Mitigation of aokusami (beany flavour) in a novel faba bean yoghurt

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Abstract

Substituting dairy products for plant-based alternatives is a more sustainable choice. Plant-based alternatives are also a viable option for those who cannot or choose not to consume dairy-based products. Consumer trends point towards an increased consumption of plant-based alternatives over conventional dairy products. Today there is no pulse-based yoghurt on the market which is made from Swedish-grown legumes. Many products are instead based on overseas cultivated soybeans. Faba beans have been identified as a possible alternative to soybeans, being suitable for Swedish conditions and having a relatively high protein content. Hence, faba bean yoghurt has been postulated as a novel product. However, as in many pulse-derived products there is an issue with beany flavour (aokusami) which negatively impacts consumer acceptability. Aokusami is the result of oxidation of unsaturated fatty acids, forming hydroperoxides which further react forming flavour compounds (e.g. hexanal). This reaction can be spontaneous (autoxidation) or enzyme-catalysed by lipid-modifying enzymes (LME) such as lipase, lipoxygenase and peroxygenase. Mitigation of beany flavour is possible by decreasing LME activity and autoxidation. The aim of this thesis was to investigate different production methods to mitigate aokusami in faba bean yoghurt. Methods employed for aokusami mitigation were: cold soaking (4 °C) of beans; thermal treatment of dry beans by microwaving; steaming of soaked beans (100 °C); blanching of soaked beans (75°C); and, hot extraction (HE) of faba bean milk (75 °C, 80 °C, 85 °C, 90 ° C). Aokusami of produced faba bean yoghurt was assessed in sensory evaluations by panellists rating the product 1-5: from most aokusami to no aokusami. It was also investigated if the aokusami mitigation methods would affect the incubation time, and if it was possible to predict aokusami of faba bean yoghurt by assessing faba bean milk flavour; results were deemed inconclusive. Cold soaking led to a slight reduction of aokusami in the finished product. Thermal microwave treatment displayed insufficient aokusami mitigation. Blanching, steaming and HE $\leq$ 80 °C resulted in moderate reduction of beany flavour. HE $\geq$ 85 °C produced a yoghurt with complete mitigation of aokusami. Yoghurt produced by HE $\geq$ 85 °C yielded a gel whereas other production methods resulted in a thickness similar to that of drinking yoghurt. To produce a satisfactory viscosity in $\geq$ 85 °C HE faba bean yoghurt, enzymatic hydrolysis of amylose and amylopectin is proposed. However, further studies of texture and mouthfeel need to be conducted before faba bean yoghurt becomes a developed product.

Keywords: aokusami, beany flavour, faba bean, faba bean yoghurt, lipid-modifying enzymes, *Vicia faba* var. *minor*

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Abbreviations

AM	Aokusami mitigation
AMM	Aokusami mitigation method
DP	Degree of polymerisation
EPS	Exopolysaccharide
FBM	Faba bean milk
FBY	Faba bean yoghurt
LB	*Lactobacillus delbrueckii* ssp. *bulgaricus*
LME	Lipid-modifying enzyme
PBM	Plant-based milk
PBMP	Plant-based milk product
PBY	Plant-based yoghurt
ST	*Streptococcus thermophilus*
UFA	Unsaturated fatty acid

1 Introduction

1.1 Background

This thesis is presented at the request of Research Institutes of Sweden (RISE), Unit for Agriculture and Food. It seeks to contribute to the investigation of faba bean yoghurt, within the larger framework of novel faba bean-based products studied at RISE.

Ingredients, equipment and the basic formulation and protocol were provided by RISE.

1.2 Aims

The aim of this thesis is to study ways to mitigate beany flavour in faba bean yoghurt produced from faba bean milk, and to experimentally identify the most promising methods by small scale lab trials. The experiments also seek to study the possibility of predicting aokusami of faba bean yoghurt by assessing the flavour of faba bean milk used as fermentation substrate, and whether fermentation can decrease the perceived beany flavour. Additionally, it is studied how different aokusami mitigation methods affect the incubation time of faba bean yoghurt production.

1.3 Limitations

A large portion of the theoretical background relates to pulses other than faba beans. This is because the faba beans utilised (*Vicia faba* var. *minor*) have conventionally been used as feed rather than for human consumption. Furthermore, faba bean milk and, consequently, faba bean yoghurt are not-yet developed products. On the other hand, soybeans, soymilk and derivatives thereof have a long history as foodstuffs. Despite the difference in species, legumes belong to the same family (*Fabaceae*),

and thus contain the same families of enzymes postulated to be responsible for the beany flavour.

Applied aokusami mitigation methods (AMM) were limited to ones that were possible to carry out with basic laboratory equipment without additives, due to equipment, ingredient and time constraints. Trials were largely restricted to single factor experiments because of time limitations.

2 Theory

2.1 Plant-based yoghurt

Demand for plant-based products is increasing. In 2018 the US market for plant-based yoghurts (PBY) displayed a growth of 55%, while consumption of dairy products was on the decline (PBFA 2018). In 2015 the Swedish market saw a growth of 24 % for plant-based milk products (PBMP) (Jansson & Karell 2015). The consumption of fermented dairy products in Sweden was 36.5 kg per capita in 2014 (Lingheimer *et al.* 2016). Scandinavia is amongst the regions with highest per capita consumption of fermented dairy products in the world (Tamime 2007). The conclusion can be made that PBYs have potential to gain considerable market shares and are already a significant market.

PBMPs generally have a lower environmental footprint per unit mass than their dairy equivalents, in respect to GHG emissions, energy use, nitrogen footprint, total land use, acidification and eutrophication potential (Röös 2018).

The fat content of PBMPs consists predominantly of unsaturated fat, whereas dairy counterparts can vary in fat content, but the inherent fat profile is dominated by saturated fat. The European Union has called on its member states to support reformulation of foodstuffs to decrease saturated fat content as a way of preventing non-communicable diseases (Folkhälsomyndigheten & Livsmedelsverket 2017).

Furthermore, PBMPs are a suitable alternative to conventional dairy products for people who are lactose intolerant, suffering from milk allergy, or for those who choose to follow a diet free from dairy products or products of animal origin altogether.

2.2 Faba beans

Faba beans of the variety *Vicia faba* var. *minor,* (sometimes *Vicia faba* var. *Minuta*) also referred to as field bean, tic bean or tick bean, were used as the raw material for the faba bean milk.

Soybean-derived products are the most successful and appreciated pulse-based dairy alternatives. However, soybeans are unfit as a crop for the Swedish climate: being short-day plants (Zhang et al. 2001), they do not flower until the long summer days shift into early autumn, which does not give the seeds enough time to fully ripen. Essentially a Swedish equivalent of soybeans was sought, and faba beans were considered a possible substitute. The bean requires a cold climate for optimal development (Åberg 2014), and was selected as the basis for a PBY, being suitable for Scandinavian and Baltic conditions. The legume having low soil requirements, and a pulse that could be processed similarly to soybeans were criteria leading to the choice of faba beans (personal communication, Fredrik Fogelberg, RISE). *Vicia faba* var. *minor* is utilized mainly as feed today, making it an untapped resource with respect to food intended for direct human consumption.

The beans were of the white-flowering variety Gloria. These were chosen as varieties producing white flowers are free from tannins (Larsson & Hagman 2013). Tannins have a bitter flavour influencing consumer acceptability. Gloria also produces a bean with comparatively high protein content, 31 % of dry matter (compare Table 1) (Larsson & Hagman 2013).

