely noticable sandiness and had fruity, sweet and sour flavors.

Other types of oils can be tested such as coconut oil. Although, the use of a locally produced oil with a fairly neutral flavor was prioritized in this project.

Using sodium bicarbonate to raise pH in the beverage seemed to affect the flavour negatively. Therefore, it is of great importance to find other food grade alkaline chemicals for the adjustment of pH that does not contribute to any unwanted sensations affecting taste and mouthfeel. Such chemicals could

be phoshoric acid or potassium bicarbonate, as mentioned earlier. Adding naturally acidic fruits or berries could be a way of decreasing pH after it has been raised without having to use extra chemicals.

A great potential can be seen in further development of a fermented bean beverage since fermentation can improve the nutritional content and could serve as an alternative to yoghurt made out of cow's milk. In the future one could search for a more suitable bacterial strain with the ability of metabolizing for example dietary fibers, starch and oligosaccharides and lowering the pH enough for coagulation of proteins to occur and to increase the shelf-life. It could also be of interest to study the outcome of fermenting the bean beverage with yogurt culture (for example with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) to see if it is possible to create a more yogurt-like product in regard to the texture. Flavoring of the fermented beverage could also be explored.

It was also observed that the centrifugation of the beverage resulted in a thick sediment that had a smooth consistency and a mild flavor. This by-product could possibly be used as a base for production of a bean spread or dip. Another alternative could be to ferment this sediment or a paste of whole mashed beans in order to obtain a spread or dip.

During mixing of the bean beverage there was a large extent of foam formation. At higher pH this formation was even greater and the foam was more firm. If a defoaming agent is not used, the foam could be of use in other applications such as production of vegetable meringue.

7.4 Shelf-life

Studying the shelf life of a bean beverage will be of great importance if this product is further developed in the future. A thorough study of the potential pathogenic and spoilage microorganisms in the bean beverage, and necessary heat treatment to eliminate them, should be conducted. If a smoothie-type beverage is developed, the addition of acidic fruits could improve shelf life, since low pH is an inhospitable environment for most microorganisms.

7.5 RFO analysis

The results from the RFO analysis using the Megazyme assay kit were not reproducible. Therefore, more resources are needed to optimize this method of analysis or to use other methods such as HPLC-based analyses. Another possible treatment of the beverage to reduce the amount of RFO could be to use pure alpha-galactosidase that could break down the RFO into simple sugars.

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Appendix 1 - Fermentation

Standard protocol for microbiological cultivation

1. Inoculation of the frozen bacterial sample with a 1µm loop to 5 ml of MRS medium
2. Incubation aerobically at 37°C for 24 h
3. Centrifugation of the cells at 6000 rpm for 5 minutes at 4°C
4. Discarding the supernatant
5. Addition of 10 ml 0.85% NaCl to the cell pellets and vortex
6. Centrifugation of the cells at 6000 rpm for 5 minutes
7. Addition of 2 ml 0.085% NaCl to the cell pellet and vortex
8. Dilution of the sample with 0.085% NaCl to 1:2, 1:4, 1:8 and 1:16 dilutions
9. Dilution of the sample (10^{-1} to 10^{-11}) with 1 g/L bacteriological peptone and 8.5 g/L NaCl for spreading on agar plates
10. Measuring of the absorbance of the 1:2, 1:4, 1:8 and 1:16 dilutions at 620 nm
11. Spreading of the dilutions (10^{-1} to 10^{-11}) on MRS agar plates (0.1 ml per plate, duplicate plates) with glass beads
12. Assessing a correlation between absorbance and growth from where inoculum concentration and volume could be calculated

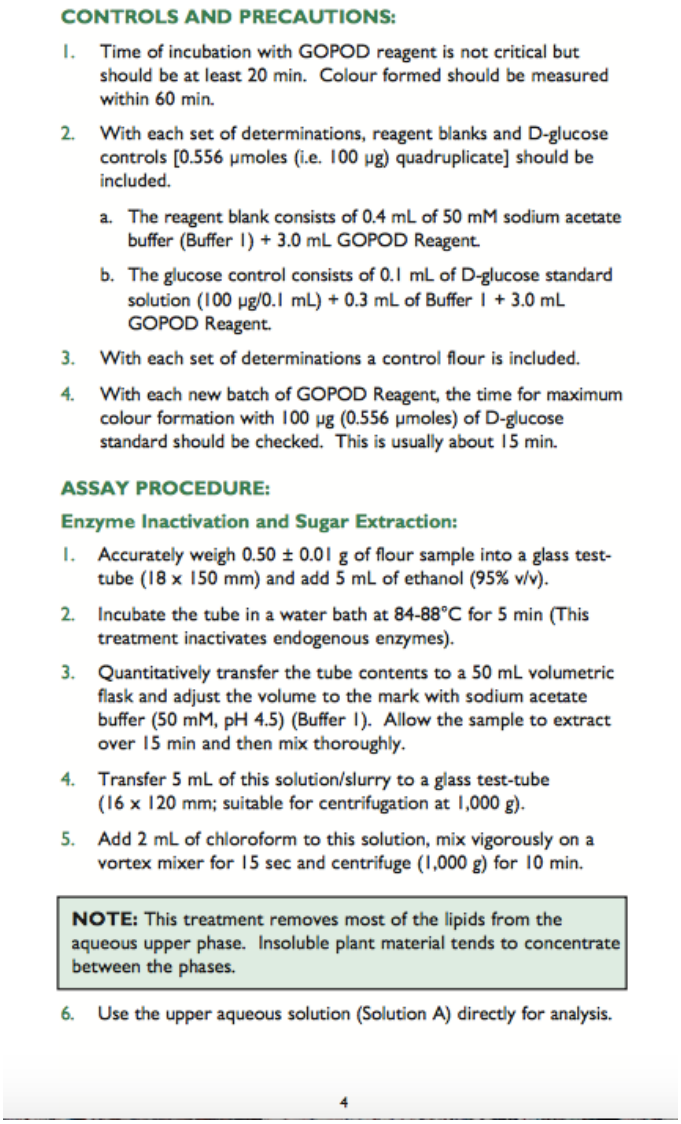
The dilutions used for spreading the sample onto the different agars as well as the incubation conditions can be seen in Table 1.

Table 12. Dilutions used for spreading on the different agar plates on day 0, 3 and 7 for L. plantarum 299v.

Agar	Day 0	Day 3	Day 7	Incubation conditions
Malt	0	0, -1	0, -1	Room temp. 7 days
VRBD	0	0, -1	0, -1	37°C 24 h
TSA	0	0, -1	-3, -4, -5	30°C 3 days
Rogosa	0	-5, -6, -7	-4, -5, -6, -7	37°C 3 days Anaerobically

Appendix 2 - RFO analysis

The assay procedure (selected pages from the instruction manual) for analysis of RFO (Megazyme assay kit) can be seen in Figures 1, 2 and 3 (instructions are read in chronological order).



CONTROLS AND PRECAUTIONS:

1. Time of incubation with GOPOD reagent is not critical but should be at least 20 min. Colour formed should be measured within 60 min.
2. With each set of determinations, reagent blanks and D-glucose controls [0.556 µmoles (i.e. 100 µg) quadruplicate] should be included.
   a. The reagent blank consists of 0.4 mL of 50 mM sodium acetate buffer (Buffer 1) + 3.0 mL GOPOD Reagent.
   b. The glucose control consists of 0.1 mL of D-glucose standard solution (100 µg/0.1 mL) + 0.3 mL of Buffer 1 + 3.0 mL GOPOD Reagent.
3. With each set of determinations a control flour is included.
4. With each new batch of GOPOD Reagent, the time for maximum colour formation with 100 µg (0.556 µmoles) of D-glucose standard should be checked. This is usually about 15 min.

ASSAY PROCEDURE:

Enzyme Inactivation and Sugar Extraction:

1. Accurately weigh 0.50 ± 0.01 g of flour sample into a glass test-tube (18 x 150 mm) and add 5 mL of ethanol (95% v/v).
2. Incubate the tube in a water bath at 84-88°C for 5 min (This treatment inactivates endogenous enzymes).
3. Quantitatively transfer the tube contents to a 50 mL volumetric flask and adjust the volume to the mark with sodium acetate buffer (50 mM, pH 4.5) (Buffer 1). Allow the sample to extract over 15 min and then mix thoroughly.
4. Transfer 5 mL of this solution/slurry to a glass test-tube (16 x 120 mm; suitable for centrifugation at 1,000 g).
5. Add 2 mL of chloroform to this solution, mix vigorously on a vortex mixer for 15 sec and centrifuge (1,000 g) for 10 min.

