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Department of Molecular Sciences

Optimising processing and sensory characteristics of Bean Blue

– a blue mould tofu

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Agronomy Program – Food Science
Independent Project in Food Science • Master Thesis • 30 hec • Advanced A2E
Molecular Sciences, 2018:21
Uppsala, 2018

Optimising processing and sensory characteristics of Bean Blue, a blue mould tofu

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Credits: 30 hec

Level: Advanced A2E

Course title: Independent Project in Food Science

Course code: EX0425

Program/education: Agronomy – Food Science

Place of publication: Uppsala

Year of publication: 2018

Cover picture: Martina Näsholm

Title of series: Molecular Sciences

Part number: 2018:21

Online publication: <http://stud.epsilon.slu.se>

Keywords: faba bean, soya bean, tofu, cheese, sensory properties

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Abstract

The increase in the global population leads to increased demand for food and especially protein sources. Legumes have potential to support the increased worldwide demand for protein. Faba bean is a legume with high protein content which can be grown in Sweden. Bean Blue is a blue mould tofu based on faba bean that has been developed by researchers at the Swedish University of Agricultural Sciences (SLU) and the Research Institutes of Sweden (RISE). The purpose of this master thesis was to optimise the processing steps and the sensory characteristics of the final product by altering different parameters. The development of Bean Blue was divided into technical and sensory trials. The result from the technical trials was evaluated during production and by sensory evaluation of the final product by the development team. The sensory trials were evaluated through both consumer- and description tests. The results showed that the water:bean ratio during processing could be decreased from 9:1 to 4:1. The original method that included the use of 70 % faba bean and 30 % soybean could be replaced by a new method including the use of 100 % faba bean and removal of the starch. The original homofermentative starter culture could not be replaced by a heterofermentative starter culture due to poor mould growth and no pore formation. Rapeseed oil could replace cooking oil without any changes in the sensory quality. The process of Bean Blue was optimised by decreasing the water:bean ratio, which enhanced handling of the bean milk during pressing to remove okara (bean solids), and shortened draining time. The sensory quality was improved and the final product was described as more creamy and less beany in taste compared with the starting recipe. All parameters together have led to a more advantageous product from an environmental point of view (usage of 100 % faba, and Swedish rapeseed oil) without negative effect on the sensory properties.

Keywords: Faba bean, soybean, tofu, cheese, sensory properties

Sammanfattning

Den globalt ökande populationen leder till ökad efterfrågan på mat och speciellt protein. Baljväxter har potential till att stödja den ökande efterfrågan på protein. Åkerböna är en baljväxt med högt proteininnehåll och som kan odlas i Sverige. Bean Blue är en blåmögel tofu baserad på åkerböna som har utvecklats av forskare på Sveriges lantbruksuniversitet (SLU) och Research Institutes of Sweden (RISE). Syftet med detta arbete var att optimera processen av Bean Blue och de sensoriska egenskaperna hos den slutliga produkten genom variation av olika parametrar. Utvecklingen av Bean Blue har delats in i tekniska- och sensoriska försök. Resultatet från de tekniska försöken har utvärderats under produktionens gång, och genom sensorisk bedömning av den slutliga produkten av utvecklingssteamet. De sensoriska försöken utvärderades genom ett konsument- och beskrivande test. Resultatet visar att förhållandet mellan vatten och bönor kan minskas från 9:1 till 4:1. Den ursprungliga metoden som inkluderat användning av 70 % åkerböna och 30 % sojaböna har kunnat ersättas med en ny metod som inkluderar 100 % åkerböna och borttagning av stärkelsen. Den ursprungliga homofermentativa starterkulturen har inte kunnat ersättas av en heterofermentativ kultur på grund av dålig mögeltillväxt och dålig hålföring. Rapsolja har kunnat ersätta matolja utan att bidra till förändringar i den sensoriska kvaliteten. Processen av Bean Blue har kunnat optimeras genom att minska mängden vatten som förenklar hantering av bönmjölken när okara pressas ut, och minskar tiden för avrinning. De sensoriska egenskaperna har förbättrats och den slutliga produkten upplevs som mer krämig med mindre smak av bönor jämfört med startreceptet. Samtliga parametrar har tillsammans lett till en mer klimatmässigt fördelaktig produkt (användning av 100 % åkerböna och svensk rapsolja) utan negativ inverkan på de sensoriska egenskaperna.

Nyckelord: Åkerböna, sojaböna, tofu, ost, sensoriska egenskaper

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1 Introduction

The increase in the global population demands increased food production. An increase from current 7.2 billion people to 9.6 billion by 2050 will in turn present challenges in food production to prevent hunger and malnutrition. These changes in population have led to a rise in the demand for food protein (Wu *et al.*, 2014). To meet the increased demand for protein sources, global meat production is projected to increase by 30% during the next 30 years (Nadathur *et al.*, 2017). Plant foods have potential to contribute to the global protein production, and support the increased worldwide demand for protein (Multari *et al.*, 2015). Development of products with protein sources from plant food can be an option for a more sustainable raw material use (Multari *et al.*, 2015).

While meat production is increasing, the demand for alternative protein sources and the proportion of individuals choosing vegan diets is also increasing. Factors influencing lifestyle and diet behavior are varied. Consumers often adopt vegetarian, and vegan life styles based on their views on animal welfare, cultural and religious beliefs, health and environmental aspects (Dyett *et al.*, 2013). A shift towards more plant based diet and reduced meat consumption is endorsed to promote sustainability, ethical and health aspects. (Graça *et al.*, 2015). Vegan diets tend to contain high levels of fibers, vitamins and minerals. The fat content is also beneficial due to its low grade of saturation. Plant food is also a good source of protein. However, the amino acid composition requires a combined intake of different plant foods to fulfill the intake of essential amino acids. (Radnitz *et al.*, 2015)

Legumes are a suitable option to replace animal protein in the diet, due to high levels of protein. Among the legumes, faba bean is a valuable crop with high content in digestible proteins and starch (Multari *et al.*, 2015). The faba bean is also a good source of fibers, vitamins and minerals (Crépon *et al.*, 2010). Faba beans (*Vicia Faba L*), also referred to as fava bean, broad bean or field bean have been an essential staple food for millennia widespread in the Mediterranean area (Duc, 1997). Faba bean provides some of the essential amino acids and intake of the legume is a good option in developing countries where the access to meat is limited. Faba bean

can be used as a vegetable replacement, or in combination with animal food products or other plant based foods to improve the nutritional quality. The majority of faba bean grown in Sweden today is used for feed. By developing new products based on faba bean, the use of this bean can be increased (Multari *et al.*, 2015).

1.1 Background to the project

To promote the consumption of vegetable foods and meet the demand for alternative protein sources, a blue mould tofu called “Bean Blue” has been developed. Bean Blue is based on faba beans and in the initial development stage, also soybeans. The production includes processing steps both similar to tofu- and blue mould cheese production. Development of Bean Blue was initiated by researchers at the Swedish University of Agricultural Sciences (SLU) and the Research Institutes of Sweden (RISE) in Uppsala, and with pilot financing by the Swedish Innovation Agency Vinnova. The purpose of the development of Bean Blue was to create a climate smart product, and increase the use of faba bean in foods. The product could also be an alternative for people with milk protein allergy. The product has so far been produced in laboratory scale by the development team at SLU.

1.2 Aim

The aim of this project was to investigate how varying processing and formulation parameters could improve the processing steps and sensory characteristics of the blue mould tofu “Bean Blue”. The development was divided into technical and sensory modification. Modification of the technical parameters was to improve efficiency of the processing steps, and changes to the sensory parameters was to improve sensory quality of the final product.

Specific parameters tested in the project included:

Technical modification

- Water:bean ratio: Adjustment of water:bean ratio from 9:1 to 4:1 to see which is most efficient to simplify the processing steps during blending and straining to obtain bean milk.
- Type of method: Test a new method that includes removal of starch from the bean milk and use of 100 % faba bean, and compare with the original method that includes starch and with 70 % faba bean and 30 % soybean.
- Type of lactic acid bacteria: compare homofermentative with heterofermentative starter cultures and their effect on pore formation and blue mould growth inside the curd mass.

Sensory modifications:

- Type of fat: investigate if cooking oil and coconut oil could be replaced by another type of oil (Swedish rapeseed oil)
- Type of mould starter culture: compare two different blue mould starters with different protease- and lipase activity and aroma profiles.

2 Literature review

2.1 Legumes in foods

Legumes have high nutritional value and are of current interest as replacements for animal based foods (Duc, 1997). However, production of functional foods using legumes is a challenge for the food industry. The biggest challenge is to maintain the sensory properties of the product and to gain consumers' acceptance. (Multari *et al.*, 2015). Legumes can be used to improve nutritional value in products, and can support the production of gluten- and milk protein free products. (Multari *et al.*, 2015) They could also provide economic advantages and a functional- and resource saving component (Chechetkina *et al.*, 2016).

Much research has focused on the use of legumes as partial or whole substitutes for traditional food ingredients. Research shows that the use of legume flour as a replacement of wheat flour is an option in cereal products such as baked goods and pasta. (Multari *et al.*, 2015). Legumes have also been introduced in some meat products, where they have been used as binders and extenders to reduce fat content, increase nutritional value and sensory properties (Serdaroğlu *et al.*, 2005). In cheese production, legumes have been used in combination with milk bases for the purpose of creating a more wholesome product. Chickpea flour has been used as a filler in soft cheese from goats' milk. The vegetable substitute in the cheese can contribute to a source of fibers, vitamins, minerals, essential fat and phospholipids that the milk does not contain (Chechetkina *et al.*, 2016).