Pulses are known to contain anti-nutritional factors which can hinder nutrient uptake. Faba beans have been shown to contain low amounts of several anti-nutritional factors, such as α-amylase inhibitors, protease inhibitors and saponins, but medium-high levels of phytate and lectins (Henriksson 2017). Lectins are reportedly denatured during thermal processing (Henriksson 2017). Phytate content has displayed conflicting results from processing, both increased and decreased content. However, fermentation has been reported as decreasing the amount (Henriksson 2017). FBY production comprises these processing steps, constructing the hypothesis of decreased amount of anti-nutritional factors in the finished product compared to unprocessed beans.

Table 1. *Nutritional content of Vicia faba var. minor (Johansson 2015)*

Macronutrient	% of DM
Protein	28-29
Starch	38-50
Fat	1.7-2.1

2.3   Plant-based milk

Soymilk is the archetype of many plant-based milks (PBM). Therefore, production of PBM often rely on processes similar to those in soymilk manufacture.

Traditional soymilk is produced accordingly (Giri & Mangaraj 2012):

- Soaking of soybeans in water
- Draining and rinsing of well-soaked soybeans
- Grinding of soybeans with water using a stone mill
- Cooking of the soybean slurry
- Pressing of soybean slurry to extract soymilk

2.4   Yoghurt

Yoghurt is a dairy product traditionally produced from bovine milk by fermentation of lactic acid bacteria (LAB). The production of yoghurt consists of four essential steps (Tamime 2007):

- Pasteurization of milk to eliminate pathogens and spoilage organisms.
- Inoculation of the milk with a thermophilic LAB culture
- Incubation of the inoculated milk under required conditions to achieve adequate fermentation; leading to formation of flavour compounds and desired viscosity of coagulum.
- Cooling of the yoghurt to end fermentation.

Yoghurt is divided into three main categories: set, stirred and drinking yoghurt. Set yoghurt is incubated in the vessel intended for retail. This approach produces a thicker yoghurt with an intact coagulum (gel). Stirred yoghurt is incubated in bulk and either stirred during fermentation, or cooled whereafter the coagulum is broken by stirring (Celeste Karam *et al.* 2013). Agitation of the coagulum makes the stirred yoghurt a viscous fluid with a smooth texture (Tamime 2007). Drinking yoghurt is produced in the same way as stirred yoghurt with the addition of water after stirring to reach desired viscosity (Tamime 2007).

2.4.1   Fermentation

In conventional yoghurt manufacture fermentation is the essential step of production. It causes the formation of coagulum while contributing with flavour from bacterial metabolic compounds (Sfakianakis & Tzia 2014). However, fermentation is not necessary for the makeup of a yoghurt-like structure in PBY. This has prompted the production of "false" PBY which contains no bacterial culture (Johansson 2019); however, without fermentation the characteristic yoghurt flavour will not be

achieved. Yoghurt culture typically consists of *Lactobacillus delbrueckii.* ssp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST) (Chen *et al.* 2017) . In the fermentation of milk ST and LB act symbiotically in their metabolism of lactose into lactic acid (Sfakianakis & Tzia 2014). Lactic acid is responsible for the low pH in the yoghurt and the sour flavour. Fermentation also produces flavour compounds such as acetaldehyde, acetoin and diacetyl (Tamime 2007). In the fermentation of soymilk to soy yoghurt LAB utilize sucrose and oligosaccharides as substrates rather than lactose (Mital & Steinkraus 1979). This is a different symbiotic relationship than in the production of dairy yoghurt. LB is unable to ferment sucrose (Amoroso *et al.* 1989), and is dependent on hydrolysis products produced by ST (Mital & Steinkraus 1979). Strains of ST and LB have shown moderate growth on the trisaccharide raffinose and delayed growth on tetrasaccharide stachyose (Zartl *et al.* 2018). Faba bean kernels contain less raffinose and stachyose compared to the content of soybean kernels (Table 2) (Fan *et al.* 2015). Faba beans have a high content of the pentasaccharide verbascose in comparison to soybeans (Table 2) (Fan *et al.* 2015), although LB and ST are unable to utilize this oligosaccharide (Zartl *et al.* 2018). Sufficient fermentation is indicated by a decrease in pH to 4.6 within a set time frame, and in the production of FBY, this can be ensured by addition of a monosaccharide such as glucose, which is readily available for the starter culture (Zartl *et al.* 2018).

Table 2. *Content of sucrose and oligosaccharides in kernel of soybean and faba bean (Fan et al. 2015)*

Pulse	Sucrose (g/kg)	Raffinose (g/kg)	Stachyose (g/kg)	Verbascose (g/kg)
Soybean	75.6	9.6	34.0	2.2
Faba bean	38.2	4.8	13.9	32.3

2.4.2 Texture

Textural attributes of yoghurt, e.g. viscosity, smoothness, thickness and structural resistance to stress are important for consumer acceptability (Han *et al.* 2016). These attributes are dependent on the production process, i.e. set or stirred yoghurt manufacture (Celeste Karam *et al.* 2013). Other factors which affect the mouthfeel of dairy yoghurt are pasteurization temperature, solids content, starters and additives (Langton 1991). The same factors of influence are relevant with regard to PBY texture. However, it is important to note that there is a substantial difference in the composition of dairy milk and PBM, and consequently in dairy yoghurt and PBY, and their structural characteristics.

PBM generally has a lower fat content than bovine milk. Low fat content in the plant matter used as raw material for the PBMP can result in a lack of creaminess

(Sethi *et al.* 2016). This undesirable texture can be compensated by addition of a small amount of vegetable oil which may increase the viscosity and provide a smoother mouthfeel (Guo & Yang 2015).

Certain strains of ST and LB have the ability to produce exopolysaccharides (EPS) (Cerning 1995), these yoghurt cultures are referred to as "ropy cultures" (Marshall & Rawson 1999). EPS can contribute to increased viscosity and enhance the mouthfeel of yoghurt (Han *et al.* 2016). Furthermore, it can prevent syneresis, which is considered a defect in yoghurt manufacture (Marshall & Rawson 1999).

PBM lacks the caseins present in bovine milk which build the aggregates forming the continuous gel network in dairy yoghurt (Jaros & Rohm 2003). To produce the desired texture of PBY hydrocolloids can be employed. Hydrocolloids comprise a range of polysaccharides and proteins which can function as thickening, stabilising or gelling agents, depending on the concentration and hydrocolloid used (Phillips & Williams 2000). Examples of common hydrocolloids used for food applications are starch (e.g. potato, maize), pectin, agar, gelatin and gums (e.g. xanthan, guar, locust bean). Starches are a ubiquitous hydrocolloid in commercial food applications, owing to their low cost of production and ready availability. Starches high in amylopectin, such as potato starch and waxy maize starch, are suitable for use as thickeners and stabilisers. In contrast, starches high in amylose (e.g. unmodified maize starch) are used in applications where a gel is the intended product. As the starch granule undergoes gelatinisation and pasting in a heated aqueous solution, amylose leaches out while amylopectin largely remains in crystalline structure in the granule. The swelling of the granules and the leached amylose contribute to the viscosity of the liquid. Upon cooling retrogradation of the starch dispersion occurs; the reassociation of amylose molecules happens quickly, and the transition from a viscous paste to a rigid gel takes place. Retrogradation involves insolubilisation of amylose molecules leading to precipitation, and results in a gel prone to syneresis (Damodaran *et al.* 2017).