NOTE: This treatment removes most of the lipids from the aqueous upper phase. Insoluble plant material tends to concentrate between the phases.

6. Use the upper aqueous solution (Solution A) directly for analysis.

4

Figure 1. The first page of the instructios for the assay procedure from the manual from Megazyme assay kit for analysis of Raffinose/Sucrose/D-Glucose.

Assay for Glucose, Sucrose and Raffinose-Series Oligosaccharides:

1. Treat 0.20 mL aliquots of Solution A as follows:
   - 0.2 mL of Soln A + 0.2 mL Buffer 1 [D-Glucose] ..... A
   - 0.2 mL of Soln A + 0.2 mL invertase [D-Glucose + Sucrose] .. B
   - 0.2 mL of Soln A + 0.2 mL α-galactosidase + invertase [D-Glucose + Sucrose + Galactosyl-sucrose oligosaccharides] .C
2. Incubate all solutions at 50°C for 20 min.
3. Add 3.0 mL of GOPOD Reagent to solutions A, B and C, as well as to the Reagent Blank and the D-glucose controls, and incubate all at 50°C for 20 min.
4. Read the absorbance of all solutions against the Reagent Blank at 510 nm:

   Absorbances: ΔA = GOPOD absorbance for A
   ΔB = GOPOD absorbance for B
   ΔC = GOPOD absorbance for C

The **reagent blank** consists of 0.4 mL of 50 mM sodium acetate buffer (Buffer 1) plus 3.0 mL of GOPOD Reagent.

The **glucose control** consists of 0.1 mL of D-glucose standard solution (100 µg/0.1 mL) plus 0.3 mL of Buffer 1 and 3.0 mL of GOPOD Reagent.

CALCULATIONS:

D-Glucose, millimoles/100 grams:

$$= \Delta A \times F \times 250 \times 200 \times \frac{1}{1000}$$

$$= \Delta A \times F \times 50$$

Sucrose, millimoles/100 grams:

$$= (\Delta B - \Delta A) \times F \times 250 \times 200 \times \frac{1}{1000}$$

$$= (\Delta B - \Delta A) \times F \times 50$$

Raffinose-series oligosaccharides (RSO), millimoles/100 grams:

$$= (\Delta C - \Delta B) \times F \times 250 \times 200 \times \frac{1}{1000}$$

$$= (\Delta C - \Delta B) \times F \times 50$$

5

Figure 2. The second page of the instructios for the assay procedure from the manual from Megazyme assay kit for analysis of Raffinose/Sucrose/D-Glucose.

where:

ΔA = GOPOD absorbance for 0.2 mL of samples + acetate buffer.

ΔB = GOPOD absorbance for 0.2 mL of samples + invertase.

ΔC = GOPOD absorbance for 0.2 mL of samples + α-galactosidase and invertase.

F = a factor to convert from absorbance to μmoles of glucose

$$= \frac{0.556 \text{ (μmoles of glucose)}}{\text{GOPOD absorbance for 0.556 μmoles of glucose}}$$

250 = conversion to 50 mL of extract (i.e. to 0.5 g of sample).

200 = conversion from 0.5 to 100 g of sample.

$\frac{1}{1000}$ = conversion from μmoles to millimoles.

The concentrations of D-glucose and sucrose can be represented as millimoles/100 g, or can simply be calculated as g/100 g of flour, as shown below. However, it is not possible to calculate galactosyl-sucrose oligosaccharides as g/100 g of flour because these oligosaccharides are a mixture of raffinose, stachyose and verbascose. If the major component of this mixture for a given seed material is known, then it is possible to use the molecular weight of this compound and calculate an approximate value in grams/100 grams of flour.

D-Glucose (g/100 g flour) = D-Glucose (millimoles)/100 g x 0.1799.

Sucrose (g/100 g flour) = Sucrose (millimoles)/100 g x 0.3425.

Galactosyl-sucrose oligosaccharides (GSO) (g/100 g flour)
= (GSO)/100 g x MW/1000.

where:

0.1799 = the MW of D-glucose (180)/1000 mg of D-glucose.

0.3425 = the MW of sucrose (342)/1000 mg of sucrose.

MW/1000 = the average MW for GSO/1000 mg of RSO.

Figure 3. The third page of the instructios for the assay procedure from the manual from Megazyme assay kit for analysis of Raffinose/Sucrose/D-Glucose.

Appendix 3 - Sensory analysis

Sensory evaluation questionnaire

The questionnaire from the sensory evaluation can be seen below.

Panelist number: ___

Sensory analysis – bean beverage

Age: ▢ 20-30
▢ 31-40
▢ 41-50
▢ 51-65
▢ 65+

Gender: ▢ Male
▢ Female

Nationality: _____

Test 1

There are 4 samples to your left at the table. Please rank each sample according to general liking on a scale of 1 to 9 (1= dislike extremely, 9 = like extremely), as well as your percieved sandinesss/graininess of the sample (1= very smooth/not sandy at all, 9 = extremely sandy)

Please rinse your mouth with water in between the samples!

Sample 385

	dislike extremely								like extremely
General liking	1	2	3	4	5	6	7	8	9

	dislike extremely								like extremely
Sandiness/ graininess	1	2	3	4	5	6	7	8	9

Sample 762

	dislike extremely								like extremely
General liking	1	2	3	4	5	6	7	8	9

	dislike extremely								like extremely
Sandiness/ graininess	1	2	3	4	5	6	7	8	9

Sample 891

	dislike extremely								like extremely
General liking	1	2	3	4	5	6	7	8	9

	dislike extremely								like extremely
Sandiness/ graininess	1	2	3	4	5	6	7	8	9

Sample 423

	dislike extremely								like extremely
General liking	1	2	3	4	5	6	7	8	9

	dislike extremely								like extremely
Sandiness/ graininess	1	2	3	4	5	6	7	8	9

Which sample did you prefer?

(Circle the sample number)

385 762 891 423

Test 2

To your right, you are presented with 2 groups of 3 samples each. Please taste samples from group 1 first and taste each sample from left to right.

Try to distinguish which one is different from the other two and answer the questions below.

Please rinse your mouth with water in between the samples!

Group 1:

Sample number _____ is different from the other two.

In what way was the sample different?

_____

_____

_____

Group 2:

Sample number _____ is different from the other two.

In what way was the sample different?

_____

_____

_____

Summary of comments from sensory evaluation

A summary of all the comments that the panlists gave in the triangle test can be seen in table 2 below.

Table 13. Summary of comments from triangle test.

	Correct responses	Incorrect responses
Sample	**Comment**	**Comment**
Treated with sodium bicarbonate	Less sandy, more oily	Lighter texture
	Less sandy - I also felt less aftertaste	Higher degree of sandiness
	Smooth mouth feel, less grainy	There is something like "fresh" or mynt or greent
	Less bitter	Dont know - warmer?
	Less bitter, lot of foam, more viscous	More viscous
	More smooth texture	More oily
	More viscous and more grainy	Thicker
	Less bitter, less solid parts, feels smoother	
	Less bean-ish aftertaste	
	Something green	
	More viscous	
Control sample	Less visous	Less graininess
	More grainy	Lighter feel
	Less viscous and less sandy	Less bitter
	More viscous	
	Minimum sandiness, less bitterness, no foam, good concistency	
	More bitter	
	More bitter, others feel smooth	

Statistical analysis

ANOVA and Tukey test

The results from the ANOVA calculations performed in Excel can be seen in tables 3, 4, 5 and 6. Tukey test was performed based on the ANOVA on the hedonic evalation of the sandiness in Table 5, since these results were the only ones that showed significant difference between the samples. In order to compare the mean values, a tukey w-value was calculated as follows:

$$w = q_{\alpha(k,df)} \cdot \sqrt{\frac{SE}{r}} = 3.765 \cdot \sqrt{\frac{3.9766}{14}} = 2.0066$$

where

$$q_\alpha = value\ obtained\ from\ a\ table\ of\ Tukey\ q - values = 3.765$$
$$df = degrees\ of\ freedom\ (within\ groups) = 52$$
$$k = number\ of\ treatment\ levels = 4$$
$$SE = standard\ error\ of\ a\ treatment\ mean$$
$$= square\ root\ of\ sum\ of\ variances\ from\ ANOVA = 3.9766$$
$$r = number\ of\ replications = 14$$

The calculated w-value was then compared to the differences of each sample pair compared. If the difference in mean values is higher than the w-value, there is a significant difference.