2.1.1 Legumes in Bean Blue

Soybean is the traditional base in tofu production and has also been used in initial production of Bean Blue. Soy is a popular legume consumed worldwide, and the soybean has a high protein content (Panchal *et al.*, 2016). Products such as soy milk, soy ice cream and frozen soy yoghurt are examples of acceptable products based on

soybean (O'Toole, 2004). The soybean contains small amounts of saturated fat (approximate 20 % fat, of which 2 % is saturated), high levels of protein (40 %) and contains no cholesterol (Zee *et al.*, 1988).

Despite the favorable nutritional properties of soy bean, a raw material with more environmental advantages from a Swedish perspective was a reason for investigating other options in production of Bean Blue. Research has investigated alternative raw material for producing tofu, such as winged bean, peas and faba beans. Studies using faba bean have shown similar functional properties to soybean tofu. (Zee *et al.*, 1988).

Considering the fact that soybean is not suitable for widespread cultivation in the climate of Sweden (only certain areas in southern Sweden, e.g. Gotland), makes faba bean a good option. The prior knowledge of good results using faba bean in tofu production and the desire to develop a product with raw material produced in Sweden makes faba bean a suitable partial or total replacement for soybean in the production of Bean Blue.

Faba Bean

Faba bean belongs to the *Fabaceae* family and occurs in several varieties. Faba is an annual plant and well adapted to most climate areas of Europe (Crépon *et al.*, 2010). The seeds of faba bean can be yellow, green, black, brown or violet, with flowers in white, brown or violet (Multari *et al.*, 2015). The root system contains *Rhizobium leguminosarum* which is a nitrogen fixing bacteria. The plants of faba bean fix nitrogen to a greater extent than other legumes. The nitrogen is converted to ammonia which can act as a natural fertilizer for the plant. This cooperation between the plant and the bacteria excludes the requirement to add nitrogenous fertilizer (Duc, 1997).

The beans of faba have a low content of saturated fat and are a good source of proteins, fibers and minerals. The protein content of faba bean varies from 27-34% of dry matter depending on genotype. Faba bean contain 55-62 % carbohydrates, of which 28-41% is starch, and 10-20 % is fibre (Bhatty, 1973). The lipid content in faba bean is low; approximate 1.5-2 % of dry matter. Faba bean is rich in vitamins such as Vitamin C and A, and minerals such as calcium, phosphorus and magnesium. Levels of nutrients vary depending on cultivar, growth season, fertilization and analysis method. (Zee *et al.*, 1988) The majority (70-80%) of the proteins are globulin proteins, where vicilin and legumin are the major proteins. Both vicilin and legumin have similar composition of amino acids. They are generally rich in aspartic and glutamic acid, leucine and arginine. Faba bean contains a balanced amino acid composition except for low levels of methionine and cysteine. (Multari *et al.*, 2015)

The raw beans of faba have been found to contain factors that may exert anti-nutritional effects. Factors such as tannins, vicine and convicine can have an antinutritional function and reduce digestibility of the seed. Tannins are polyphenols binding different protein compounds together and have been considered as the factor that reduces faba bean protein digestibility and decreases the bioavailability of amino acids. Tannins also contribute to a bitter and unwanted taste in the final product. Vicine and convicine occur mainly in the seed; they are glycosides and involved in plant defense mechanisms against pathogens. Derivatives of vicine and convicine named divicine and isouramil have been found to be toxic to humans who carry a genetic defect. (Crépon *et al.*, 2010). In Europe, cultivars of faba bean are divided in four different groups depending on content of tannins (low or high), and the content of vicine and convicine (low or high). Breeding of the Faba bean has led to improved yield and seed composition. Cultivars that have been shown to be free or low in tannins are suitable for food production. Removal of hull and other processing such as soaking and heat treatment can reduce the amount of antinutritional factors and reduce the detrimental consequences. (Duc, 1997)

2.2 Tofu- and blue mould cheese production

Bean Blue can be called a blue mould tofu due to similarities to the process of tofu- and blue mould cheese production. In tofu production, a coagulant is added after boiling followed by pressing of the curd. In production of Bean Blue, starter culture, microbial coagulant and mould culture is added after boiling followed by draining of the curd and addition of salt similar to blue mould cheese production.

2.2.1 Tofu

Tofu is a protein-rich food widely consumed in eastern countries (Kamizake *et al.*, 2016). The popularity of tofu can be explained by the product's inexpensive cost and versatility. (Panchal *et al.*, 2016). Tofu is a vegan product with soybean as the traditional starting material, and is obtained from bean milk with addition of coagulant. The final product is rich in protein, unsaturated fatty acids and minerals. Also, unlike other common animal-derived protein foods, tofu is cholesterol free (Zee *et al.*, 1988).

The traditional method for making tofu involves specific stages. The first step is to obtain bean milk. This is done by soaking the beans, followed by rinsing and grinding in water, and finally, filtration to separate the soymilk from okara. Okara is the residue of fibers and is not used in tofu production. The bean milk is heated

to 100°C and cooled down to approximately 80°C at which point the coagulant is added and the formation of a gel is started. The coagulation involves interaction between the coagulant and protein and non-protein components in the milk, such as carbohydrates, lipids and phytates (O'Toole, 2004). The proteins at neutral pH in soymilk are negatively charged. By addition of a coagulant, the surface of the proteins become neutralized, which lead to aggregation of the proteins and a gel is formed. Common coagulants used are calcium sulphate, magnesium chloride and gluco-delta-lactone. (Peng *et al.*, 2016) The last steps in traditional tofu production include pressing and shaping of the curd. The curd is placed in forms and pressed, and then cut into cakes and stored under cold water. (O'Toole, 2004)

2.2.2 Blue mould cheese

The most common raw material in cheese manufacturing is milk from cows or goats. Different types of cheese have different processing steps. The process in blue mould cheese production typically includes pasteurization and standardization of the milk and addition of starter culture, coagulant and mould culture. Later the curd is shaped, pressed and ripened. Optimum maturation and storage conditions for soft cheeses are approximately 10-12°C and relative humidity 70 % which protects the cheese from spoilage or losing flavor. (Walstra *et al.*, 2005).

Starter culture – lactic acid bacteria

The starter culture is added after heat treatment of the milk. The culture provides the product with specific texture and aroma compounds, as well as decreasing the pH via conversion of lactose to lactic acid, which promotes coagulation. A starter culture is one or more species or strains of lactic acid bacteria (LAB) which are added to ferment the milk. There are currently 12 genera of different LAB and five of them are commonly used in dairy fermentation: *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Lactobacillus* and *Enterococcus*. (Walstra *et al.*, 2005)

Lactose is the major energy source in milk for LAB. The LAB convert glucose into lactic acid and other products. Homofermentative LAB produce solely lactic acid as fermentation product, whereas heterofermentative LAB produce additional fermentation products such as gas (CO₂) and diacetyl, among others. LAB differ in optimal growth temperatures, and the thermophilic LAB prefer higher temperatures compared with the mesophilic. Both meso- and thermophilic LAB are used in cheese production. (Walstra *et al.*, 2005)

The starter culture has a major impact on the properties of the final product. The rate of lactic acid production is dependent on the activity of the starter culture. Pro-

teolysis is important for ripening and affects the texture. Production of CO₂ is essential for the texture in many blue mould cheeses since the formation of openings is important for mould growth inside the cheese mass. (Walstra *et al.*, 2005)

Coagulant

The addition of coagulant is essential to the curd formation of the cheese. Sometimes only LAB is used as coagulant and provides the product with lactic acid as the primary coagulant, and sometimes additional enzymes or acids are used for coagulation.

Rennet is used in enzymatic coagulation and is provided from the stomach of the calf and is often used in hard cheese. The rennet enzymes cut the negatively charged kappa caseins on the neutral charged micelle. The neutralization lead to aggregation of the casein micelles and lead to coagulation. (Walstra *et al.*, 2005) Acids are often used in production of soft cheeses like cottage cheese, quark and chevre. The addition of an acid neutralizes the negative charged kappa casein surrounding the micelle. The neutralized casein micelles start to aggregate and coagulation occurs. (Walstra *et al.*, 2005) Coagulants based on vegetables and microbial coagulants can also be used in cheese production (García *et al.*, 2012) as an option for vegan products such as Bean Blue.

Mould

The mould culture is often added after heating and before the curd is placed in forms. The cheese is also needled to provide the mould with oxygen for optimum growth. *Penicillium roqueforti* (PR) is the blue mould species commonly used to give flavor and ripen the blue mould cheese. The mould cultures will upon germination develop a mycelium in the cheese and contribute to the typical flavor (by lipolysis) and texture (by proteolysis) of the cheese. (Walstra *et al.*, 2005)

2.3 Sensory tests

Development of new food products is a long and intensive process (Naes & Nyvold, 2004). Evaluation through sensory tests is an important step before the product is launched, as an investigation of the product's potential on the market (Fuller, 2011). Products can be evaluated through different types of sensory methods which are divided into analytical and affective test methods. Consumer test is an affective test method where the degree of liking or disliking of a product is quantified. Through a consumer test, knowledge can be obtained about which among several samples the consumer prefers and if the samples differ from each other. Affective methods can be used in combination with analytical methods where the actual differences

between the samples are investigated. An examples of an analytical sensory method is a description test. The test is performed by a trained panel (6-14 participants) judging the intensity of different properties of the product. The result can be used in marketing to describe the product, or to suggest necessary changes in a recipe (Lawless & Heymann, 2010).

3 Method

The method used is based on the development team's experience of tofu- and cheese making and relevant literature. The development of Bean Blue is divided into technical and sensory trials based on specific parameters. All development and production of Bean Blue was performed at laboratory scale at SLU. The starting recipe (Appendix 1) and original method of Bean Blue were based on previous trials performed by the researchers of the development team at SLU and RISE. The processing steps of Bean Blue can be seen in appendix 2 with an overview of all trials that have been performed.