Furthermore, other characteristics of a starch must be considered. These include granule size (having a positive correlation with paste viscosity), clarity of the starch paste (opaqueness or translucence), and flavour (impartment thereof to the finished product).

2.4.3 Aokusami (beany flavour)

Pronounced aokusami is an issue in the production of soy milk and has a large impact on consumer acceptability (Endo *et al.* 2004). However, aokusami is not limited to soybeans and soybean-derived products but is also a problem in other pulses, such as peas (Lan *et al.* 2019), Bambara groundnut (Kudre & Benjakul 2014) and faba

beans (Jiang *et al.* 2016). Lipid-derived compounds formed by oxidation of unsaturated fatty acids (UFA) are the cause of aokusami (Roland *et al.* 2017).The oxidation can be enzyme-catalysed, by lipid-modifying enzymes (LME), or spontaneous (Yang *et al.* 2017). Off-flavours can also be a consequence of maillard reactions, thermal break-down of phenolic acids, thermal and oxidative break-down of carotenoids and thermal break-down of thiamine (Roland *et al.* 2017).

Lipase
Lipase is a lipid-associated enzyme, hydrolysing free fratty acids (FFA) from their esters. FFA are susceptible to oxidation and thereby aokusami formation. Endogenous lipase hydrolyses acyl lipids when the seed structure breaks down and enzymes encounter the substrates (Yang *et al.* 2017). Lipase has been identified in *Vicia faba* var. *minor* (Dundas *et al.* 1978). Faba beans are reported as having a high lipase activity (Yang *et al.* 2017).

Lipoxygenase
Lipoxygenase (LOX) is a key enzyme in the formation of aokusami compounds, and believed to be the major cause of beany flavour development in pulses (Jiang *et al.* 2016). LOX catalyses the oxidation of UFA (e.g. linoleic and linolenic acids) (Jiang *et al.* 2016) to flavour- and odourless hydroperoxides (Yang *et al.* 2017). The hydroperoxides further react forming volatile off-flavours (e.g. hexanal) and non-volatile compounds (e.g. oxoacids and dimers) (Yang *et al.* 2017). Aokusami is regarded as principally the effect of LOX activity due to the degradation products of hydroperoxides (Roland *et al.* 2017). LOX-derived compounds, such as hexanal, 3-cis-hexenal, 2(1-pentenyl)furan, *n*-pentylfuran and ethyl vinyl ketone contribute to beany, grassy and green flavours (Roland *et al.* 2017). Faba beans were found to have a medium-level LOX activity in a comparison of 14 legumes (Chang & McCurdy 1985). Two LOX isoenzymes (BBL-1, BBL-2), with a pH-optimum around 5.8, have been identified in faba beans (Clemente *et al.* 2000) .

Peroxygenase
Peroxygenase (POX) is responsible for the conversion of UFA to non-volatile flavour compounds, hydroxy and epoxy fatty acids (Hamberg & Hamberg 1996). The mechanism relies on the transfer of an oxygen from a fatty acid hydroperoxide to an UFA, generating an epoxy fatty acid and a hydroxy fatty acid (Yang *et al.* 2017). These POX-derived compounds are proposed to be responsible for bitter flavour (Hamberg & Hamberg 1996). Epoxidation of UFA associated with hydroperoxides has been identified in faba beans (Hamberg & Fahlstadius 1992). Faba beans were found to have a low POX activity (Yang *et al.* 2017). Furthermore, peroxidases are present in plant material, which are responsible for different oxidation-reduction

reactions which modify lipids. Peroxidase is more heat stable than lipase and LOX, thereby inactivating peroxidase indicates inactivation of other LME (Jiang *et al.* 2016).

Aokusami mitigation

As the formation of some aokusami compounds occurs spontaneously it is relevant to consider mitigation strategies already in pre-processing. One cause of aokusami, among others, is autoxidation which can be reduced by optimising storage conditions. Higher temperatures and exposure to light and oxygen are conditions which promote autooxidation and therefore measures should be taken to minimize these detrimental factors. However, the lipid content of faba beans is low (Table 1) possibly limiting the effectiveness and impact of storage conditions.

A positive correlation between soaking temperature and aokusami compound concentration has been observed in soybeans (Li *et al.* 2019). Therefore, by implementing a cold soaking process aokusami can be diminished. However, a lower soaking temperature also decreased water absorption and thereby the extraction of fat, solids and protein in soymilk (Li *et al.* 2019). Li et al. (2019) reported an adequate balance between soymilk flavour and quality employing soaking conditions of 25 °C and pH 9. However, the writers also reported that soaking at pH 3 or with deionized water produced soymilk with the lowest odour profile.

Khaleque et al. (1970) reported that soaking soybeans with 0.4 M or 0.5 M Na_2CO_3 at room temperature (no other temperature was investigated) resulted in a soymilk with the least aokusami compared to lower concentrations and additives (NaOH, Na_2SO_4 Na_2HPO_4, NaCl, $NaHCO_3$, Na_3PO_4), soaking without additive serving as control. Soaking in sodium carbonate solution also increased the viscosity and protein content of the soymilk comparing to soaking in water (Khaleque *et al.* 1970).

Heat treatment is vital in controlling the enzymatic reactions leading to formation of aokusami compounds (Yang *et al.* 2017). Adequate heat treatment leads to inactivation of LME (lipase, LOX and POX) by denaturation (Jiang *et al.* 2016). However, heat treatment decreases the protein solubility and causes a reduced protein extraction in soybeans (Endo *et al.* 2004). The effect of heat-treatment of soybeans has been researched thoroughly, while there is little literature concerning its effects on pulses high in starch, such as faba beans (Jiang *et al.* 2016). Jiang et al. (2016) proposed a thermal treatment prior to soaking to reduce the aokusami of faba beans. They found that employing a microwave treatment of 1.5 min at 950 W for 200 g dry faba beans resulted in effective inactivation of endogenous POX and LOX, while providing an "excellent balance of improved milling properties [and] preserved protein solubility" (Jiang *et al.*, 2016). Jiang et al. (2016) also reported

that conventional oven heating at 170 °C for 30 min had a similar effect while maintaining a greater protein solubility. Both heat treatments reduced LOX and POX activity to undetectable levels, but also increased formation of water-insoluble protein-starch aggregates.

Thermal treatment has also been extensively researched on soaked soybeans intended for production of soymilk with minimized aokusami. The hypothesis is that treating the hydrated beans allows the heat to rapidly penetrate the bean and inactivate the lipid-modifying enzymes (Endo *et al.* 2004). Methods such as hot water-added grinding where soybeans are soaked in 70 °C and homogenized in 95 °C water, blanching in 99.3 °C, and microwave heating have been tried for LOX inactivation, but dismissed as the high temperature leads to water-insoluble proteins and less protein extraction in soymilk (Endo *et al.* 2004). Endo et al. (2004) instead proposed a blanching method where hydrated soybeans are steamed at 75 °C (relative humidity 80-90%) for 10 minutes and thereby extracted with a "hot-squeezing method" (Endo *et al.* 2003). They found that soymilk prepared by this method had a significantly lower peroxide content than soymilk prepared from varieties of soybeans that lack LOX. Yuan et al. (2008) on the other hand employed a blanching process of 80 °C and 2 min for soybeans. The resulting soymilk contained no hexanal, indicating total inactivation of LOX (Yuan *et al.* 2008).