Table 14. Results from ANOVA on raw data from the hedonic evaluation of general liking.

Analysis of Variance (One-Way) - RAW DATA – HEDONIC EVALUATION - LIKING

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
Sample 385	14	56,	4,	2,76923
Sample 762	14	55,	3,92857	3,76374
Sample 891	14	50,	3,57143	4,72527
Sample 423	14	61,	4,35714	3,47802

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	4,35714	3	1,45238	0,39423	0,75766	2,7826
Within Groups	191,57143	52	3,68407			
Total	195,92857	55				

Table 15. Results from ANOVA on centered and normalized data from the hedonic evaluation of general liking.

Analysis of Variance (One-Way) - CENTERED AND NORMALIZED – HEDONIC EVALUATION - LIKING

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
Sample 385	13	-0,03097	-0,00238	0,49389
Sample 762	13	1,834	0,14108	0,64175
Sample 891	13	-1,80303	-0,13869	0,98862
Sample 423	13	9,64566	0,74197	4,9094

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	5,8765	3	1,95883	1,11398	0,3527	2,79806
Within Groups	84,40389	48	1,75841			
Total	90,28039	51				

Table 16. Results from ANOVA on raw data from the hedonic evaluation of sandiness.

Analysis of Variance (One-Way) - RAW DATA - SANDINESS

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
Sample 385	14	82,	5,85714	5,20879
Sample 762	14	65,	4,64286	5,63187
Sample 891	14	27,	1,92857	2,68681
Sample 423	14	26,	1,85714	2,28571
				15,81319

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	168,14286	3	56,04762	14,17744	7,13215E-7	2,7826
Within Groups	205,57143	52	3,9533			
Total	373,71429	55				

Table 17. Results from ANOVA on centered and normalized data from the hedonic evaluation of sandiness.

Analysis of Variance (One-Way) - CENTERED AND NORMALIZED - SANDINESS

Summary

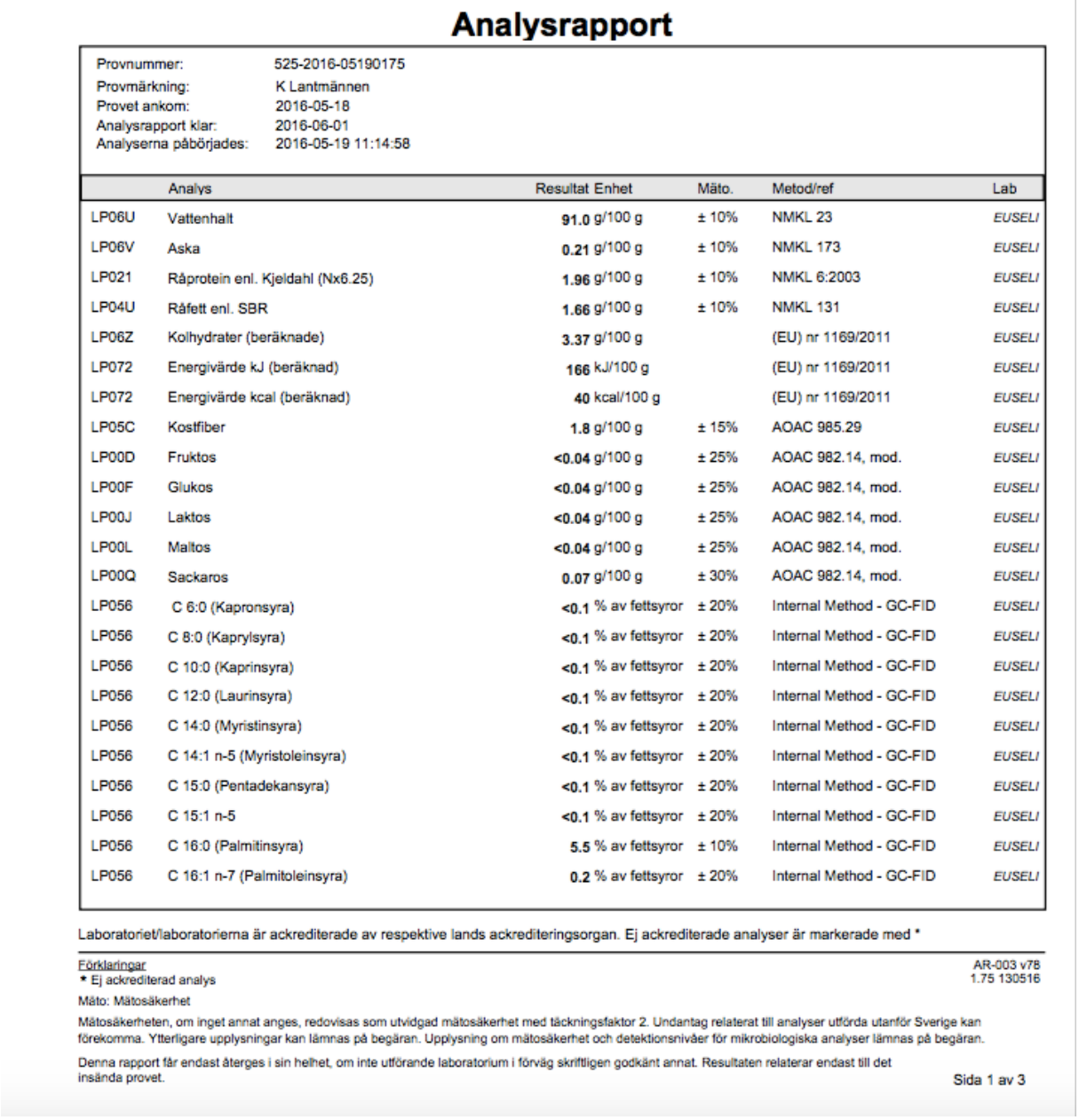
Groups	*Sample size*	*Sum*	*Mean*	*Variance*
Sample 385	13	11,10602	0,85431	0,04934
Sample 762	13	1,51762	0,11674	0,21358
Sample 891	13	-12,62364	-0,97105	0,0768
Sample 423	13	-13,30465	-1,02343	0,10795
				0,44767

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	32,13566	3	10,71189	95,71277	0,	2,79806
Within Groups	5,37202	48	0,11192			
Total	37,50767	51				

Appendix 4 - External analysis - raw data

The raw data from the external analysis for the five samples can be seen in Figures 1-10.



Analysrapport

Provnummer: 525-2016-05190175
Provmärkning: K Lantmännen
Provet ankom: 2016-05-18
Analysrapport klar: 2016-06-01
Analyserna påbörjades: 2016-05-19 11:14:58

	Analys	Resultat	Enhet	Mäto.	Metod/ref	Lab
LP06U	Vattenhalt	91.0	g/100 g	± 10%	NMKL 23	EUSELI
LP06V	Aska	0.21	g/100 g	± 10%	NMKL 173	EUSELI
LP021	Råprotein enl. Kjeldahl (Nx6.25)	1.96	g/100 g	± 10%	NMKL 6:2003	EUSELI
LP04U	Råfett enl. SBR	1.66	g/100 g	± 10%	NMKL 131	EUSELI
LP06Z	Kolhydrater (beräknade)	3.37	g/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kJ (beräknad)	166	kJ/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kcal (beräknad)	40	kcal/100 g		(EU) nr 1169/2011	EUSELI
LP05C	Kostfiber	1.8	g/100 g	± 15%	AOAC 985.29	EUSELI
LP00D	Fruktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00F	Glukos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00J	Laktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00L	Maltos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00Q	Sackaros	0.07	g/100 g	± 30%	AOAC 982.14, mod.	EUSELI
LP056	C 6:0 (Kapronsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 8:0 (Kaprylsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 10:0 (Kaprinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 12:0 (Laurinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:0 (Myristinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:1 n-5 (Myristoleinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:0 (Pentadekansyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:1 n-5	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 16:0 (Palmitinsyra)	5.5	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 16:1 n-7 (Palmitoleinsyra)	0.2	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI

Laboratoriet/laboratorierna är ackrediterade av respektive lands ackrediteringsorgan. Ej ackrediterade analyser är markerade med *

Förklaringar
* Ej ackrediterad analys

AR-003 v78
1.75 130516

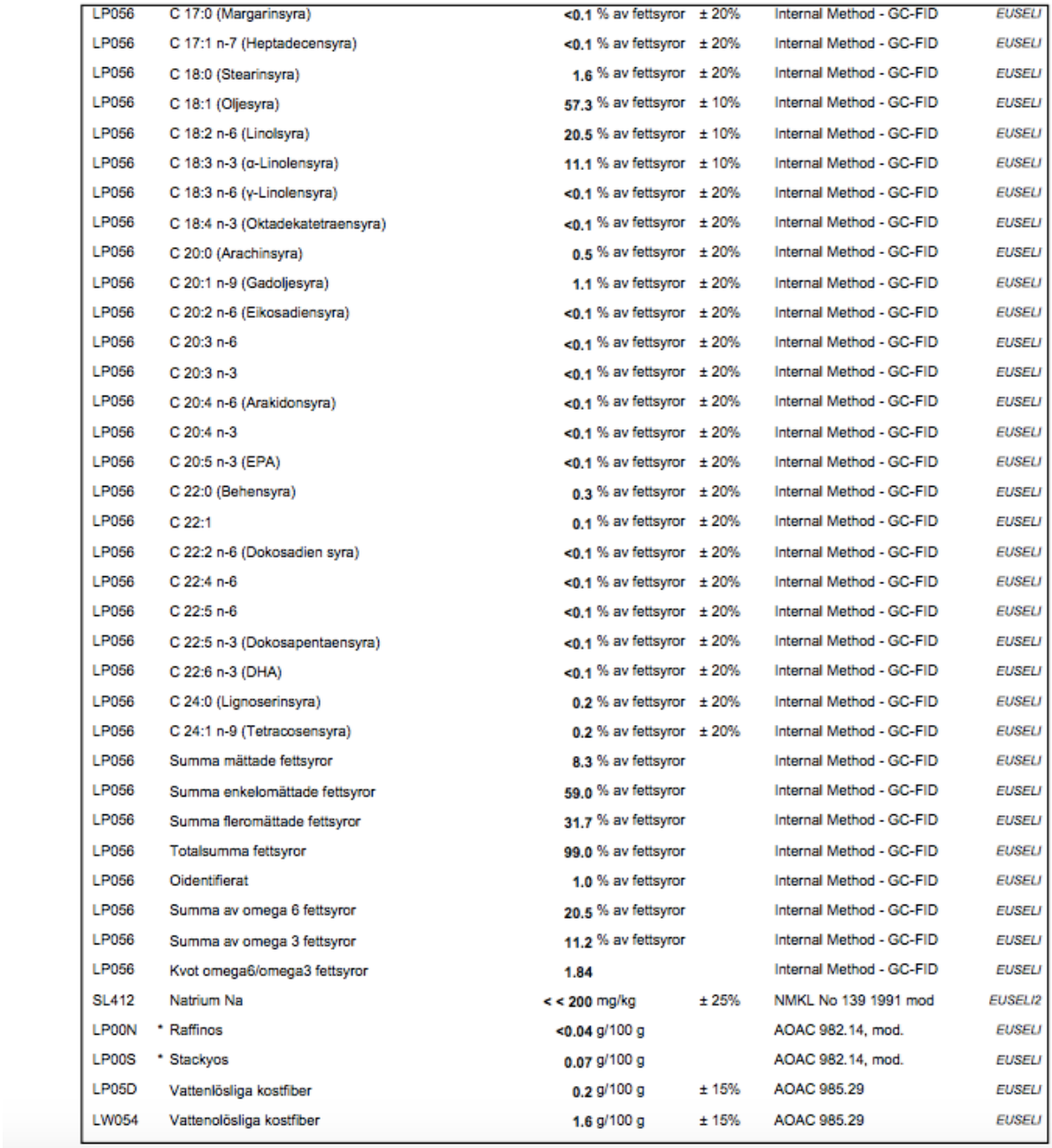
Mäto: Mätosäkerhet

Mätosäkerheten, om inget annat anges, redovisas som utvidgad mätosäkerhet med täckningsfaktor 2. Undantag relaterat till analyser utförda utanför Sverige kan förekomma. Ytterligare upplysningar kan lämnas på begäran. Upplysning om mätosäkerhet och detektionsnivåer för mikrobiologiska analyser lämnas på begäran.

Denna rapport får endast återges i sin helhet, om inte utförande laboratorium i förväg skriftligen godkänt annat. Resultaten relaterar endast till det insända provet.

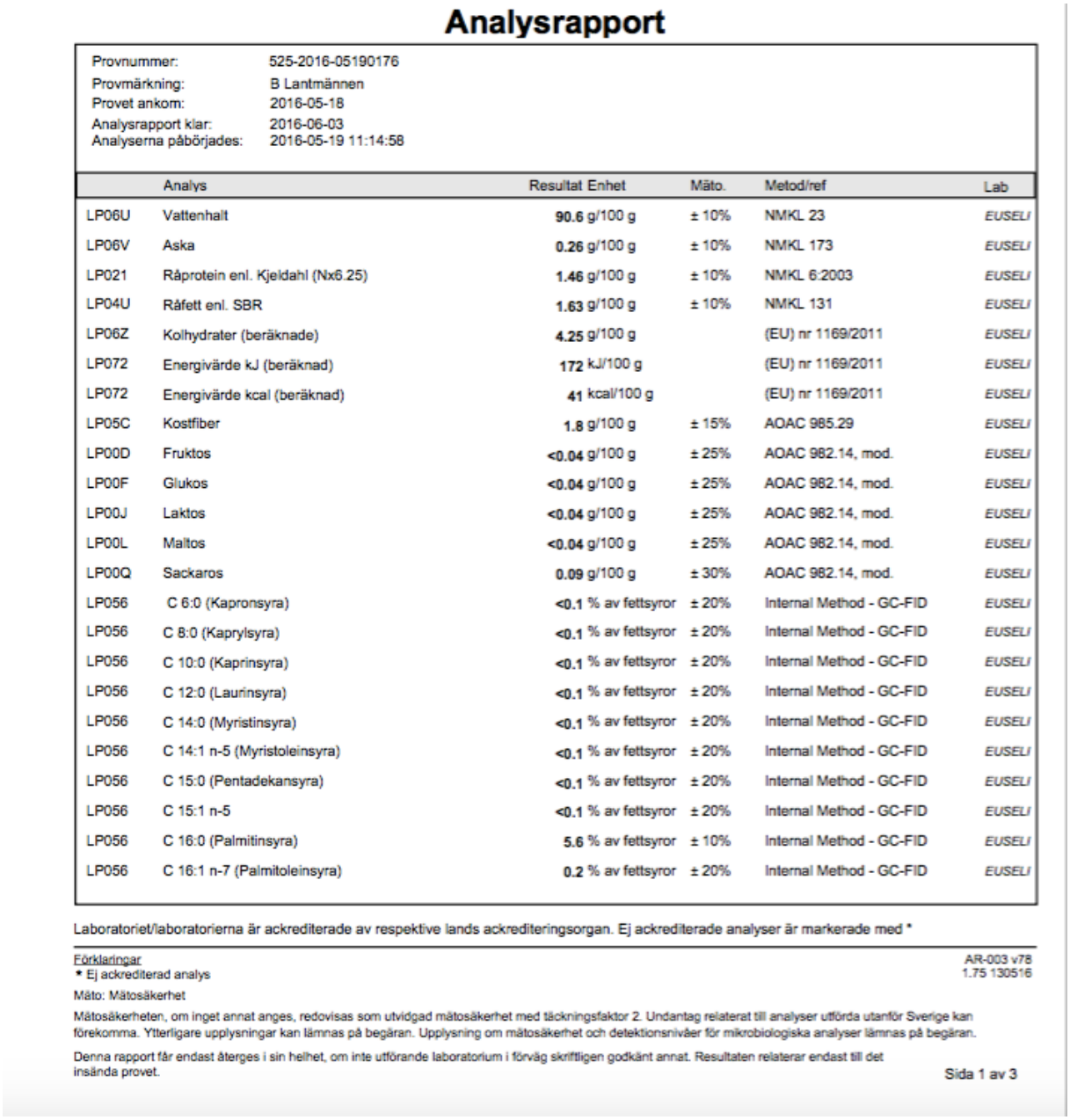
Sida 1 av 3

Figure 1. Data from the external analysis of nutritional content for sample 1 (basic bean beverage), page 1.