3.1 Material

The main raw material used in Bean Blue production is the Swedish faba bean cultivar 'Gloria'. In the initial stage of the development, Canadian soybean was also used. Additional ingredients in the production are fat, lactic acid bacteria starter culture, glucose monohydrate, mould culture, microbial coagulant, lactic acid, sodium hydroxide and sodium citrate. Different amounts and combinations of the ingredients vary in the different trials. Chemicals used were food grade.

The equipment used for production of Bean Blue is a kitchen blender, heating plate, water bath, cheese cloths, cheese forms, pH meter and incubator with temperature and humidity control. All the equipment was washed thoroughly and cheese forms and cloths were boiled before use.

3.1.1 Fat

Two different types of oils were used: cooking oil and rapeseed oil. Fat used in earlier development in production of Bean Blue was cooking oil and coconut oil. Coconut oil was decided to be eliminated (for environmental sustainability reasons), due to good results only using cooking oil in the first trial. The cooking oil is a

mixture of sunflower oil, rapeseed oil and soy oil and with a content of 13.3 g saturated fat per 100 g. The rapeseed oil is produced in Sweden and contains 7 g saturated fat per 100 g oil.

3.1.2 Starter cultures

In the production of Bean Blue, the LAB and the lactic acid / pH drop that they generate are essential to the coagulation and formation of the curd. The pH needs to be below 5 within 12 hours after inoculation to secure a safe product i.e. prevent growth of pathogenic bacteria. Two different starter cultures were used, which we will designate LAB-C and LAB-L. Amount of the starter culture used depended on the volume of bean milk. The cultures used are adopted for dairy products and the amount used in Bean blue was decided to be 10 x more than the recommended inoculation ratio to obtain a strong fermentation. Glucose monohydrate was added to provide the LAB with glucose to be able to start fermentation and produce lactic acid. Amount of glucose monohydrate was added according to the volume of bean milk, and had been previously shown by the development team to support the necessary decrease in pH within the given time frame.

LAB-C is a thermophilic starter culture with culture composition of *Lactobacillus delbrueckii* subsp *bulgaris*, *Lactobacillus rhamosus* and *Streptococcus thermophilus*. The starter culture comes in frozen pellets form and is suitable for stirred and set fermented milk products i.e. yogurts. LAB-C is a homofermentative culture and produces lactic acid with mild creamy flavor, extra high viscosity and low post-acidification. The culture is based on dairy ingredients. Optimum fermentation occurs at 40°C.

LAB-L is a freeze dried mesophilic culture with a high content of aroma and gas (CO₂) producing lactic acid bacteria: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp *lactis*, and defined strains of *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis* and *Leuconostoc* ssp. LAB-L comes in powder form and is used in production of fresh- and soft cheese. Optimum fermentation of the culture occurs at 32°C. The culture is heterofermentative and produces lactic acid and CO₂; it requires sodium citrate as a substrate for producing aroma and CO₂. The CO₂ is needed to produce pores in the curd to permit better mould growth inside. Tri-Sodium citrate dihydrate was added in batches where LAB-L was used.

3.1.3 Mould cultures

Two different types of *Penicillium roqueforti* cultures were used (denoted PR1 & PR2). Amount of culture used was adjusted according to g dry beans. The two cultures are similar in flavor profile and both are used in cheeses such as Stilton and

Danablu. PR1 is a liquid mould culture consisting of a single strain of dark blue-green *Penicillium roqueforti*. The culture has a high/medium proteolytic activity and medium lipolytic activity. PR2 is a single strain culture and provides the product with a blue mycelium. The culture is in powder form and has a high effect on proteolysis, and medium effect on lipolysis.

3.1.4 Microbial coagulant

A microbial coagulant denoted MC was used. The coagulant is produced by fermentation of the mould *Rhizomucor miehei* to extract an aspartic proteinase. MC is recommended to be used in soft cheeses, and was added according to g dry beans. MC was not mainly used in this product as a coagulant, since the bean milk does not contain casein proteins, and thereby its effect on coagulation of the bean milk proteins is unknown. Earlier trials by the development team showed that Bean Blue with MC resulted in a more creamy texture and taste than without addition of MC, hence MC was used in all trials in the current study.

3.2 Technical trials

The technical trials included testing of different parameters with the purpose to optimise the processing steps of Bean Blue. The technical trials investigated one factor at a time. Six different technical trials were performed and the results from each trial were evaluated from a technical point of view during the production. Also sensory properties were evaluated by tasting by the development team. The evaluation of the sensory properties was done using free language and the overall experience of the samples was noted.

The different parameters that the technical trials included were:

- Different water:bean ratio used.
- Two different methods including removal of starch or not; testing if it was possible to achieve suitable curd formation based on 100 % faba bean.
- Different types of LAB and their effect on pore formation in the product.

3.2.1 Water:bean ratio

The first trial included comparison between the original water:bean ratio (9:1) and decreased ratio (4:1). The purpose of the trial was to reduce the water content to enhance handling of the product in the different processing steps. A decrease in

water ratio could hypothetically improve efficiency and shorten time in handling during pressing out okara, draining of the curd and coagulation.

Trial 1

The first trial was made according to the original method and two different batches were made (Table 1). Batch 1 was made with the original water:bean ratio (9:1) and batch 2 with reduced water:bean ratio (4:1). 70 % faba beans and 30 % soybeans was used in both batches.

Table 1. *Trial 1 with water:bean ratio 9:1 and 4:1.*

Batch	Faba:soy ratio	With / without starch	Water:bean ratio	Coagulant
1	70:30	With	9:1	LAB-C
2	70:30	With	4:1	LAB-C

3.2.2 New method including removal of starch and 100 % faba beans

Earlier development of Bean Blue used 70 % faba bean and 30 % soybean. The new method (Zee *et al.*, 1988) is similar to the original method and can be seen in appendix 2. The main differences between the methods is removal of the starch, and the use of 100% faba beans. To create separation of the starch, 5 M Sodium hydroxide (NaOH) was used to adjust the pH of the bean milk. The initial pH of 6.3-6.6 in the bean milk was adjusted to pH 8.0. The increase in pH makes the proteins soluble which prevents them from precipitating with the starch sediment. The solid phase containing starch was separated from the rest of the bean milk by decanting. 90 % lactic acid was used to adjust the pH to 6.2 after removal of the starch. The separated starch sediment was dried at 105° for 24 hours and analyzed by adding iodine to ensure the removed sediment primarily contained starch. Samples of final product with 100 % faba bean, and 70 % faba bean were also analyzed for starch content. The starch analysis including staining and microscopy was performed by Daniel Johansson at SLU.

Trial 2

The second trial included production of Bean Blue according to the new method for starch removal. Two batches were made (Table 2); batch 1 with water:bean ratio 9:1, and batch 2 with water:bean ratio 4:1. Both batches were made without LAB as coagulant and lactic acid was used instead combined with the new method.

Table 2. *Trial 2, new method for starch removal, water:bean ratio 9:1 and 4:1, lactic acid as coagulant*

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	Coagulant
1	100:0	Without	9:1	Lactic acid
2	100:0	Without	4:1	Lactic acid

Trial 3

Since LAB is an important ingredient in blue mould cheese production, two batches with LAB-C as coagulant instead of lactic acid also were prepared according to the new method (without starch) with two different water:bean ratios (Table 3).

Table 3. *Trial 3, water:bean ratio 9:1 and 4:1, with LAB-C as coagulant*

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	coagulant
1	100:0	Without	9:1	LAB – C
2	100:0	Without	4:1	LAB – C

Trial 4

After evaluation of the water:bean ratio in trial 1, and the new method (without starch, 100 % faba bean) in trials 2-3, three different batches based on the original method and the new method were made to be able to compare them with each other. The water:bean ratio was selected as 4:1 and LAB-C was used as a coagulant based on evaluation from trials 1-3.

Three batches were made in trial 4 (Table 4). Batch 1 was made according to the original method (with starch, 70 % faba bean and 30 % soybean). Batch 2 was made according to the new method (without starch and 100 % faba bean) and batch 3 was also made according to the new method (without starch) but with 70 % faba bean and 30 % soybean. A batch with 100% faba according to the original method (with starch) was excluded due to earlier trials by the development team in which the bean milk failed to coagulate. Trial number 4 was repeated two times.

Table 4. *Three batches were made in trial 4; one using 70 % faba and 30 % soy (with starch), one using 100 % faba (without starch), and one using 70 % faba and 30 % starch (without starch). All with water:bean ratio 4:1 and LAB-C as coagulant.*

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	Coagulant
1	70:30	With	4:1	LAB – C
2	100:0	Without	4:1	LAB – C
3	70:30	Without	4:1	LAB – C

3.2.3 Heterofermentative starter culture

Trials 5 and 6 were performed with a heterofermentative LAB (LAB-L). This was to investigate differences in pore formation and mould growth inside the final product. Mould growth has so far mostly appeared on the outside of the product, with occasional growth along the needle-holes after extended incubation. By using LAB-L, the possibility to create a porous structure inside the curd mass permitting mould growth inside Blue Bean was investigated.

Trial 5

The batches were produced according to the new method (without starch and 100 % faba bean), water:bean ratio 4:1 and LAB as coagulant based on evaluation from previous trials. Two batches with different amounts of LAB-L culture were made (Table 5). The difference in amount LAB added was to investigate if the increased amount of starter culture could increase the rate of the pH drop. In batch 1, LAB-L was added at 10x more than the recommended inoculum rate (similar to LAB-C in previous trials, also added at 10x), and in batch 2, 50x more than the recommended inoculum rate was added.