Lv *et al.* (2011) have researched not only the reduction of undesirable beany flavours in soybeans through thermal treatment, but also the simultaneous decrease in desirable non-beany flavour compounds. It was concluded that with increased thermal treatment both beany and non-beany flavours decrease and a balance between the two must be maintained (Lv *et al.* 2011).

Other researchers have achieved aokusami mitigation by blanching with different additives. Kudre & Benjakul (2014) decreased the aokusami of Bambara ground nut protein isolate significantly by thermal treatment at 70 °C for 30 min in 100 mM EDTA solution. Peng & Guo (2015) found that using a blanching temperature ≥70 °C for 5 min in 3‰ (w/v) $NaHCO_3$ decreased aokusami in soy yoghurt. A blanching temperature of 70 °C produced a 60% decrease in beany flavour and panellists could not distinguish a difference in aokusami between soy yoghurt produced by this method and commercial yoghurt. Soy yoghurt produced from beans blanched at 80 °C was perceived as having even less aokusami although panellists could not distinguish between 70 °C and 80°C blanch-treated soy yoghurt. A majority of panellists agreed that the 80 °C treated yoghurt and commercial yoghurt were in the same textural category. Blanching temperatures ≥70 °C also significantly decreased the chalky taste of soy yoghurt. The findings also indicated fermentation of soy products can increase consumer acceptability as the fermentation flavours mask aokusami (Peng & Guo 2015).

Giri & Mangaraj (2012) claim that while traditional production of soymilks yields a rancid odour, the processes of hot grinding and hot blanching result in a soy milk with a chalky mouthfeel. Instead, they propose what they call the Canadian airless cold-grind method: by grinding hydrated beans in an anaerobic environment, LOX is unable to catalyse aokusami compounds and the product is a non-chalky soy milk with a slight cereal taste. Processes such as membrane separation, pulsed electric field treatment and membrane separation are also recommended as methods which destroy or inactivate LOX in soymilk production (Giri & Mangaraj 2012).

3 Method

3.1 Faba bean milk production

To produce FBY with reduced beany flavour several processing techniques were applied to achieve aokusami mitigation (Table 3). Faba beans (*Vicia faba* var. *minor*) variety Gloria, harvested in Sweden 2017, were used for the production of FBY. The beans had been dehulled, split and stored at room temperature. Logistical limitations only allowed for batches of four FBY/FBM being produced per occasion (Table 3), where a control (Table 3) was produced each time as a reference.

Table 3. *Aokusami mitigation methods employed for faba bean milk and faba bean yoghurt*

FBM/FBY	Batch	Aokusami mitigation method				
		Pre-treat-ment	Cold soaking	Blanching	Steaming	Hot extraction
Control	A, B, C	No	No	No	No	No
Microwaved (M)	A	Yes	No	No	No	No
Room blanched (RB)	A	No	No	Yes	No	No
Cold blanched (CB)	A	No	Yes	Yes	No	No
Cold control (CC)	B	No	Yes	No	No	No
Steamed (S)	B	No	Yes	No	Yes	No
Hot extrac-tion T °C (HET)	B, C	No	Yes	No	No	Yes

Unless otherwise noted all FBYs were subject to processing as subsequently described.

3.1.1 Pre-treatment

200 g of faba beans were heat treated in a microwave for 2 min at 700 W (M). At 30 s intervals the beans were stirred to achieve homogenous heat treatment.

3.1.2 Soaking

100 g faba beans were soaked in 300 ml tap water for 10-12 hours. Cold soaked faba beans were soaked at 4 °C (CC, CB, S, HET). Room temperature soaked faba beans were soaked at 21 °C (C, M, RB). After soaking beans were drained and rinsed.

3.1.3 Treatment

Blanching
Blanching was carried out by placing hydrated beans in a beaker with 800 ml 75 °C tap water (RB, CB). The beaker was placed in a 99 °C water bath until the temperature of the water in the beaker reached 75 °C, whereby it was transferred to a 75 °C water bath and kept at 75 ± 1 °C for 5 min.

Steaming
Hydrated faba beans were steamed for 10 min in a strainer over a pot of boiling tap water (S).

3.1.4 Extraction

Faba beans were ground for 2 min at speed 3 in an Electrolux ESB9300 Masterpiece blender with 900 ml tap water and 59 g of warm-pressed rapeseed oil. Hot extracted FBM (HET) was produced using tap water at T (75 °C, 80 °C, 85 °C, 90 °C). Bean milk was extracted by manually pressing the bean slurry in a cheese cloth, thereby removing the okara.

3.1.5 Heat treatment

A 500 ml aliquot of bean milk was transferred to a Duran bottle. 12 g of glucose monohydrate was dissolved in the bean milk. The sweetened bean milk was placed

in a 99 °C shaking water bath (130 rpm) until the temperature reached 95 °C and kept at ≥ 95 °C for 5 min. Heat treated milk was cooled to 43 °C in a cold-water bath.

3.2 Faba bean yoghurt production

3.2.1 Yoghurt culture

A thermophilic culture (Lyofast Y 450 B) consisting of *L. bulgaricus* and *S. thermophilus* was used for the FBY production. The culture was acquired from Kemikalia. According to the manufacturer attributes of the culture were high EPS-formation, robust consistency and low post-acidification; yielding a result suitable for stirred yoghurts.

3.2.2 Inoculation and incubation

A 250 ml aliquot of FBM was transferred to a baffled E-flask. 0.05 g of LAB culture was added to the FBM. The E-flask was placed in a 43 °C shaking water bath (130 rpm) to produce the stirring motion required for stirred yoghurt. The nascent yoghurt was left to incubate until pH 4.6. pH readings were conducted with a digital pH meter (VWR pHenomenal® pH 1100 H). Incubation of yoghurt was allowed to proceed for a maximum of 6 hours. Upon reaching desired pH the FBY was removed from the water bath and stored at 4 °C. Duration of the incubation was recorded for the FBY.

3.3 Sensory evaluation

Aokusami of FBM and FBY was assessed by conducting a taste test with a trained sensory panel rating the product 1-5 (Table 4). The 6 panellists were trained in respect to being familiar with the flavour of FBY, and comprised of staff and students at SLU, and staff at RISE. At each evaluation 1-4 panellists were present. During the evaluation the aokusami mitigation method (AMM) of each FBM and FBY tasted was unknown to the panellists except for the conductor. Hence, where there was only one panellist the AMM of each FBY was known. As FBY production was limited to 4 FBM/FBY per occasion, aokusami was assessed in three evaluations consisting of batch A, B and C respectively. The sensory evaluations were carried out 12-18 h after production of FBY with the product stored at 4 °C.