LP056	C 17:0 (Margarinsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 17:1 n-7 (Heptadecensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:0 (Stearinsyra)	1.6 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:1 (Oljesyra)	57.3 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:2 n-6 (Linolsyra)	20.5 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-3 (α-Linolensyra)	11.1 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-6 (γ-Linolensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:4 n-3 (Oktadekatetraensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:0 (Arachinsyra)	0.5 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:1 n-9 (Gadoljesyra)	1.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:2 n-6 (Eikosadiensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-6 (Arakidonsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:5 n-3 (EPA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:0 (Behensyra)	0.3 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:1	0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:2 n-6 (Dokosadien syra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:4 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-3 (Dokosapentaensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:6 n-3 (DHA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:0 (Lignoserinsyra)	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:1 n-9 (Tetracosensyra)	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	Summa mättade fettsyror	8.3 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa enkelomättade fettsyror	59.0 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa fleromättade fettsyror	31.7 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Totalsumma fettsyror	99.0 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Oidentifierat	1.0 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 6 fettsyror	20.5 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 3 fettsyror	11.2 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Kvot omega6/omega3 fettsyror	1.84		Internal Method - GC-FID	EUSELI
SL412	Natrium Na	< < 200 mg/kg	± 25%	NMKL No 139 1991 mod	EUSELI2
LP00N	* Raffinos	<0.04 g/100 g		AOAC 982.14, mod.	EUSELI
LP00S	* Stackyos	0.07 g/100 g		AOAC 982.14, mod.	EUSELI
LP05D	Vattenlösliga kostfiber	0.2 g/100 g	± 15%	AOAC 985.29	EUSELI
LW054	Vattenolösliga kostfiber	1.6 g/100 g	± 15%	AOAC 985.29	EUSELI

Figure 2. Data from the external analysis of nutritional content for sample 1 (basic bean beverage), page 2.



Analysrapport

Provnummer: 525-2016-05190176
Provmärkning: B Lantmännen
Provet ankom: 2016-05-18
Analysrapport klar: 2016-06-03
Analyserna påbörjades: 2016-05-19 11:14:58

	Analys	Resultat	Enhet	Mäto.	Metod/ref	Lab
LP06U	Vattenhalt	90.6	g/100 g	± 10%	NMKL 23	EUSELI
LP06V	Aska	0.26	g/100 g	± 10%	NMKL 173	EUSELI
LP021	Råprotein enl. Kjeldahl (Nx6.25)	1.46	g/100 g	± 10%	NMKL 6:2003	EUSELI
LP04U	Råfett enl. SBR	1.63	g/100 g	± 10%	NMKL 131	EUSELI
LP06Z	Kolhydrater (beräknade)	4.25	g/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kJ (beräknad)	172	kJ/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kcal (beräknad)	41	kcal/100 g		(EU) nr 1169/2011	EUSELI
LP05C	Kostfiber	1.8	g/100 g	± 15%	AOAC 985.29	EUSELI
LP00D	Fruktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00F	Glukos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00J	Laktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00L	Maltos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00Q	Sackaros	0.09	g/100 g	± 30%	AOAC 982.14, mod.	EUSELI
LP056	C 6:0 (Kapronsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 8:0 (Kaprylsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 10:0 (Kaprinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 12:0 (Laurinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:0 (Myristinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:1 n-5 (Myristoleinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:0 (Pentadekansyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:1 n-5	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 16:0 (Palmitinsyra)	5.6	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 16:1 n-7 (Palmitoleinsyra)	0.2	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI

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Förklaringar
* Ej ackrediterad analys

AR-003 v78
1.75 130516

Mäto: Mätosäkerhet

Mätosäkerheten, om inget annat anges, redovisas som utvidgad mätosäkerhet med täckningsfaktor 2. Undantag relaterat till analyser utförda utanför Sverige kan förekomma. Ytterligare upplysningar kan lämnas på begäran. Upplysning om mätosäkerhet och detektionsnivåer för mikrobiologiska analyser lämnas på begäran.

Denna rapport får endast återges i sin helhet, om inte utförande laboratorium i förväg skriftligen godkänt annat. Resultaten relaterar endast till det insända provet.

Sida 1 av 3

Figure 3. Data from the external analysis of nutritional content for sample 2 (alkali treated bean beverage), page 1.

LP056	C 17:0 (Margarinsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 17:1 n-7 (Heptadecensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:0 (Stearinsyra)	1.5 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:1 (Oljesyra)	57.4 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:2 n-6 (Linolsyra)	20.6 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-3 (α-Linolensyra)	11.2 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-6 (γ-Linolensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:4 n-3 (Oktadekatetraensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:0 (Arachinsyra)	0.5 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:1 n-9 (Gadoljesyra)	1.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:2 n-6 (Eikosadiensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-6 (Arakidonsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:5 n-3 (EPA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:0 (Behensyra)	0.3 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:1	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:2 n-6 (Dokosadien syra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:4 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-3 (Dokosapentaensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:6 n-3 (DHA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:0 (Lignoserinsyra)	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:1 n-9 (Tetracosensyra)	0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	Summa mättade fettsyror	8.3 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa enkelomättade fettsyror	59.1 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa fleromättade fettsyror	31.9 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Totalsumma fettsyror	99.3 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Oidentifierat	0.7 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 6 fettsyror	20.7 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 3 fettsyror	11.2 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Kvot omega6/omega3 fettsyror	1.85		Internal Method - GC-FID	EUSELI
SL412	Natrium Na	240 mg/kg	± 25%	NMKL No 139 1991 mod	EUSELI2
LP05D	Vattenlösliga kostfiber	0.1 g/100 g	± 15%	AOAC 985.29	EUSELI
LW054	Vattenolösliga kostfiber	1.7 g/100 g	± 15%	AOAC 985.29	EUSELI

Figure 4. Data from the external analysis of nutritional content for sample 2 (alkali treated bean beverage), page 2.

Analysrapport

Provnummer: 525-2016-05190177
Provmärkning: KC Lantmännen
Provet ankom: 2016-05-18
Analysrapport klar: 2016-05-30
Analyserna påbörjades: 2016-05-19 11:14:58

	Analys	Resultat	Enhet	Mäto.	Metod/ref	Lab
LP06U	Vattenhalt	97.9	g/100 g	± 10%	NMKL 23	EUSELI
LP06V	Aska	0.12	g/100 g	± 10%	NMKL 173	EUSELI
LP021	Råprotein enl. Kjeldahl (Nx6.25)	<0.30	g/100 g	± 20%	NMKL 6:2003	EUSELI
LP04U	Råfett enl. SBR	1.23	g/100 g	± 10%	NMKL 131	EUSELI
LP06Z	Kolhydrater (beräknade)	0.75	g/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kJ (beräknad)	58	kJ/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kcal (beräknad)	14	kcal/100 g		(EU) nr 1169/2011	EUSELI
LP05C	Kostfiber	<1.0	g/100 g	± 15%	AOAC 985.29	EUSELI
LP00D	Fruktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00F	Glukos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00J	Laktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00L	Maltos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00Q	Sackaros	0.08	g/100 g	± 30%	AOAC 982.14, mod.	EUSELI
LP056	C 6:0 (Kapronsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 8:0 (Kaprylsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 10:0 (Kaprinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 12:0 (Laurinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:0 (Myristinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:1 n-5 (Myristoleinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:0 (Pentadekansyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:1 n-5	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 16:0 (Palmitinsyra)	5.2	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 16:1 n-7 (Palmitoleinsyra)	0.2	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI

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Förklaringar
* Ej ackrediterad analys

AR-003 v78
1.75 130516

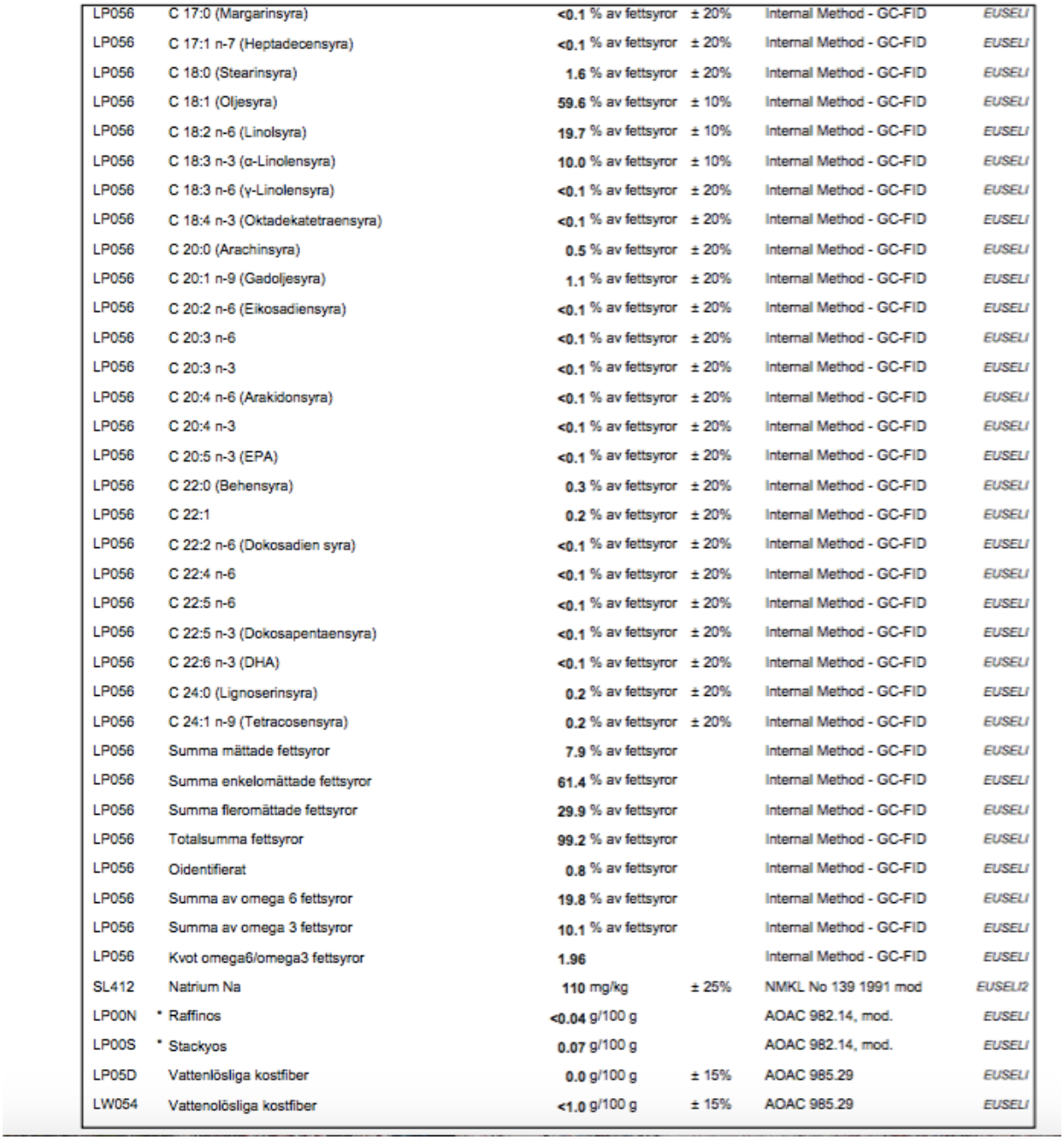
Mäto: Mätosäkerhet

Mätosäkerheten, om inget annat anges, redovisas som utvidgad mätosäkerhet med täckningsfaktor 2. Undantag relaterat till analyser utförda utanför Sverige kan förekomma. Ytterligare upplysningar kan lämnas på begäran. Upplysning om mätosäkerhet och detektionsnivåer för mikrobiologiska analyser lämnas på begäran.

Denna rapport får endast återges i sin helhet, om inte utförande laboratorium i förväg skriftligen godkänt annat. Resultaten relaterar endast till det insända provet.

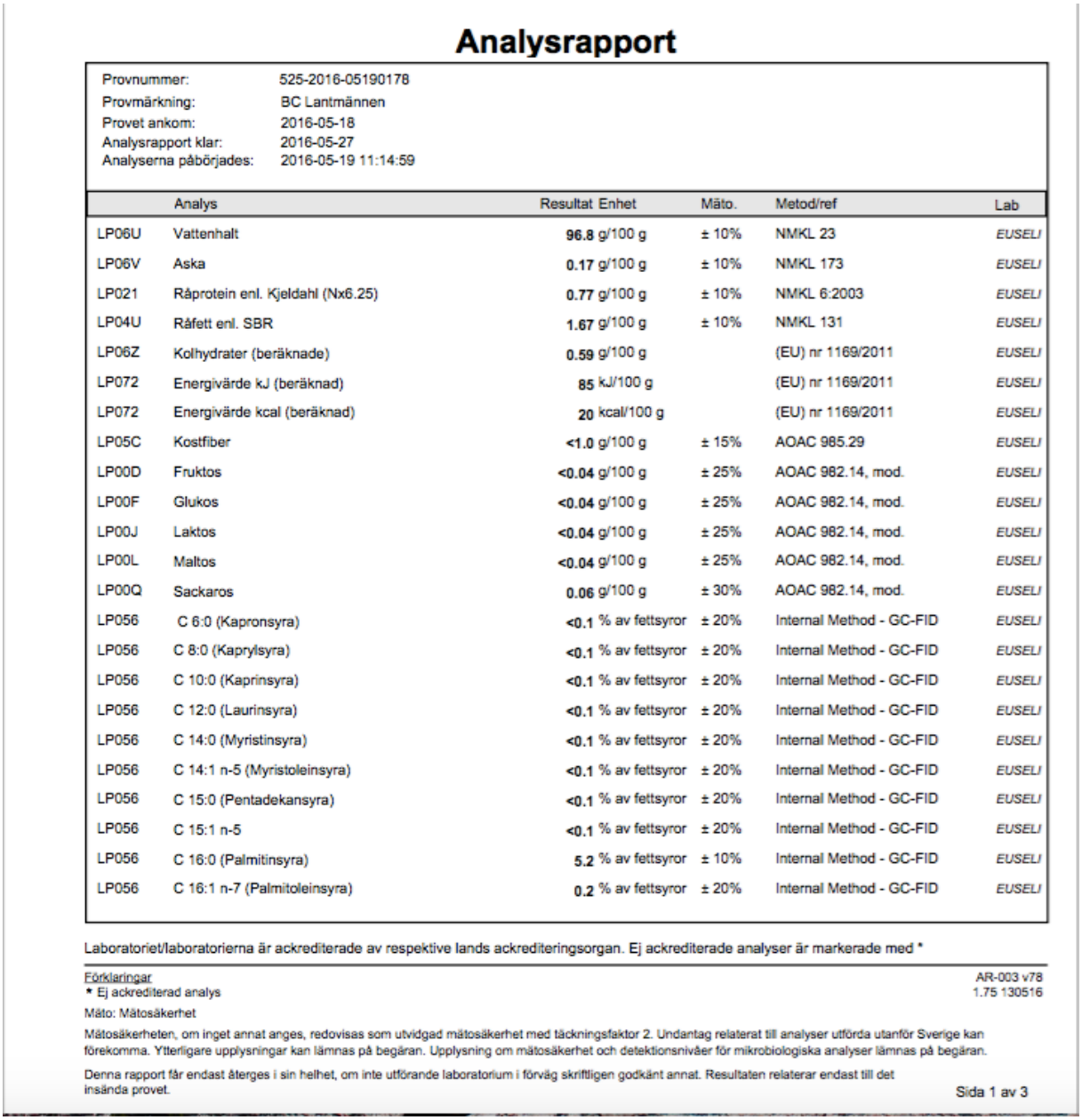
Sida 1 av 3

Figure 5. Data from the external analysis of nutritional content for sample 3 (centrifuged bean beverage), page 1.