Table 5. *Trial 5 with different amount LAB-L used (x10 and x50). Both with 100 % faba, without starch, water:bean ratio 4:1*

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	Coagulant
1	100:0	Without	4:1	LAB – L (x10)
2	100:0	Without	4:1	LAB – L (x50)

Trial 6

The 6th trial was performed after observations that the decreased water:bean ratio (4:1) and the new method that include addition of lactic acid to adjust pH to 6.2 before heating resulted in a thicker milk after boiling, with different coagulation and curd properties compared with the original water:bean ratio (9:1) and method. The difference in properties of the bean milk and coagulation and curd properties can have an effect on the properties of the final product.

Trial 6 included three different batches with addition of lactic acid at different stages (Table 6). In batch 1, lactic acid was added before heating to bring the pH to the initial 6.2 (after solubilisation of protein via addition of NaOH according to the new method). In batch 2, lactic acid was added after heating, and no lactic acid was added in batch 3, i.e. all reduction in pH was due to activity of the LAB-L starter culture. The batches were performed according to the new method (without starch and 100 % Faba bean) and water:bean ratio was 4:1. LAB-L was used for further

investigation of the effect of a heterofermentative LAB. Amount LAB used was according to original amount (10× recommended inoculation rate) due to results from trial 5. To investigate if the decreased water:bean ratio (4:1) also could be a contributing factor for changes in the milk properties, the Trial 6 was repeated but with water:bean ratio 6:1 (Table 7).

Table 6. Trial 6. Lactic acid for adjustment of pH was added at different stages; before boiling, after boiling and in one batch was lactic acid omitted.

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	Coagulant	Addition of lactic acid
1	100:0	Without	4:1	LAB – L (x10)	Before boiling
2	100:0	Without	4:1	LAB- L (x10)	After boiling
3	100:0	Without	4:1	LAB- L (x10)	No lactic acid

Table 7. Trial 6 with water:bean ratio 6:1 instead of 4:1.

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	Coagulant	Addition of lactic acid
1	100:0	Without	6:1	LAB – L (x10)	Before boiling
2	100:0	Without	6:1	LAB – L(x10)	After boiling
3	100:0	Without	6:1	LAB- L (x10)	No lactic acid

3.3 Sensory trials

The sensory trials included two different parameters that could help improve the sensory quality of the product. Two different trials were included in the sensory modification. Trial 7 included two different types of fat and was evaluated by a consumer test. Trial 8 was performed using factorial design with two factors at two levels. The samples were evaluated by a descriptive test with a panel.

Parameters that the sensory trials tested were:

- Type of fat
- Type of mould

3.3.1 Type of fat – consumer test

Trial 7

Two batches with two different fats were made in trial 7. The purpose of this trial was to investigate if cooking oil could be replaced with rapeseed oil without changes

in sensory properties. The batches were made according to the new method (without starch, 100 % faba bean) and with water:bean ratio 4:1. LAB-C was used due to undesirable results using LAB-L in trials 5-6. In batch 1, cooking oil was used, and in batch 2, rapeseed oil was used (Table 8).

The samples were evaluated through a consumer test at an event arranged by the Royalty Institute of Technology (KTH) in Stockholm. The number of consumers who participated in the test was 118. Each consumer was asked to taste the two different samples one by one, and estimate on a scale from “dislike very much” to “like very much” their opinion of the taste of the samples (answer forms can be seen in appendix 3). The consumer also filled in if they experienced any difference between the samples. They were also asked to fill in the overall experience of the product. The samples were served on the same plate and marked “1” and “2”. Number 1 was the formulation with cooking oil, and 2, with rapeseed oil for the first participants, and the order of the samples was reversed after 77 participants. The result was analyzed by calculating mean, minimum, median, maximum and quartile values (Q1 and Q3) and presented in a box and whiskers plot.

Table 8. *Trial 7, consumer test with cooking oil and rapeseed oil.*

Batch	Faba:soy ratio	With/without starch	Water:bean ratio	Coagulant	Type of fat
1	100:0	Without	4:1	LAB – C	Cooking oil
2	100:0	Without	4:1	LAB – C	Rapeseed oil

3.3.2 Type of fat and mould – descriptive test

Trial 8

Two different types of fat (cooking oil and rapeseed oil) and two different types of mould (PR1 and PR2) were used in trial 8. In total, four different batches were made according to factorial design with two factors at two levels (Table 9). Factor number one was type of fat, at two different levels (level 1 = cooking oil, level 2= rapeseed oil). Factor two was type of mould at two different levels (level 1= PR1, level 2= PR2). This resulted in four different batches where each factor was represented at each level (Table 10). The batches were prepared according to the new method (without starch and 100 % faba beans), 4:1 water:bean ratio and with LAB-C as coagulant.

The samples were judged by a descriptive test. Only two of the batches (3 and 4) were included in the test due to yeast contamination of batches 1 and 2. Batches 3 and 4 representing the two different oils with mould culture PR1. The test panel consisted of 14 participants with people from the development team, students and

employees at SLU and RISE. The panel was asked to estimate the intensity on a scale from “low” to “high” of six different properties. The panel was also asked to comment on overall impression of the product (see form in appendix 4). The properties evaluated were: mouldy odor, creaminess, salt-, sour-, beany-, and blue mould taste. The properties were predetermined before the test (see properties and definition in appendix 4). The properties and definition were presented for the panel before the tasting, they also had the definitions available on a separate paper during the test. The samples were judged individually by each panelist, and both the samples were served on the same plate with a three number code (see serving order in kitchen list in appendix 4). The test was performed at a conference room at SLU. The result was analyzed by calculating mean, minimum, Q1, median, Q3 and maximum values and presented in a spider diagram.

Table 9. *Factorial design with two factors (type of fat and type of mould) at two levels (cooking oil/rapeseed oil and PR1/PR2).*

Factor	Level 1	Level 2
Type of fat	Cooking oil	Rapeseed oil
Type of mould	PR1	PR2

Table 10. *Four different batches representing each factor at each level for descriptive sensory analysis.*

Batch	Type of fat	Type of mould
1	Rapeseed oil	PR2
2	Cooking oil	PR2
3	Rapeseed oil	PR1
4	Cooking oil	PR1

4 Results

4.1 Technical trials

The results of the technical trials are divided into aspects regarding a) handling of the bean milk during removal of okara and curd draining, b) coagulation, pH and properties of the curd, and c) sensory properties of the final product. The samples were evaluated during production and after 12-16 days of incubation.

4.1.1 Water:bean ratio

Trial 1 (9:1 vs 4:1)

Handling: The result showed that water:bean ratio 4:1 is easier to handle in the step where the okara is removed due to less water to press out. Using 4:1 resulted in a thicker curd with shorter coagulation and draining time compared with 9:1. However, a decreased water:bean ratio cause problems during the heating (boiling) step and was easily burned compared with 9:1. This problem was addressed in subsequent trials by vigorous stirring during heating.

Coagulation and curd properties: The coagulation started directly after inoculation of LAB in the batch with water:bean ratio 4:1. Ratio 4:1 resulted in a more homogeneous and thicker curd than 9:1, where the coagulation was more similar to tofu production with a distinct coagel and separation between the curd and the water phase. The curd of 4:1 was like a thick yogurt with no distinct coagel (Figure 1). The pH after adding LAB showed a faster drop in the batch where water:bean ratio 9:1 was used compared with 4:1. In the batch with 9:1, the pH decreased from 6.7 to 4.9 within 3 hours. In the batch with 4:1 water:bean ratio, the pH only decreased from 6.5 to 5.8 within the same time period. It took 14 hours for the batch with ratio 4:1 to reach below pH 5.

Sensory properties: The two batches were tasted after 14 days incubation. The result showed that sample with 4:1 had a more intensive and creamy taste and was less watery in the texture compared with 9:1. Ratio 4:1 also had less beany taste than 9:1. The outer appearance of the two batches was similar (Figure 2).



Figure 1. Curd properties after adding LAB, To the left; water:bean ratio 4:1. To the right; water:bean ratio 9:1. (Photo: Martina Näsholm)



Figure 2. Tasting after 14 days incubation. To the left: water:bean ratio 9:1. To the right: water:bean ratio 4:1. (Photo: Martina Näsholm)

Conclusion water:bean ratio: The decrease in water:bean ratio was done to improve efficiency in the step where the okara is removed, and to shorten the draining step. Water:bean ratio 4:1 was easier to handle regarding removal of the okara, and had shorter draining and coagulation time. The curd in 4:1 did differ in curd properties and was more homogeneous like thick yogurt. Ratio 4:1 got easily burned during the heating step, and the pH drop was slower compared in the batch where ratio 9:1 was used. The sensory properties were better in 4:1, perceived as less beany taste and creamier taste and texture.

4.1.2 New method including removal of starch and 100 % faba beans

Trial 2 (new method, coagulant - lactic acid)

Handling: The new method included extra steps and required longer time to implement compared with the original method due to removal of the starch. The removal of starch by manually adjusting pH with NaOH and lactic acid requires accuracy and can lead to errors. The starch sediment was easily removed and a distinct separation in the bean milk appeared.

Coagulation and curd properties: Lactic acid were used for coagulation instead of LAB as a test to see if coagulation was possible with 100 % faba beans, if starch had been removed. The coagulation started directly after adding lactic acid in both batches, and both had a clear coagulation with separation of the coagel and water phase, similar to the original recipe and batch with water:bean ratio 9:1 in trial 1. The pH was adjusted to below 5 before draining.

Sensory properties: The final samples were never evaluated by the development team due to sour taste and dry texture. The yield from an equivalent mass of dry beans was almost half compared with cheeses in trial 1. The samples can be seen in Figure 3.