Table 4. *Rating system for sensory evaluation of faba bean yoghurt*

Rating	Aokusami perception
1	Strong
2	Moderately strong
3	Weak
4	Almost none
5	None whatsoever

4   Results

4.1   Aokusami of faba bean yoghurt

Results from the taste test of FBY show that hot extracted FBY (HE85, HE90) scored the highest with average ratings of 5 (Figure 1). The lowest scores were recorded for the control for FBY and FBM with ratings of 1 (Figure 1). Microwave treated FBM and FBY (M) displayed the second to lowest ratings with average scores of 1.25 and 2 respectively (Figure 1). The individual ratings of FBY and FBM can be found in Appendix 1. The differences in number of panellists participating in sensory evaluation was due to time constraints and the panellists not being available. Data is missing for FBMs CC, S and HE due to oversight during evaluation of batch B and C. Results from sensory evaluation of RBY, RBM contra CBY, CBM suggested that cold soaking had a small effect on perceived aokusami therefore the subsequent batches B and C employed this AMM, except for the control. CC produced in batch B supported this hypothesis (Figure 1).
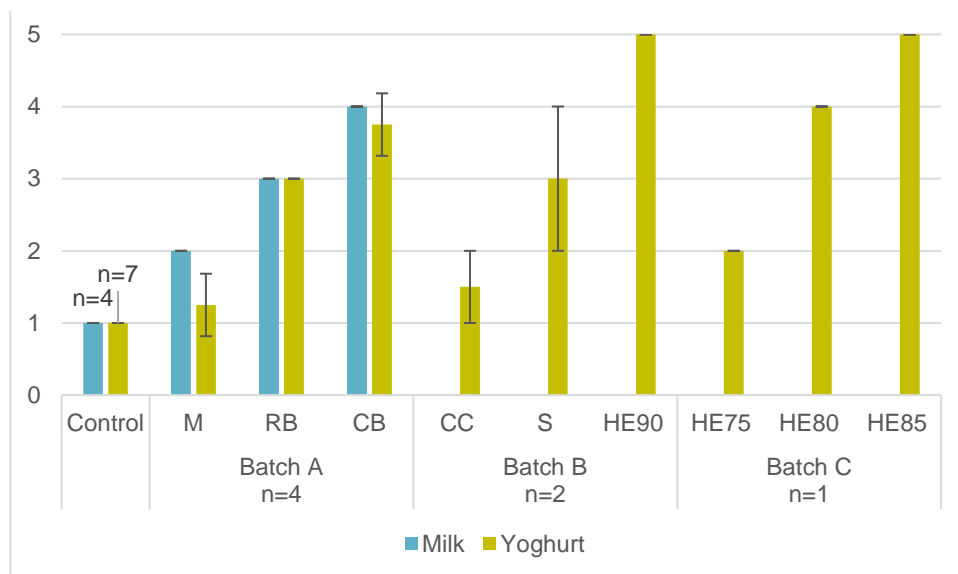
Figure 1. Average rating of beany flavour (1: strong, 2: moderately strong, 3: weak, 4: almost none, 5: none whatsoever) in faba bean milk and faba bean yoghurt: CC: cold control, M: microwaved, RB: room blanched, CB: cold blanched, S: steamed, HE*T*: Hot extraction *T* °C; where n = number of panellists. Errors bars represent standard deviation of ratings.

4.2   Incubation duration of faba bean yoghurt

The FBYs which employed AMM in their production yielded a faster pH decrease than the control (Table 5). CC required a considerably longer incubation than other AMM produced FBYs (Table 5).

Table 5. *Incubation duration of faba bean yoghurt with termination pH 4.6*

	Faba bean yoghurt									
	Control	M	RB	CB	CC	S	HE90	HE75	HE80	HE85
Time (min)	345[1]	260	210	220	330	240	230	220	220	220

1. Average of 2 batches of Control. First batch of Control reached insufficient pH 4.7 during set time limit.

4.3   Texture

FBYs produced without hot extraction method ≥ 80 °C yielded a product with a low viscosity, resembling a drinking yoghurt. The corresponding FBMs formed a precipitate which adhered to the bottle during heat treatment. Hot extracted ≥ 80 °C FBYs displayed a cohesive curd which would break following the agitation required

to remove the yoghurt from the vessel. When HE $\geq$ 80 °C FBYs were examined 2 weeks after production, considerable syneresis had occurred. FBYs produced without HE $\geq$ 80 °C did not show signs of syneresis with increased storage time.

5 Discussion and conclusion

5.1 Aokusami mitigation

5.1.1 Thermal pre-treatment by microwaving

Contrary to the results reported by Jiang et al. (2016) (being the only study presented here which studied inferred reduction of aokusami in faba beans) the panellists did not find the beany flavour of MM and MY to be noticeably reduced compared to the control (Table 2). It is possible that the modified treatment of 700 W for 2 min, an effort to compensate the lower output with longer treatment, did not sufficiently reduce the LME activity. However, Jiang et al. executed no sensory analysis on their findings. Instead, they analysed volatile compounds by GC-MS, finding that microwave treatments of 1.5 min or longer lead to a major content of hexanal in the finished product, and increased hexanal concentration with longer treatment. The proposed explanation was that this kind of treatment promoted spontaneous autoxidation (Jiang *et al.* 2016). This hypothesis could explain the results from sensory evaluation of MM and MY. Regardless of the reason being autoxidation from heat treatment or insufficient LME inactivation, this implies that the type of thermal pre-treatment by microwave tested in our study is an ineffective AMM.

5.1.2 Cold soaking

Cold soaking appeared to slightly reduce aokusami (Figure 1), as reported by Li et al. (2019). It could be explained by the low temperature decreasing LME activity in faba beans.

5.1.3 Blanching and steaming

Blanching and steaming proved to be a somewhat effective AMM, indicating some inactivation of LME (Figure 1). It is possible that the blanching temperature was lacking in respect to achieving sufficient LME inactivation, or that the addition of cold beans to 75 °C water enabled LME activity for a period of time. The same can be assumed for steaming. All processes where LME inactivation is unsatisfactory can lead to an exacerbated aokusami as the temperature drops during grinding and extraction, possibly approaching the temperature optimum of an LME. This is a problem especially encountered with manual extraction, as the slurry cannot be too hot to press.

5.1.4 Hot extraction

HE ≥85 °C proved to be the most effective AMM (Figure 1), corresponding to the findings of Endo et al. (2013) and Giri & Mangaraj (2012). HE 80 °C did not completely mitigate aokusami, and HE 75 °C resulted in rather poor AM (Figure 1). This infers that HE 85 °C causes sufficient inactivation of LME, whereas HE 80 °C results in partial LME inactivation. It should be noted that the soaked beans had a temperature of 4 °C when mixed with water for the HE. The actual temperature of the mixture would be lower than temperatures listed for the HE. It would be of value to measure the temperature of the mixture to ascertain the true LME inactivation temperature. However, this was not done as grinding was undertaken immediately after the addition of hot water to ensure LME inactivation. As the intact beans were immersed in hot water there would be a lower internal temperature of the bean, possibly facilitating increased LME activity. Swift inactivation is crucial as lipids are susceptible to lipolysis as soon as the bean structure is broken (Yang *et al.* 2017).

5.2 Texture

The difference between the gel texture of ≥ 80 °C HE and drinking yoghurt consistency other FBY produced can be explained by the precipitate formed during heat treatment of FBM not subject to ≥ 80 °C hot extraction. Due to inadequate agitation of the suspension during heat treatment, the precipitate was most likely caused by starch precipitating and gelatinization occurring in the FBM. As the precipitate adhered to the bottle it could not be transferred to the aliquot used for FBY production. Whereas, in ≥ 80 °C HE FBM the starch was presumably gelatinized uniformly as the starch suspension was sufficiently homogenised by the grinding. In FBM pro-

duced by thermal treatment prior to extraction (microwaving, blanching and steaming) it can be assumed a fraction of starch was gelatinized in the bean rendering it insoluble.