LP056	C 17:0 (Margarinsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 17:1 n-7 (Heptadecensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:0 (Stearinsyra)	1.6 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:1 (Oljesyra)	59.6 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:2 n-6 (Linolsyra)	19.7 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-3 (α-Linolensyra)	10.0 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-6 (γ-Linolensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:4 n-3 (Oktadekatetraensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:0 (Arachinsyra)	0.5 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:1 n-9 (Gadoljesyra)	1.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:2 n-6 (Eikosadiensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-6 (Arakidonsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:5 n-3 (EPA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:0 (Behensyra)	0.3 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:1	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:2 n-6 (Dokosadien syra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:4 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-3 (Dokosapentaensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:6 n-3 (DHA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:0 (Lignoserinsyra)	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:1 n-9 (Tetracosensyra)	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	Summa mättade fettsyror	7.9 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa enkelomättade fettsyror	61.4 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa fleromättade fettsyror	29.9 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Totalsumma fettsyror	99.2 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Oidentifierat	0.8 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 6 fettsyror	19.8 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 3 fettsyror	10.1 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Kvot omega6/omega3 fettsyror	1.96		Internal Method - GC-FID	EUSELI
SL412	Natrium Na	110 mg/kg	± 25%	NMKL No 139 1991 mod	EUSELI2
LP00N	* Raffinos	<0.04 g/100 g		AOAC 982.14, mod.	EUSELI
LP00S	* Stackyos	0.07 g/100 g		AOAC 982.14, mod.	EUSELI
LP05D	Vattenlösliga kostfiber	0.0 g/100 g	± 15%	AOAC 985.29	EUSELI
LW054	Vattenolösliga kostfiber	<1.0 g/100 g	± 15%	AOAC 985.29	EUSELI

Figure 6. Data from the external analysis of nutritional content for sample 3 (centrifuged bean beverage), page 2.



Analysrapport

Provnummer: 525-2016-05190178
Provmärkning: BC Lantmännen
Provet ankom: 2016-05-18
Analysrapport klar: 2016-05-27
Analyserna påbörjades: 2016-05-19 11:14:59

	Analys	Resultat	Enhet	Mäto.	Metod/ref	Lab
LP06U	Vattenhalt	96.8	g/100 g	± 10%	NMKL 23	EUSELI
LP06V	Aska	0.17	g/100 g	± 10%	NMKL 173	EUSELI
LP021	Råprotein enl. Kjeldahl (Nx6.25)	0.77	g/100 g	± 10%	NMKL 6:2003	EUSELI
LP04U	Råfett enl. SBR	1.67	g/100 g	± 10%	NMKL 131	EUSELI
LP06Z	Kolhydrater (beräknade)	0.59	g/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kJ (beräknad)	85	kJ/100 g		(EU) nr 1169/2011	EUSELI
LP072	Energivärde kcal (beräknad)	20	kcal/100 g		(EU) nr 1169/2011	EUSELI
LP05C	Kostfiber	<1.0	g/100 g	± 15%	AOAC 985.29	EUSELI
LP00D	Fruktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00F	Glukos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00J	Laktos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00L	Maltos	<0.04	g/100 g	± 25%	AOAC 982.14, mod.	EUSELI
LP00Q	Sackaros	0.06	g/100 g	± 30%	AOAC 982.14, mod.	EUSELI
LP056	C 6:0 (Kapronsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 8:0 (Kaprylsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 10:0 (Kaprinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 12:0 (Laurinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:0 (Myristinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 14:1 n-5 (Myristoleinsyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:0 (Pentadekansyra)	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 15:1 n-5	<0.1	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 16:0 (Palmitinsyra)	5.2	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 16:1 n-7 (Palmitoleinsyra)	0.2	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI

Laboratoriet/laboratorierna är ackrediterade av respektive lands ackrediteringsorgan. Ej ackrediterade analyser är markerade med *

Förklaringar
* Ej ackrediterad analys

AR-003 v78
1.75 130516

Mäto: Mätosäkerhet

Mätosäkerheten, om inget annat anges, redovisas som utvidgad mätosäkerhet med täckningsfaktor 2. Undantag relaterat till analyser utförda utanför Sverige kan förekomma. Ytterligare upplysningar kan lämnas på begäran. Upplysning om mätosäkerhet och detektionsnivåer för mikrobiologiska analyser lämnas på begäran.

Denna rapport får endast återges i sin helhet, om inte utförande laboratorium i förväg skriftligen godkänt annat. Resultaten relaterar endast till det insända provet.

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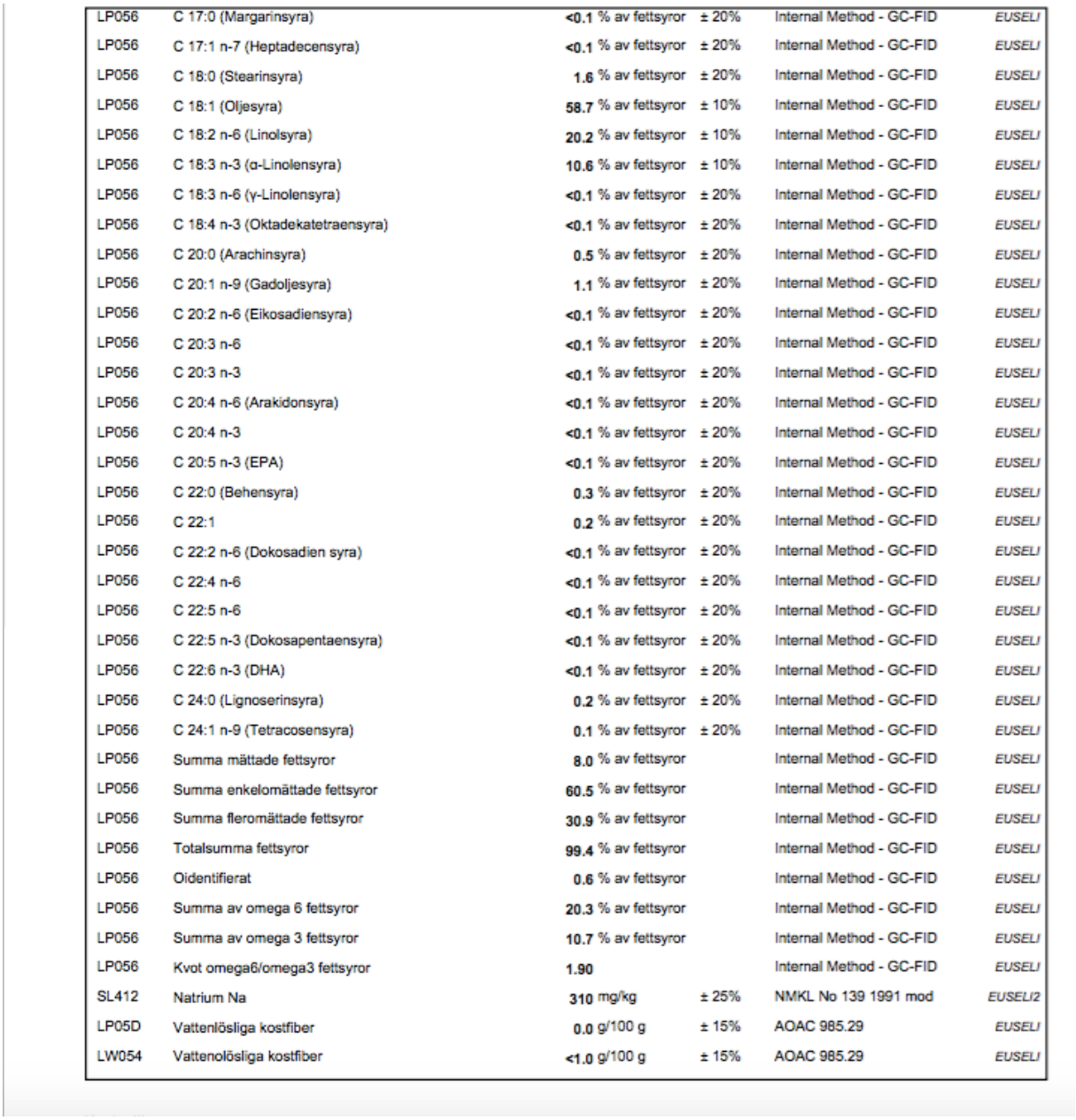
Figure 7. Data from the external analysis of nutritional content for sample 4 (centrifuged alkali treated bean beverage), page 1.