Figure 3. Samples according to the new method (100 % Faba beans, without starch) after 13 days incubation. To the right; water:bean ratio 9:1. To the left: 4:1. (Photo: Martina Näsholm)

Trial 3 (new method, coagulant – LAB-C)

Handling, coagulation and curd properties: The handling in the processing steps was similar to trial 2. The coagulation started directly after addition of LAB and the pH decreased to below 5.0 in both batches during coagulation and before draining the curd (within 3 hours). The properties of the curd was similar to the curd in trial 1 with water:bean ratio 4:1 and resulted in a more homogeneous yogurt-like curd compared with 9:1, that had a more distinct separation of the coagel and the water phase.

Sensory properties: The two batches were tasted after 12 days' incubation. The batch with water:bean 4:1 ratio was more appealing regarding taste and texture to the informal panel. The batch with 4:1 was less watery and more creamy in the taste compared with batch with water:bean ratio 9:1.

Overall conclusion water:bean ratio and new method: The technical evaluation of the samples from trial 1-3 showed that water:bean ratio 4:1 was easier to handle and had shorter draining and coagulation time. However, the bean milk with decreased water:bean ratio got easily burned in the heating step; this was avoided by careful monitoring and vigorous stirring. Ratio 4:1 resulted in different properties of the curd compared with 9:1, where 4:1 was more homogenous and creamy.

The new method including removal of the starch requires some extra steps in the process and takes longer time, since the bean milk was required to stand for 2 hours for the starch to sediment. Lactic acid as coagulant resulted in sour taste and dry texture and almost half the yield compared with the original method, however it was positive that acid coagulation of 100 % faba beans was possible when starch is removed. The result of the sensory properties of samples 1-3 showed that sample from trial 3 (without starch and 100 % faba beans, water:bean ratio 4:1, and with LAB as coagulant) was preferred due to creamy taste and texture, and being less watery. After the evaluation of the samples from trial 1-3, it was decided to go further with water:bean ratio 4:1 and with LAB-C as coagulant for trial number 4 where the two methods were compared.

Analyse of starch

The analysed dried starch sediment appeared to be mainly starch. Dried starch in the sediment colored in purple; final product with 100 % faba bean (without starch) shows primarily protein colored in green with no starch is present; and final product containing 70 % faba bean and 30 % soybean (with starch) clearly shows both starch granules colored in purple and protein colored in green (Figure 4).



Figure 4. To the left: Dried starch colored in purple. In the middle: Sample with 100 % Faba bean, protein colored in green. To the right: Sample with 70 % Faba bean and 30 % Soybean. Protein colored in green and starch in purple. (Photo: Daniel Johansson)

Trial 4 (original vs new method)

Handling: The handling in the process steps and curd property was similar to earlier trials when 4:1 was used.

Coagulation, curd properties: Coagulation started direct after adding LAB, but the decrease in pH was slow in both batches and did not reach below pH 5 during coagulation or before draining, and nor within the first 12 hours. The pH was 5.1 (12 hours after addition of LAB) when the samples were incubated. The pH reached 4.3-4.8 after one day incubation (48 hours after addition of LAB). The slow pH drop resulted in bacterial contamination visible on the surface of the product after 16 days in incubation.

Sensory properties: The batches were evaluated by tasting after 13 days incubation. The result showed that batch with 100 % faba bean (without starch) compared with batch with 70 % faba bean and 30 % soybean (with starch) did not differ in appearance, taste and texture. Neither did the batch with 70 % faba bean and 30 % soybean without starch differ.

Trial 4 was repeated, however, a slow pH drop was again observed. This resulted in contamination and bacteria growth after 12 days in incubation and the samples were discarded without tasting (Figure 5).



Figure 5. Contaminated samples after 12 days incubation from trial 4 due to high pH. (Photo: Martina Näsholm)

Overall conclusion original vs new method: Using the new method that included removal of the starch with 100 % faba beans did not differ much in sensory properties compared with the original method with 70 % faba bean and 30 % soybean. Since previous trials by the development team that included the use of 100 % faba bean according to the original method resulted in no coagulation, the removal of starch seems to enable the use of 100 % faba for the product. Since the use of 100 % faba bean is more favorable considering environmental factors compared to soybean, 100 % faba bean according to the new method (without starch) was chosen for trial 5, although the high pH that allowed bacterial contamination to occur was of concern.

4.1.3 Heterofermentative starter culture

Trial 5 (LAB-L x10 and x50)

Handling, coagulation and curd: The handling, properties of the curd and coagulation was similar in both batches and was similar to previous trials using 4:1 water:bean ratio and 100 % faba beans. The pH did not decrease below 5 before curd draining, but reached below 5 within 12 hours. The result showed that 50x inoculum compared with standard 10x inoculum of LAB-L did not result in faster pH drop.

Sensory properties: The samples were not evaluated by the whole development team due to no mould growth in both batches after 14 days incubation. After 23 days

incubation, the mould growth was poor and uneven (Figure. 6). Figure 6 also shows samples after 36 days incubation. The LAB-L products were similar in texture to those previously made using LAB-C; however, pore formation and mould growth inside the samples was not observed. The samples with 50x inoculum rate showed less mould growth than those with 10x inoculum, and were drier in texture. All samples had a sour and metallic taste.



Figure 6. Batches with LAB-L incubated for 23 days. To the left: 10x times inoculum rate. In the middle: 50 x inoculum rate. To the right: Batches with LAB-L incubated for 36 days. (Photo: Martina Näsholm)

Trial 6: Adjustment of pH before boiling, after boiling, or omitted. (Water:bean ratio 4:1 and 6:1)

Handling, coagulation and curd properties: The result of addition of lactic acid at different stages in batches with water:bean ratio 4:1 showed that batch 1 (adding lactic acid to pH 6.2 before heating) had similar properties of the curd as in previous trials using 4:1 water:bean ratio (homogeneous curd like thick yogurt). The bean milk was thicker during heating compared with when lactic acid was added after heating. In batch 2 (adding lactic acid to pH 6.2 after heating), the curd had properties similar to cottage cheese with small coagel formation, but no clear separation of the coagel and water phase as with 9:1 water bean ratio in earlier trials was observed. The coagulation required longer time in batch 3 (no lactic acid added, pH after heating 7.7) and the properties of the curd was similar to batch 1. In all batches, the pH was not below 5 before curd draining, but had attained this level within 12 hours. Batch number 3 required almost double the coagulation time to reach the same pH as batches 1 and 2.

The three batches with water:bean ratio 6:1 did not show substantial differences in coagulation or curd properties compared with the corresponding batches using water:bean ratio 4:1. However, the increased amount of water resulted in a more watery curd and thus a longer draining step. The use of 6:1 water:bean ratio did not result in a faster pH drop.

Sensory properties: The samples showed poor mould growth after 12 days in incubation for both 4:1 and 6:1. No changes were observed in texture compared with

using LAB-C. The samples were not evaluated by the whole development team. The taste of the samples was sour with metallic taste similar to samples in trial 5. Mould growth was uneven and poor during the first 30 days in incubation. The samples were discarded after 40 days incubation.

Overall conclusion using heterofermentative LAB-L: The LAB-L starter culture did not show any difference in texture or mould growth inside the product. The mould growth was poor in all batches and the incubation time before visible mould growth was double compared with LAB-C in earlier trials. Increased amount LAB added did not result in faster pH drop. No further trials were performed with LAB-L due to the poor and irregular mould growth, no changes in texture compared with LAB-C (i.e. no porous structure in the product was observed), and sour and metallic taste of the final product. In subsequent trials, it was decided to continue with addition of lactic acid before heating (as according to the method), due to no difference in texture between the final products being observed when lactic acid was added after boiling or omitted.

The key result from the technical trials is summarised in figure 7.

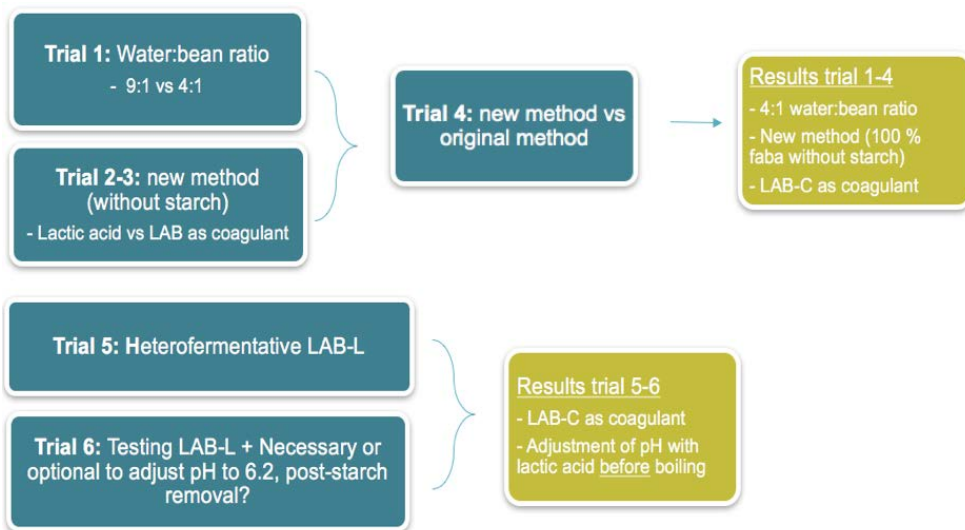


Figure 7. Overview of key results from the technical trials.

4.2 Sensory trials

The sensory modification was evaluated by a consumer- and a descriptive test after 21-22 days incubation of the final product.

4.2.1 Type of fat – consumer test

Trial 7 (cooking oil vs rapeseed oil)

In total, 118 persons participated in the consumer test. The results of the consumer test were summarised by measuring the scores on the scale from each participant. The scale was 65 mm, where the midpoint was assumed to be equal to 0 “neither dislike or like”, 32.5 mm as the highest value “like very much”, and -32.5 mm as the lowest value on the scale “dislike very much”. The scores of each sample were summarized, and mean, minimum, maximum, median and quartile values were calculated (Table 11).