5.3 Prediction of aokusami in faba bean yoghurt

Unfortunately, data was not collected on all FBMs to give a more definitive answer regarding the aokusami relationship between substrate FBM and FBY. However, sensory evaluations of FBM were carried out for the control, CC, RB, CB and M (Figure 1). Contrary to what has been suggested, the fermentation appeared to increase the aokusami in the cases of M and CB, whereas control and RB FBM scored equally as their corresponding FBY. More pronounced aokusami could be a result of panellists perceiving the sour fermentation flavour as exacerbating an already negative sensory experience. It is possible that the panellists did not strictly distinguish between aokusami and adverse flavour when rating the product. Even if the undesirable flavour was not what is specifically considered aokusami, the poor ratings give an indication about consumer acceptability. Furthermore, it can be assumed that the task of only determining the aokusami without regarding mouthfeel proved a challenge. HE ≥ 80 °C FBY were texturally more akin to dairy yoghurt, which may have yielded a more positive rating.

5.4 Incubation time and aokusami mitigation method

It is evident from the incubation times that thermal treatments had a positive effect on LAB growth (Table 5). For HE ≥ 80 °C it might be explained by the cooked starch remaining in the FBY. A faster pH decrease and increased survival of LAB by addition of hydrocolloids has been observed in reconstituted sweetened yoghurt (Seth *et al.* 2018) and MRS broth (Chatterjee *et al.* 2016). This does not sufficiently explain how less viscous thermally treated FBYs which did not retain their starch displayed a shorter incubation time. However, faba beans contain favin (also called *Vicia faba* agglutinin [VFA]); a glucose- and mannose-binding lectin (Reeke & Becker 1986) (Barre *et al.* 2019). It is possible favin was denatured in the thermally treated FBY, while in the control and CC a fraction of glucose bound to the favin and was made inaccessible for the LAB.

5.5   Further studies

5.5.1   Standardisation and optimisation

For pressing of FBM from the bean slurry a method where the pressing is reproducible needs to be developed. Manual pressing by hand is not optimal regarding neither reproducibility nor is it fit for handling a hot bean slurry as is the result of e.g. HE. A simple tool for this would be a juice press, such as a press used for production of apple juice, or a more appropriate instrument would be a centrifuge.

If an FBM produced without HE ≥ 85 °C would be manufactured, such as a control, more vigorous stirring during heat treatment would be required to avoid the formation of a precipitate. This could be achieved by using a magnetic stirrer.

A process closer to commercial production of stirred yoghurt should be considered, where incubation is left unagitated and the coagulum is only broken after completed fermentation, as industrial equipment is built for this process. Sendra et al. (2010) and Marshall & Rawson (1999) suggest stirring a yoghurt sample 10 times clockwise and 10 times anti-clockwise following fermentation for simulating stirred yoghurt manufacture.

5.5.2   Analyses

The composition of FBM and/or FBY should be analysed to better understand the nutritional value, structure, presence of anti-nutritional factors, and rheology of the product. In the theory presented here, thermal treatment is reported to cause loss of protein solubilisation. High protein content was a factor for choosing faba bean as the basis for a PBY. However, it remains unknown how different AMM affected the protein solubility and protein extraction.

More extensive sensory analyses have to be carried out. These should be conducted as a true blind taste test. Mouthfeel is an important factor in consumer acceptability and should be examined by sensory analysis. The presence of a chalky mouthfeel should be investigated, as this has been a reported quality defect in soy-derived products.

5.5.3   Product development

Further aokusami mitigation and consumer preference
Production of FBY without added (or less) rapeseed oil is a possible development for processes other than HE ≥ 85 °C, where LME are assumed to be rapidly denatured. Rapeseed oil is high in UFA which are susceptible to LME pathway reactions,

leading to aokusami. FBY which has a fat content lower than 1.5 % would qualify for the keyhole labelling (LIVSFS 2015:1), which could impact consumer preference. However, the low fat content of FBY without added rapeseed oil could result in inferior texture and mouthfeel.

Cold storage and storage time of unprocessed faba beans should be studied. This could lead to less autoxidation and possibly reduced aokusami in the finished product.

A starter culture containing bacteria which can utilize the high verbascose content of faba beans could be used as inoculum. *Bifidobacterium lactis* HNO19 has displayed rapid growth with verbascose as the only carbohydrate source (Zartl *et al.* 2018). Employing a culture with verbascose-utilizing bacteria could eliminate the need of added glucose and affect consumer preference. Regulation (EC) 1924/2006 states that a food can be labelled "with no added sugars" if the product contains no added mono- or disaccharides, an attribute preferred by consumers (Valio 2018).

Improved texture

The results presented here show that HE ≥ 85 °C FBY achieved what would be considered sufficient aokusami mitigation. What would still be regarded a quality defect is the texture and thickness of the FBY. The high viscosity and gel structure of the HE ≥ 85 °C FBY are in keeping with the fact that the faba beans are high in starch content (Table 1). The substantial retrogradation and syneresis observed in these yoghurts suggest that a considerable fraction of the starch consists of amylose. This issue could be resolved by filtration or precipitation to remove starch from FBM. A suitable hydrocolloid would have to be added to the FBM to produce desired viscosity. However, filtration and precipitation are not viable in the process of hot extraction. As the starch is cooked an irreversible physiochemical modification occurs, forming a water-binding network structure that can no longer be separated from the liquid. Instead, a proposed method for decreasing amylose content is by enzymatic hydrolysis. β-amylase could be considered for this as it cleaves the α-(1 → 4) linkages from the reducing end of amylose and amylopectin, releasing maltose units (Damodaran *et al.* 2017). Complete hydrolysis of amylose into maltose is possible. These maltose units can be metabolized by LAB (Gänzle & Follador 2012) and added glucose would not be necessary. Consequently, a finished product could be labelled as containing no added sugar. Application of β-amylase would also cause hydrolysis of amylopectin. It is incapable of cleaving α-(1 → 6) linkages or the α-(1 → 4) linkages surrounding it, leaving amylopectin residues with a degree of polymerisation (DP) ∼20,000, so called β-limit dextrins (Tester & Qi 2011) (Izydorczyk & Edney 2003). β-limit dextrins behave as a hydrocolloid similar to amylopectin, yielding high viscosity and resisting retrogradation due to their branched nature (Tester & Qi 2011). Employing α-amylase as the hydrolytic enzyme would

instead cleave α-limit dextrins from amylopectin. As α-amylase can cleave α-(1 →
4) linkages anywhere along the polysaccharide chain it produces a limit dextrin with
a far lower DP, e.g. an average DP of 7.06 has been reported from waxy-maize
amylopectin after full hydrolysis (Roberts & Whelan 1960). High DP is required for
a polysaccharide to function as a hydrocolloid (Tester & Qi 2011). Using β-amylase
could eliminate the need of adding another hydrocolloid. β-amylase application
would be carried out at the enzyme temperature optimum of 55-70 °C (Tester & Qi
2011). Upon achieving desired polysaccharide breakdown, the enzyme would be
denatured by thermal treatment which could coincide with pasteurization of FBM.

Decreasing starch content could also be achieved by inoculation with a yoghurt
culture containing amylolytic LAB. Strains of e.g. *L. fermentum*, *L. acidophilus*, *L.
plantarum* have shown amylolytic activity (Reddy *et al.* 2008). However, fermen-
tation by amylolytic LAB could lead to significant post-acidification. Reducing the
starch content of FBY has to be studied further, e.g. how enzymatic hydrolysis of
starch (e.g. by α-amylase and β-amylase) affects the texture, syneresis, flavour, in-
cubation time and mouthfeel of FBY. The cost-effectiveness must also be consid-
ered.