LP056	C 17:0 (Margarinsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 17:1 n-7 (Heptadecensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:0 (Stearinsyra)	1.6 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:1 (Oljesyra)	58.7 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:2 n-6 (Linolsyra)	20.2 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-3 (α-Linolensyra)	10.6 % av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056	C 18:3 n-6 (γ-Linolensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 18:4 n-3 (Oktadekatetraensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:0 (Arachinsyra)	0.5 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:1 n-9 (Gadoljesyra)	1.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:2 n-6 (Eikosadiensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:3 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-6 (Arakidonsyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:4 n-3	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 20:5 n-3 (EPA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:0 (Behensyra)	0.3 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:1	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:2 n-6 (Dokosadien syra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:4 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-6	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:5 n-3 (Dokosapentaensyra)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 22:6 n-3 (DHA)	<0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:0 (Lignoserinsyra)	0.2 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	C 24:1 n-9 (Tetracosensyra)	0.1 % av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056	Summa mättade fettsyror	8.0 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa enkelomättade fettsyror	60.5 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa fleromättade fettsyror	30.9 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Totalsumma fettsyror	99.4 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Oidentifierat	0.6 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 6 fettsyror	20.3 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Summa av omega 3 fettsyror	10.7 % av fettsyror		Internal Method - GC-FID	EUSELI
LP056	Kvot omega6/omega3 fettsyror	1.90		Internal Method - GC-FID	EUSELI
SL412	Natrium Na	310 mg/kg	± 25%	NMKL No 139 1991 mod	EUSELI2
LP05D	Vattenlösliga kostfiber	0.0 g/100 g	± 15%	AOAC 985.29	EUSELI
LW054	Vattenolösliga kostfiber	<1.0 g/100 g	± 15%	AOAC 985.29	EUSELI

Figure 8. Data from the external analysis of nutritional content for sample 4 (centrifuged alkali treated bean beverage), page 2.

Analysrapport

Provnummer:	525-2016-05190179
Provmärkning:	F Lantmännen
Provet ankom:	2016-05-18
Analysrapport klar:	2016-06-01
Analyserna påbörjades:	2016-05-19 11:14:59

	Analys	Resultat	Enhet	Mäto.	Metod/ref	Lab
LP06U	Vattenhalt	**93.1**	g/100 g	± 10%	NMKL 23	*EUSELI*
LP06V	Aska	**0.18**	g/100 g	± 10%	NMKL 173	*EUSELI*
LP021	Råprotein enl. Kjeldahl (Nx6.25)	**1.48**	g/100 g	± 10%	NMKL 6:2003	*EUSELI*
LP04U	Råfett enl. SBR	**1.02**	g/100 g	± 10%	NMKL 131	*EUSELI*
LP06Z	Kolhydrater (beräknade)	**4.22**	g/100 g		(EU) nr 1169/2011	*EUSELI*
LP072	Energivärde kJ (beräknad)	**135**	kJ/100 g		(EU) nr 1169/2011	*EUSELI*
LP072	Energivärde kcal (beräknad)	**32**	kcal/100 g		(EU) nr 1169/2011	*EUSELI*
LP05C	Kostfiber	**<1.0**	g/100 g	± 15%	AOAC 985.29	*EUSELI*
LP00D	Fruktos	**<0.04**	g/100 g	± 25%	AOAC 982.14, mod.	*EUSELI*
LP00F	Glukos	**<0.04**	g/100 g	± 25%	AOAC 982.14, mod.	*EUSELI*
LP00J	Laktos	**<0.04**	g/100 g	± 25%	AOAC 982.14, mod.	*EUSELI*
LP00L	Maltos	**<0.04**	g/100 g	± 25%	AOAC 982.14, mod.	*EUSELI*
LP00Q	Sackaros	**<0.04**	g/100 g	± 30%	AOAC 982.14, mod.	*EUSELI*
LP056	C 6:0 (Kapronsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 8:0 (Kaprylsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 10:0 (Kaprinsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 12:0 (Laurinsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 14:0 (Myristinsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 14:1 n-5 (Myristoleinsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 15:0 (Pentadekansyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 15:1 n-5	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*
LP056	C 16:0 (Palmitinsyra)	**5.7**	% av fettsyror	± 10%	Internal Method - GC-FID	*EUSELI*
LP056	C 16:1 n-7 (Palmitoleinsyra)	**0.2**	% av fettsyror	± 20%	Internal Method - GC-FID	*EUSELI*

Laboratoriet/laboratorierna är ackrediterade av respektive lands ackrediteringsorgan. Ej ackrediterade analyser är markerade med *

Förklaringar
* Ej ackrediterad analys
Mäto: Mätosäkerhet

AR-003 v78
1.75 130516

Mätosäkerheten, om inget annat anges, redovisas som utvidgad mätosäkerhet med täckningsfaktor 2. Undantag relaterat till analyser utförda utanför Sverige kan förekomma. Ytterligare upplysningar kan lämnas på begäran. Upplysning om mätosäkerhet och detektionsnivåer för mikrobiologiska analyser lämnas på begäran.

Figure 9. Data from the external analysis of nutritional content for sample 5 (fermented bean beverage), page 1.

LP056		C 17:0 (Margarinsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 17:1 n-7 (Heptadecensyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 18:0 (Stearinsyra)	**1.6**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 18:1 (Oljesyra)	**57.4**	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056		C 18:2 n-6 (Linolsyra)	**20.4**	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056		C 18:3 n-3 (α-Linolensyra)	**11.0**	% av fettsyror	± 10%	Internal Method - GC-FID	EUSELI
LP056		C 18:3 n-6 (γ-Linolensyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 18:4 n-3 (Oktadekatetraensyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:0 (Arachinsyra)	**0.5**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:1 n-9 (Gadoljesyra)	**1.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:2 n-6 (Eikosadiensyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:3 n-6	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:3 n-3	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:4 n-6 (Arakidonsyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:4 n-3	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 20:5 n-3 (EPA)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:0 (Behensyra)	**0.3**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:1	**0.2**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:2 n-6 (Dokosadien syra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:4 n-6	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:5 n-6	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:5 n-3 (Dokosapentaensyra)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 22:6 n-3 (DHA)	**<0.1**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 24:0 (Lignoserinsyra)	**0.2**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		C 24:1 n-9 (Tetracosensyra)	**0.2**	% av fettsyror	± 20%	Internal Method - GC-FID	EUSELI
LP056		Summa mättade fettsyror	**8.4**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Summa enkelomättade fettsyror	**59.1**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Summa flеromättade fettsyror	**31.5**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Totalsumma fettsyror	**99.0**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Oidentifierat	**1.0**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Summa av omega 6 fettsyror	**20.5**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Summa av omega 3 fettsyror	**11.0**	% av fettsyror		Internal Method - GC-FID	EUSELI
LP056		Kvot omega6/omega3 fettsyror	**1.85**			Internal Method - GC-FID	EUSELI
SL412		Natrium Na	**< < 200**	mg/kg	± 25%	NMKL No 139 1991 mod	EUSELI2
LP00N	*	Raffinos	**<0.04**	g/100 g		AOAC 982.14, mod.	EUSELI
LP00S	*	Stackyos	**0.05**	g/100 g		AOAC 982.14, mod.	EUSELI
LP05D		Vattenlösliga kostfiber	**0.0**	g/100 g	± 15%	AOAC 985.29	EUSELI
LW054		Vattenolösliga kostfiber	**<1.0**	g/100 g	± 15%	AOAC 985.29	EUSELI

Figure 10. Data from the external analysis of nutritional content for sample 5 (fermented bean beverage), page 2.