The result shows that sample 2 (rapeseed oil) has a higher mean value (6.5) compared with sample 1 (cooking oil) that has mean value 6.3. However sample 1 had a higher median (7.5) than mean value, which indicated that the data is “skewed to the left”, with a long tail of low scores which are “pulling” the mean down more than the median. Both samples had the lowest possible minimum value (-32.5) as well as the highest possible maximum value (32.5). The value of the first quartile (Q1) represents 25 % of the answers and was 0 in both samples. This means that 25 % of the answers were 0 or below i.e. on the “dislike-side” of the scale. The third quartile (Q3) represents 75 % of the answers and was 17.5 for sample 1, and 18 for sample 2. This means that 75% of the answers were 17.5 or below for sample 1, and 18 or below for sample 2. High Q3 demonstrates overall higher scores on the scale. The p-value was 0.93 which indicates no significant difference between the two samples at 95 % significance level. The result is also presented in a box and whiskers plot (Figure 8).

Table 11. Mean, minimum, Q1, median, Q3, maximum, standard deviation and p-value of samples 1 (cooking oil) and 2 (rapeseed oil).

Sample	Mean	Min	Q1	Median	Q3	Max	Standard deviation	P-value
1 (Cooking oil)	6.3	-32.5	0	7.5	17.5	32.5	15.8	0.93
2 (Rapeseed oil)	6.5	-32.5	0	6.3	18	32.5	15.9	

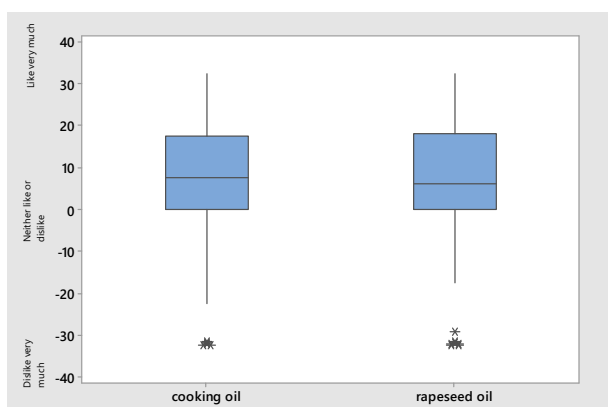


Figure 8. Box- and whiskers plot showing median in the box, quartiles denoted by a line, minimum and maximum (whiskers), and outliers as stars.

4.2.2 Type of fat and mould – descriptive test

Trial 8 (cooking oil, rapeseed oil, PR1, PR2)

The panel consisted of 14 participants in the descriptive test. The result of the test was summarized by calculating the mean from the score values for each property for both samples. The scale was 0-100 mm where 0 mm is equal to low intensity and 100 mm is equal to high intensity. Table 12 presents mean, minimum, Q1, median, maximum, Q3, standard deviation and p-values of each property for sample 1 (cooking oil + PR1) and sample 2 (rapeseed oil + PR1). The mean of the properties is similar for each sample. However mouldy odor is the property that differs the most between the samples, where sample 1 had mean 34.1 and sample 2 had mean 25.5. Blue mould odor also had the lowest mean values compared with other properties. Creaminess had the highest mean value for both samples (73.8 respectively 75.7). The p-value for each property indicates that there were no significant differences between the two samples for each property at significance level 95 %.

Table 12. Mean, minimum, Q1, median, maximum, Q3, standard deviation and p-value of sample 1 (cooking oil) and 2 (rapeseed oil).

Category	Property	Sample	Mean	Min	Q1	Median	Q3	Max	Standard deviation	P-value
Odor	Blue mould	1	34.1	10	15	31.3	50.6	70	19.3	0.265
		2	25.5	0	8.8	17.5	43.8	60	20.5	
Texture	Creaminess	1	73.8	30	68.8	76.3	87.5	95	18.3	0.796
		2	75.7	20	62.5	78.8	93.1	100	21.3	
Taste	Salt	1	62.9	27,5	43.8	68.8	79.4	90	20.3	0.828
		2	61.4	32,5	50	63.8	72.5	80	13.25	

Sour	1	50.2	10	30	57.5	71.3	77.5	24.2	0.705
	2	53.2	25	38.8	56.3	66.3	80	17.1	
Beany	1	49.1	15	30	50	66.3	82.5	20.8	0.886
	2	47.7	10	10	57.5	70	90	30.3	
Blue mould	1	45.6	7.5	26.9	50	62.5	75	21.8	0.529
	2	40.1	0	25.6	45	52.5	75	22.6	

The result is also presented in a spider diagram where the mean of each property is shown for samples 1 and 2 (Figure 9). Figure 10 shows key result from the sensory trials with suggested recipe for further development of Bean Blue including 100 % faba bean, 4:1 water:bean ratio, rapeseed oil, LAB-C, PR1 and microbial coagulant.

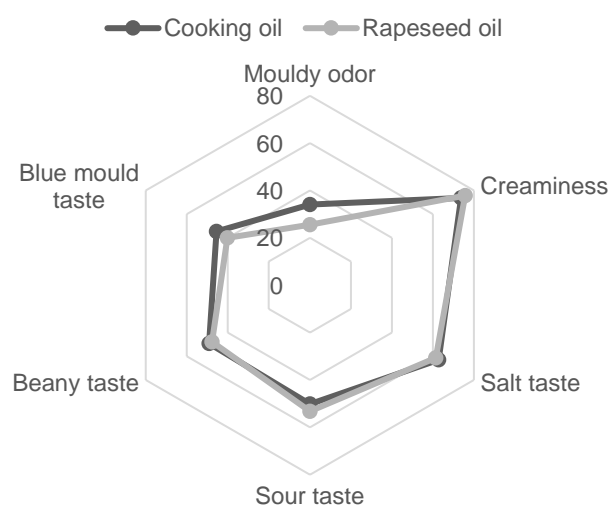


Figure 9. The spider diagram shows mean value of each property of samples 1 (cooking oil + PR1) and 2 (rapeseed oil + PR1).

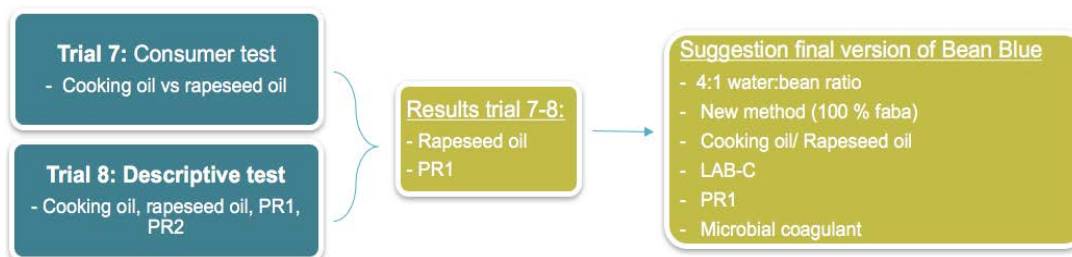


Figure 10. Key results from the sensory trials with suggestion of final version of Bean Blue.

5 Discussion

Since the purpose of this project was to optimise both the process and sensory quality, the different parameters were divided into technical- and sensory trials. The purpose with the technical trials was to optimise the process, and the purpose with the sensory trials was to optimise the sensory quality. However the technical trials can have effect on the sensory quality, and the sensory trials can have effect on the process. An important goal was to optimise the technical parameters without having negative effects on the sensory properties, therefore the technical modifications were evaluated considering not only a technical point of view, but also the sensory properties of the final product.

The technical modifications were performed by testing one parameter at a time. This was due to limitations in manufacturing, since the product is made on a small scale as a “handcrafted” product. Production of several different batches simultaneously was not possible. The size of the batches was adapted to the capacity of the equipment and number of manufacturers (team members) involved. Reasonable and standard amount of dry beans used in the trials was 250 g dry beans per batch, which yields a round product of approximately 250 g (approx. diameter 9 cm × 7 cm high). By testing one parameter at a time, the interaction between the different parameters was not included. It would have been preferable to test several parameters according to factorial design, in order to get an indication about how the different parameters interact with each other and their individual and combined effects (Lawless & Heymann, 2010). Factorial design was not performed due to limitations to manufacture several batches at the same time, apart from in the last trial for the sensory modification tests, however the interactions between the different factors was not possible to evaluate due to yeast contamination of two batches.

Water:bean ratio

The result from using water:bean ratio 4:1 instead of 9:1 optimised the technical parts in the production by pressing out the okara easier and faster and decreasing curd draining time. However, using less water caused some problems due to the

bean milk more easily getting burnt during the heating step. This was solved with more stirring during the heating step in the production of Bean Blue, and should not be a problem in larger scale productions due to additional options for heating equipment (e.g. not just heat applied to the bottom of the vessel via a hotplate), automated stirring, and larger volumes of bean milk.

The decreased water:bean ratio also changed the sensory properties in the final product. By using 4:1, the properties of the curd and coagulation were changed, which resulted in a more creamy product. This is a key result for further trials, that if a creamy texture is desirable, water:bean ratio 4:1 can be used. If the curd and coagulation should be more similar to tofu with a less creamy texture of the final product, a water:bean ratio of 9:1 can instead be used. However after sensory evaluation of the two different water:bean ratios, 4:1 was preferred due to the creaminess and less beany taste.

Coagulation of Bean Blue was not the same as in blue mould cheese made from cow milk based on previous experience of regular cheese making, and the result was overall a more creamy and liquid texture of the curd. Coagulation and curd formation in cow milk is influenced by the acidification, but also by composition of the milk, especially protein, water and calcium phosphate content (Walstra *et al.*, 2005). Since bean milk does not contain the same nutrients as cow milk, the influence of these factors on the curd formation is not certain. However, Bean Blue is clearly influenced by the acidification, due to the observed coagulation during acidification by addition of lactic acid and/or activity of LAB starter culture. Also the use of a microbial coagulant affects the texture based on results from trials performed by the development team. This indicates that interactions with the proteins occur, but not to the same extent as with caseins in cow milk where the coagulant (together with LAB) is crucial for coagulation in most types of cheese.