5.6   Conclusion

A hot extraction process at 85 °C or 90 °C used in the production of faba bean milk
(yoghurt) effectively mitigated the beany flavour of the finished product while also
decreasing the incubation time needed. Soaking beans at 4 °C as opposed to room
temperature also seemed to slightly prevent aokusami. The hot extraction method
resulted in an undesirable thickness and syneresis due to the high starch content of
faba beans. A proposed method for reducing firmness in ≥ 85 °C HE yoghurt is
enzymatic hydrolysis of the starch-derived polysaccharides amylose and amylopec-
tin. For this β-amylase is suggested as its hydrolysis products maltose and β-limit
dextrin could benefit the product. Maltose can replace glucose as the energy source
for LAB and β-limit dextrin can possibly contribute to the desired viscosity of the
yoghurt while preventing syneresis.

However, rigorous sensory analyses need to be conducted to confirm these
results as well as study how different production methods affect the mouthfeel of
the product and consumer preference in general. In conclusion, faba bean yoghurt
has potential of becoming a developed product although much research has to be
done.

References

Amoroso, M.J., Manca De Nadra, M.C. & Oliver, G. (1989). The growth and sugar utilization by Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus salivarius ssp. thermophilus isolated from market yogurt. *Le Lait*, vol. 69 (6), pp. 519–528

Barre, A., Bourne, Y., Van Damme, E.J.M. & Rougé, P. (2019). Overview of the Structure–Function Relationships of Mannose-Specific Lectins from Plants, Algae and Fungi. *International Journal of Molecular Sciences*, vol. 20 (2), p. 254

Celeste Karam, M., Gaiani, C., Hosri, C., Burgain, J. & Scher, J. (2013). Effect of dairy powders fortification on yogurt textural and sensorial properties: A review. *The Journal of dairy research*, vol. 80, pp. 400–9

Cerning, J. (1995). Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. *Le Lait*, vol. 75 (4–5), pp. 463–472

Chang, P.R.Q. & McCurdy, A.R. (1985). Lipoxygenase activity in fourteen legumes. *Canadian Institute of Food Science and Technology Journal*, vol. 18 (1), pp. 94–96

Chatterjee, E., Manuel, S.G. & Hassan, S.S. (2016). Effect of Fruit Pectin on Growth of Lactic Acid Bacteria. *Journal of Probiotics & Health*, vol. 4 (2), pp. 1–4

Chen, C., Zhao, S., Hao, G., Yu, H., Tian, H. & Zhao, G. (2017). Role of lactic acid bacteria on the yogurt flavour: A review. *International Journal of Food Properties*, vol. 20 (sup1), pp. S316–S330

Clemente, A., Olias, R. & Manuel Olías, J. (2000). Purification and Characterization of Broad Bean Lipoxygenase Isoenzymes. *Journal of agricultural and food chemistry*, vol. 48, pp. 1070–5

Damodaran, S., Parkin, K.L. & Fennema, O.R. (2017). *Fennema's food chemistry*. Fifth edition. Boca Raton: CRC Press.

Dundas, D.G.A., Henderson, H.M. & Eskin, N.A.M. (1978). Lipase from Vicia faba minor. *Food chemistry*, vol. July (3), pp. 171–178

Endo, H., Ohno, M., Tanji, K., Shimada, S. & Kaneko, K. (2003). Effect of Variety of Soybean Seeds and Processing of Soy Milk on the Isoflavone Content of Tofu. *food preservation science*, vol. 29, pp. 165–172

Endo, H., Ohno, M., Tanji, K., Shimada, S. & Kaneko, K. (2004). Effect of Heat Treatment on the Lipid Peroxide Content and Aokusami (Beany Flavor) of Soymilk. *Food Science and Technology Research*, vol. 10 (3), pp. 328–333

Fan, P.-H., Zang, M.-T. & Xing, J. (2015). Oligosaccharides composition in eight food legumes species as detected by high-resolution mass spectrometry. *Journal of the Science of Food and Agriculture*, vol. 95 (11), pp. 2228–2236

Folkhälsomyndigheten och Livsmedelsverket (2017). Förslag till åtgärder för ett stärkt, långsiktigt arbete för att främja hälsa relaterad till matvanor och fysisk aktivitet. Available: https://www.folkhalsomyndigheten.se/contentassets/5514b381077f4175b13fca5fe1089abe/forslag-till-atgarder-matvanor-fysiskaktivitet.pdf [2019-05-28]

Giri, S.K. & Mangaraj, S. (2012). Processing Influences on Composition and Quality Attributes of Soymilk and its Powder. *Food Engineering Reviews*, vol. 4 (3), pp. 149–164

Guo, J. & Yang, X.-Q. (2015). 11 - Texture modification of soy-based products. I: Chen, J. & Rosenthal, A. (red.) *Modifying Food Texture*. Woodhead Publishing, pp. 237–255.

Gänzle, M.G. & Follador, R. (2012). Metabolism of Oligosaccharides and Starch in Lactobacilli: A Review. *Frontiers in Microbiology*, vol. 3. DOI: https://doi.org/10.3389/fmicb.2012.00340

Hamberg, M. & Fahlstadius, P. (1992). On the Specificity of a Fatty Acid Epoxygenase in Broad Bean (Vicia faba L.). *Plant Physiology*, vol. 99 (3), pp. 987–995

Hamberg, M. & Hamberg, G. (1996). Peroxygenase-Catalyzed Fatty Acid Epoxidation in Cereal Seeds (Sequential Oxidation of Linoleic Acid into 9(S),12(S),13(S)-Trihydroxy-10(E)-Octadecenoic Acid). *Plant Physiology*, vol. 110 (3), pp. 807–815

Han, X., Yang, Z., Jing, X., Yu, P., Zhang, Y., Yi, H. & Zhang, L. (2016). Improvement of the Texture of Yogurt by Use of Exopolysaccharide Producing Lactic Acid Bacteria. *BioMed Research International*, vol. 2016. DOI: https://doi.org/10.1155/2016/7945675

Henriksson, Y. (2017-08-15). *Antinutritionella faktorer i baljväxter*. Available: https://stud.epsilon.slu.se/10621/ [2019-05-19]

Izydorczyk, M.S. & Edney, M.J. (2003). MALT | Chemistry of Malting. I: Caballero, B. (red.) *Encyclopedia of Food Sciences and Nutrition (Second Edition)*. Oxford: Academic Press, pp. 3677–3685.

Jansson, A. & Karell, L. (2015). Framtida möjligheter på den växtbaserade mejerimarknaden. Available: http://lup.lub.lu.se/student-papers/record/7869354 [2019-05-19]

Jaros, D. & Rohm, H. (2003). 13 - The rheology and textural properties of yoghurt. I: McKenna, B.M. (red.) *Texture in Food*. Woodhead Publishing, pp. 321–349.

Jiang, Z., Pulkkinen, M., Wang, Y., Lampi, A.-M., Stoddard, F.L., Salovaara, H., Piironen, V. & Sontag-Strohm, T. (2016). Faba bean flavour and technological property improvement by thermal pre-treatments. *LWT - Food Science and Technology*, vol. 68, pp. 295–305

Johansson, B. (2015). *Proteinfoder till mjölkkor*. Uppsala: Centrum för ekologisk produktion och konsumtion EPOK, Sveriges lantbruksuniversitet.