The higher creaminess in 4:1 can be explained by less water used, and less water lost in the draining step. Using less water resulted in less water loss and thereby less fat loss. Since the amount of fat added was the same as in 9:1, fat content should be higher in the product and can be a contributing factor to the higher creaminess. Earlier analyses of fat content using 60 % added fat (mixed in bean:water 9:1) resulted in a fat content of 21-24 % in the final product. The fat content should theoretically be higher using water:bean ratio 4:1 and this should be analysed in the new formulation. The target fat content in Bean Blue is around 35-40 %, similar to a dairy cheese.

Further trials must be done to investigate how the coagulation of the bean milk can be increased which would result in a firmer, and less liquid product (if that is desirable). A drier product can lead to more similar properties as a blue mould cheese rather than a soft spreadable cheese, and a firmer, more stable structure could

also favor mould growth inside the product. Mould growth inside the product could provide Bean Blue with more blue mould taste and cover the beany taste.

Type of LAB and mould

A less liquid curd is also desired to optimise the conditions for the heterofermentative LAB. The use of the heterofermentative LAB-L did not succeed in its purpose to optimise the texture of the product, neither were sensory properties (flavor) improved. Heterofermentative LAB produce not only lactic acid and CO₂, but also acetic acid which could be a contributing factor to the undesirable taste. The product at this stage seems to be too wet for the LAB-L to produce gas and in turn form pores in the curd-mass to make mould growth inside possible. Further trials using LAB-L or alternative starter cultures are required to investigate the use of a heterofermentative LAB and the potential effect on pore formation and subsequent mould growth. Also since both of the LAB cultures are produced on dairy ingredients, alternative LAB produced from non-dairy ingredients should be investigated to be able to market the product as a complete vegan product.

The PR2 mould culture did not succeed in the last trial due to yeast contamination. Further trials must be performed to be able to evaluate this mould culture, and if it contributes to a better flavor profile.

New method without starch and 100 % faba beans

Removal of the starch in the new method is performed due to observations that the gelatinized starch can cause difficulty in filtering and low protein recovery (Zee *et al.*, 1988). In earlier production of Bean Blue, the formulation with 100 % faba did not coagulate, so the new method was introduced in the hope to be able to eliminate the use of soy bean, or to improve the quality of the product with 70 % faba and 30 % soy. Introducing the new method did not improve the efficiency of the processing steps, due to the longer time required to perform the new method. However, it resulted in the possibility to use 100 % faba beans. The reason that 100 % faba bean did not coagulate according to the original method, but did coagulate with the new method is a matter of speculation. Coagulation depends on the aggregation between proteins, and maybe if the starch is retained, the gelatinized starch after the boiling step can prevent the proteins from aggregating. Also, removal of the starch can contribute to a less watery product. Starch binds water, and by removing the starch, a less watery product can be obtained, which can lead to a more appealing sensory quality. The use of the new method made it possible to use 100 % faba beans, and although the processing steps was not improved (shortened), the sensory quality of the final product was also not substantially altered (negatively or positively) compared with the original method and mixed faba and soy formulation. The use of faba

bean instead of soybean is favorable since it can be grown throughout Sweden and thereby excludes the reliance on imported soy.

Consumer and descriptive test

The result from the consumer and the descriptive tests showed no significant differences between the samples with cooking oil and rapeseed oil. This suggests that cooking oil can be replaced with rapeseed oil without affecting the sensory quality of the final product. The sensory quality regarding this parameter has not been improved but rather maintained. Bean Blue aims to be a climate-smart product, with ingredients produced in Sweden, and replacing cooking oil with rapeseed oil could contribute to that. Earlier informal sensory trials by the development team showed that samples with coconut oil were preferred over samples with cooking oil. The use of coconut oil was therefore eliminated in the current consumer and descriptive tests, due to the likelihood that the consumer would have preferred samples with coconut oil. The coconut oil provides the product with a higher content of saturated fat which could improve the texture and lead to a more appealing taste. Using imported coconut oil is more expensive, and more importantly, not a climate-smart ingredient in Sweden compared with rapeseed oil. The desire to create a sustainable product eliminated the use of coconut oil, even if it could potentially have helped to optimise the sensory qualities of the product.

Number of participants in the consumer test was 118, which corresponds well with the recommendations of 75-150 consumers in this type of test (Lawless & Heymann, 2010). The answer form for the consumer test was done using a scale instead of boxes, which is commonly used in consumer tests (Gustafsson *et al.*, 2014). The advantages of using a scale instead of a box is that the consumer can put the mark (x) on a relative scale of how much they like or dislike the samples.

The description test was performed with a non-trained panel, where some of the panelists have been involved in the development of the product, which can affect the result. An ideal panel for the descriptive test should be recruited from individuals who have no personal connection to the product (Lawless & Heymann., 2010). However, by using people that are familiar with the product, they can be considered to be “partially trained” due to their earlier experience of evaluating the product. The conclusion from the descriptive test shows that the product seems to have a balanced salt taste. However the salt is added by hand on the outside of the product, which can lead to less or more salt on some parts of the surface. It is desirable if the beany taste is low in the product, but it was hard to eliminate completely. Stronger profile flavour in the mould culture could hypothetically decrease the beany taste further by masking it. It would be desirable to have higher scores on the blue mould taste, since this taste can hide the bean taste.

Food safety of Bean Blue

No microbial tests were performed during this project, but some contaminations were observed. One batch was contaminated with bacteria, which can be explained by the slow pH drop which did not reach below 5. This indicates the importance of pH and the production of lactic acid by the LAB. The decrease in pH was slow using LAB-C in some of the first trials, which probably permitted the bacterial growth. Using LAB-C in later trials did not show any problem with the pH decrease. The pH drop seemed to be slower in the batches with less water in the first trials using water:bean ratio 4:1. However, later trials using 4:1 did reach pH below 5 and no bacterial growth was observed. The reason for the slow pH decrease in the earlier trials is not certain. It could have been due to errors in the amount of LAB added.

The batches contaminated with bacteria or yeast were not tasted. The contamination of yeast that occurred in batches using PR2 can have been caused by the PR2 culture itself, which could have been contaminated from the beginning or contaminated during the rehydration process by dirty glassware, measuring cylinder or pipette. The contamination could also depend on the location in the incubator since all PR2 was kept on the same tray and same shelf, separated from the batch with PR1 which was not contaminated.

Microbial tests performed and a HACCP-plan developed during earlier stages in the production of Bean Blue (Sapieja, 2017) indicate that no pathogens were identified in the product, except low levels of *B. cereus*. Analysis was performed on cheeses from three different batches and the results indicated that the product has a high hygienic status. By good personal hygiene, properly washing equipment and implementing better routines in upscaled production, hazards can be easier to control and prevent product contamination.

Overall impression and further development of Bean Blue

The overall impression based on the consumer and descriptive test development is that Bean Blue is an appealing product for people who are looking for an option to regular cheese. The target for the product can be vegan people, people with lactose intolerance or milk protein allergy. The use of faba bean as raw material is a good option, both considering economic and environmental factors compared with regular cheese, but also compared with other vegan “cheeses” produced of cashew or soy. The development of new food products based on faba bean can encourage research and technological innovation focused on development of cultivars adapted to foods, and production of food products with optimum consumer’s acceptability and nutritional value (Multari *et al.*, 2015).

Comments from consumers suggested that the product should not be marketed as a substitute to regular dairy blue mould cheese, because this raises consumer expectations that the product will taste similar to a regular blue mould cheese. These

expectations can lead to lower acceptance of the product (Costa & Jongen, 2006). One idea is to market the product more as a tofu instead of a vegan cheese.

The parameters tested were limited by this project's length. Development of new products is time-consuming (Naes & Nyvold, 2004) and further development of Bean Blue is needed before launching the product. Bean Blue has only been produced in laboratory scale, and further tests including production on a larger scale, and finding optimal storage conditions and packaging for a long enough shelf-life that enables distribution and sale of the product. Different cultivars with higher protein content and lower tannin content of faba can also be investigated. A general observation from these trials is that small scale production by hand requires accuracy to be able to achieve the same result in every batch. Replicates of batches need to be made to achieve consistent results every time.

5.1 Conclusion

The process of Bean Blue has been optimised by decreasing the water:bean ratio from 9:1 to 4:1. This decrease in water enhances handling of the bean milk during pressing out okara, and shorter curd draining time. By introducing a new method including removal of starch, coagulation of the bean milk based on 100 % faba has been possible, and the use of soybean has been eliminated. Attempts to improve mould growth inside the product via pore-formation by a heterofermentative LAB were not successful, as the strain and formulation tested yielded no difference in texture. Further trials need to be done to support pore formation inside the curd mass. Certain sensory qualities have been improved and according to the development team, the final product is more creamy and less beany in taste compared with the starting recipe. Batches with the new mould culture got contaminated by yeast and could not be evaluated. Cooking oil can be replaced by rapeseed oil without having any negative effect on sensory properties, i.e. the sensory modifications of the product were not improved by using rapeseed oil, but were maintained, as clearly demonstrated by the absence of statistically significant differences between the two samples in the consumer and descriptive tests. All parameters together have led to a more advantageous product from an environmental point of view (usage of 100 % faba, and Swedish rapeseed oil) without negative effect on the sensory properties.

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Acknowledgements

I would like to give a special thank you to my supervisors Su-lin Hedén and Albina Bakeeva for making this project possible. I would also like to thank the development team including Fredrik Fogelberg and my co-supervisors Xinmei Feng and Monika Johansson who have helped with ideas and guidance during the project. I am grateful to have been a part of the development of Bean Blue and the knowledge of food product development it has given me.

Appendix 1 – Starting recipe

- Beans (70 % faba bean, 30 % soybean)
- Water (9 L water to 1 kg dry beans)
- Cooking oil
- Lactic acid bacteria starter culture (LAB-C)
- Glucose monohydrate
- Microbial coagulant (MC)
- Mould culture: PR1

Appendix 2 - Processing steps; new and original method

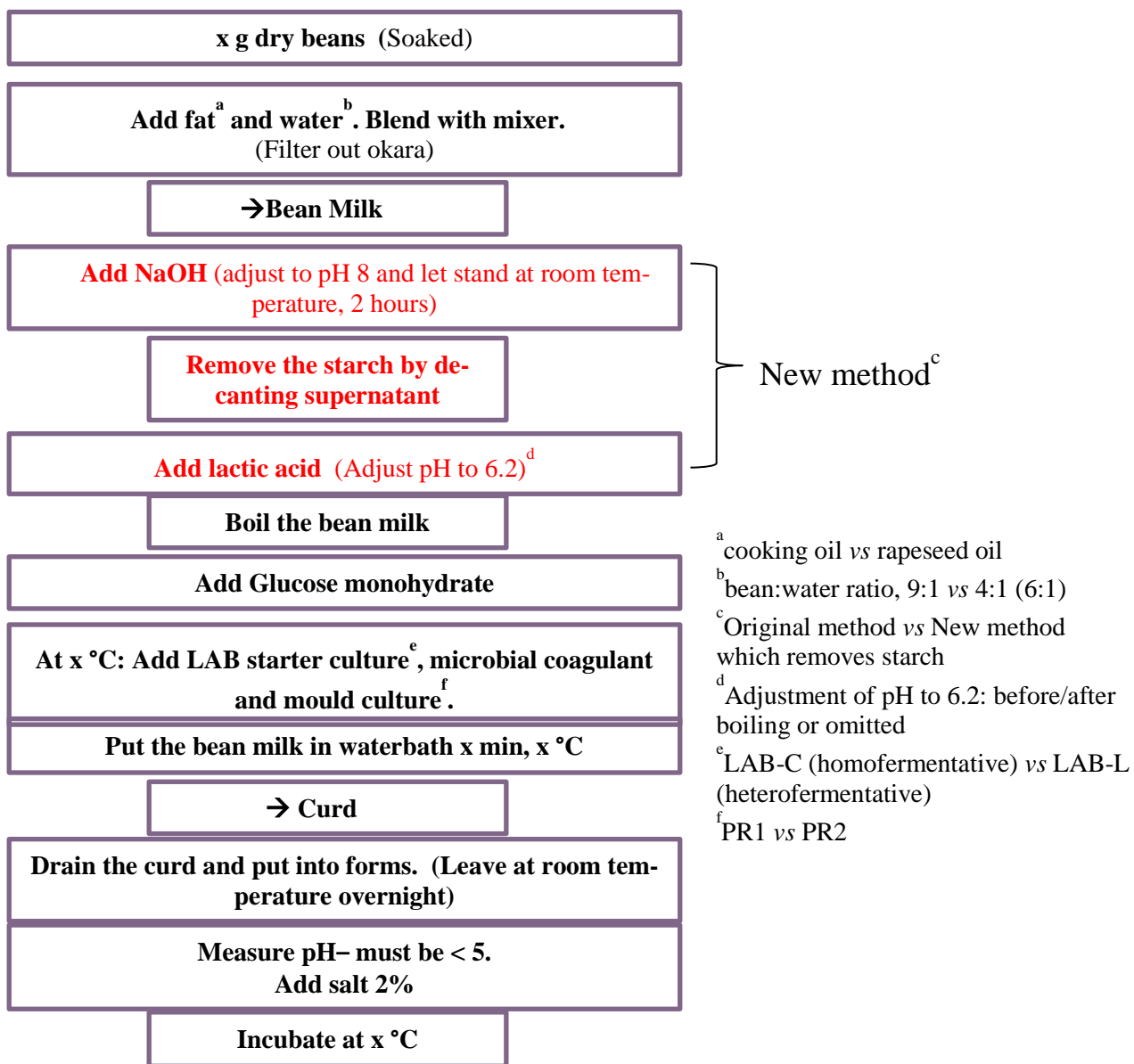


Figure 11. Original method with additional steps of New method in red. Superscript letters^{a,b} etc. denote parameters modified and tested in the technical and sensory trials.

Appendix 3 – Form (consumer test)

Konsumenttest av Bean Blue
Consumer test – Bean Blue

Du kommer nu att smaka på två olika prover av Bean Blue. Ange på en skala från "Tycker mycket illa om" till "Tycker mycket bra om" vad du tycker om produktens smak. Ange även om du kände någon skillnad mellan de två proven. Lämna gärna en kommentar på ditt övergripande intryck av produkten.

You will taste two different samples of the blue-moulded tofu Bean Blue. Fill in on the scale from "Dislike very much" to "Like very much" what you think of the taste of the product, also fill in if you experience the any difference between the two samples. Feel free to comment on your overall impression of the product.

Kön: Man Kvinna (*Woman*)

Ålder: 20 år eller yngre 20-29 år 30-39 år 40-49 år 50-59 år 60-69 år 70 år eller äldre
 Age:

Sätt ett kryss (x) på linjen där det överensstämmer bäst med vad du tycker om smak.
Put an X somewhere on the line which best expresses your opinion about the taste, e.g. {-----X-----}

Prov nummer 1 / <i>Sample nr 1</i>	Prov nummer 2 / <i>Sample nr 2</i>
<p>SMAK / TASTE</p> <p>Tycker mycket illa om Tycker varken bra eller illa om Tycker mycket bra om</p> <p><i>Dislike very much Neither like nor dislike Like very much</i></p> <p>{----- -----}</p>	<p>SMAK / TASTE</p> <p>Tycker mycket illa om Tycker varken bra eller illa om Tycker mycket bra om</p> <p><i>Dislike very much Neither like nor dislike Like very much</i></p> <p>{----- -----}</p>

Känner du någon skillnad mellan proverna? *Did you notice any difference between the two samples?* Ja Yes Nej No
Om ja, beskriv gärna / *If yes, please describe:*.....

Övrig kommentar / *Overall impression:*.....

Tack för din medverkan! *Thank you for your participation!*

Appendix 4 – Descriptive test

Description test - Bean Blue

You will try two different samples of Bean Blue. Try the samples in served order on your plate, starting to the right. Make a mark (x) on the scale from "low" to "high" intensity for each property for the first sample, then continue with the second sample. Rinse your mouth with water between the samples. Please also comment your overall impression of the product.

Definitions of "low" and "high" intensity for each property can be seen in the separate paper.

Judge no:.....

Sample:.....

ODOR

1. Mouldy *low* |-----| *high*

TEXTURE

2. Creaminess *low* |-----| *high*

TASTE

3. Salt *low* |-----| *high*

4. Sour *low* |-----| *high*

5. Beany *low* |-----| *high*

6. Blue mould *low* |-----| *high*




Figure 12. Form for description test

Table 13. Properties and definition of “low” and “high” intensity.

Category	Property	Definition low/high intensity
Odor	Mouldy	Low = little odor of mould
		High = much odor of mould
Texture	Creaminess	Low= little creaminess, dry in the texture
		High = much creaminess
Taste	Salt	Low = little salt taste High = much salt taste
	Sour	Low = little sour taste High = much sour taste
	Beany	Low = little beany taste High = much beany taste
	Blue mould	Low = little blue mould taste High = much blue mould taste

Table 14. Kitchen list with serving order for descriptive test

Judge	Sample	Sample
1	821 (rapeseed oil)	439 (cooking oil)
2	439	821
3	821	439
4	439	821
5	821	439
6	439	821
7	821	439
8	439	821
9	821	439
10	439	821
11	821	439
12	439	821
13	821	439
14	439	821

Appendix 5 – Popular summary

Development of Bean Blue – a blue mould tofu

The increase in global population leads to increased demand for food and protein sources. Plant foods and especially legumes have potential to contribute to global protein production, and support the increased worldwide demand for protein.

The majority of the global population lives on plant-based diets and development of products based on vegetables can be recommended for economic, ethical, environmental and health reasons. Legumes have high nutritional value and are of current interest as replacements for animal based foods. Faba bean is a legume with high protein content which can be grown in Sweden and can be used as a vegetable replacement, or in combination with animal food products. The majority of faba bean grown in Sweden today is used for feed. By developing new products based on faba bean, the use of this bean can be increased.

“Bean Blue” is a blue mould tofu based on faba beans that has been developed by researchers at the Swedish University of Agricultural Sciences (SLU) and the Research Institutes of Sweden (RISE) in Uppsala. The purpose of this project was to optimise the processing steps in the production of Bean Blue, and to optimise the sensory properties (taste, texture etc) of the final product by testing different ingredients and recipes. The development was divided into different trials including parameters such as: amount water used, different types of fat, starter and mould cultures, and testing a new method.

The results showed that the water used could be decreased to half volume which enhanced handling of the bean milk and contributed to a more creamy product with less beany taste. The original method that included the use of 70 % faba bean and 30 % soybean could be replaced by a new method including removal of the starch and the use of 100 % faba bean. The original starter culture, and mould culture could not be replaced by alternatives cultures due to poor mould growth and no pore formation inside the product. Rapeseed oil could replace cooking without any changes in the sensory quality.

All parameters together tested in this project have led to a more advantageous product from an environmental point of view (usage of 100 % faba, and Swedish rapeseed oil) without negative effect on the sensory properties.