Johansson, E. (2019). De är nominerade till Årets matbluff: "Man luras på en mikrosekund". Available: https://www.svt.se/nyheter/inrikes/snart-koras-en-matvara-till-antipriset-arets-matbluff [2019-05-18]

Khaleque, A., Bannatyne, W.R. & Wallace, G.M. (1970). Studies on the processing and properties of soymilk I.—Effect of preprocessing conditions on the flavour and compositions of soymilks. *Journal of the Science of Food and Agriculture*, vol. 21 (11), pp. 579–583

Kudre, T.G. & Benjakul, S. (2014). Physicochemical and functional properties of beany flavour-free bambara groundnut protein isolate. *Journal of the Science of Food and Agriculture*, vol. 94 (6), pp. 1238–1247

Lan, Y., Xu, M., Ohm, J.-B., Chen, B. & Rao, J. (2019). Solid dispersion-based spray-drying improves solubility and mitigates beany flavour of pea protein isolate. *Food Chemistry*, vol. 278, pp. 665–673

Langton, M. (1991). *The microstructure of yoghurt: a literature review*. Göteborg: SIK. (SIK-rapport, 580)

Larsson, S. & Hagman, J. (2013). *Sortval i ekologisk odling 2013 sortförsök 2008-2012*. Uppsala, Sverige: Faculty of Natural Resources and Agricultural Sciences : Dept. of Crop Production Ecology. Available: http://www.slu.se/sv/fakulteter/nl-fakulteten/om-fakulteten/ovriga-enheter/faltforsk/resultat/rapporter/ [2019-05-17]

Li, X., Liu, X., Hua, Y., Chen, Y., Kong, X. & Zhang, C. (2019). Effects of water absorption of soybean seed on the quality of soymilk and the release of flavor compounds. *RSC Advances*, vol. 9 (6), pp. 2906–2918

Lingheimer, I., Jirskog, E., Johansson, K., Lannhard Öberg, Å. & Törnquist, M. (2016). *Marknadsöversikt - mjölk och mejeriprodukter*. (2016:11)

LIVSFS 2015:1. Föreskrifter om ändring i Livsmedelsverkets föreskrifter (LIVSFS 2005:9) om användning av viss symbol. Uppsala: Livsmedelsverket

Lv, Y.-C., Song, H.-L., Li, X., Wu, L. & Guo, S.-T. (2011). Influence of Blanching and Grinding Process with Hot Water on Beany and Non-Beany Flavor in Soymilk. *Journal of Food Science*, vol. 76 (1), pp. S20–S25

Marshall, V.M. & Rawson, H.L. (1999). Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. *International Journal of Food Science & Technology*, vol. 34 (2), pp. 137–143

Mital, B.K. & Steinkraus, K.H. (1979). Fermentation of Soy Milk by Lactic Acid Bacteria. A Review. *Journal of Food Protection*, vol. 42 (11), pp. 895–899

Peng, X. & Guo, S. (2015). Texture characteristics of soymilk gels formed by lactic fermentation: A comparison of soymilk prepared by blanching soybeans under different temperatures. *Food Hydrocolloids*, vol. 43, pp. 58–65

Phillips, G.O. & Williams, P.A. (2000). *Handbook of hydrocolloids*. Boca Raton, Fla: CRC Press.

Plant Based Foods Association (2018). *Plant-based food sales grow 20 %.* Available: https://plant-basedfoods.org/wp-content/uploads/2018/07/PBFA-Release-on-Nielsen-Data-7.30.18.pdf [2019-06-04]

Reddy, G., Altaf, M., Naveena, B.J., Venkateshwar, M. & Kumar, E.V. (2008). Amylolytic bacterial lactic acid fermentation — A review. *Biotechnology Advances*, vol. 26 (1), pp. 22–34

Reeke, G.N. & Becker, J.W. (1986). Three-dimensional structure of favin: saccharide binding-cyclic permutation in leguminous lectins. *Science (New York, N.Y.)*, vol. 234 (4780), pp. 1108–1111

Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods (2006). *OJ L*. Available: http://data.europa.eu/eli/reg/2006/1924/oj/eng [2019-05-22]

Roberts, P. J., & Whelan, W. J. (1960). The mechanism of carbohydrase action. 5. Action of human salivary alpha-amylase on amylopectin and glycogen. The Biochemical journal, volk 76(2), pp. 246–253. doi:10.1042/bj0760246

Roland, W.S.U., Pouvreau, L., Curran, J., Velde, F. van de & Kok, P.M.T. de (2017). Flavor Aspects of Pulse Ingredients. *Cereal Chemistry*, vol. 94 (1), pp. 58–65

Röös, E. (2018). *The role of dairy and plant based dairy alternatives in sustainable diets*. Uppsala: Swedish University of Agricultural Sciences, the research platform Future Food. (SLU Future Food Reports ; 3)

Sendra, E., Kuri, V., Fernández-López, J., Sayas-Barberá, E., Navarro, C. & Pérez-Alvarez, J.A. (2010). Viscoelastic properties of orange fiber enriched yogurt as a function of fiber dose, size and thermal treatment. *LWT - Food Science and Technology*, vol. 43 (4), pp. 708–714

Seth, D., Mishra, H.N. & Deka, S.C. (2018). Effect of hydrocolloids on the physico-chemical and rheological properties of reconstituted sweetened yoghurt powder. *Journal of the Science of Food and Agriculture*, vol. 98 (5), pp. 1696–1702

Sethi, S., Tyagi, S.K. & Anurag, R.K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: a review. *Journal of Food Science and Technology*, vol. 53 (9), pp. 3408–3423

Sfakianakis, P. & Tzia, C. (2014). Conventional and Innovative Processing of Milk for Yogurt Manufacture; Development of Texture and Flavor: A Review. *Foods*, vol. 3 (1), pp. 176–193

Tamime, A.Y. (2007). *Tamime and Robinson's yoghurt: science and technology*. 3rd ed. Boca Raton, Florida ; CRC Press. (Woodhead Publishing in food science, technology, and nutrition)

Tester, R.F. & Qi, X. (2011). β-limit dextrin – Properties and applications. *Food Hydrocolloids*, vol. 25 (8), pp. 1899–1903 (25 years of Advances in Food Hydrocolloid Research)

Valio (2018-10-13). *Consumers want to reduce sugar in food products. Valio.com*. Available: https://www.valio.com/articles/consumers-want-to-reduce-sugar-in-food-products/ [2019-05-17]

Yang, Z., Piironen, V. & Lampi, A.-M. (2017). Lipid-modifying enzymes in oat and faba bean. *Food Research International (Ottawa, Ont.)*, vol. 100 (Pt 1), pp. 335–343

Yuan, S., Chang, S.K.C., Liu, Z. & Xu, B. (2008). Elimination of Trypsin Inhibitor Activity and Beany Flavor in Soy Milk by Consecutive Blanching and Ultrahigh-Temperature (UHT) Processing. *Journal of Agricultural and Food Chemistry*, vol. 56 (17), pp. 7957–7963

Zartl, B., Silberbauer, K., Loeppert, R., Viernstein, H., Praznik, W. & Mueller, M. (2018). Fermentation of non-digestible raffinose family oligosaccharides and galactomannans by probiotics. *Food & Function*, vol. 9 (3), pp. 1638–1646

Åberg, M. (2014-07-03). *Jämförelse mellan sojaböna och åkerböna som proteinfoder till växande grisar*. Available: https://stud.epsilon.slu.se/6970/ [2019-05-19]

Appendix 1. Sensory evaluation data

Appendix 1. *Compilation of sensory evaluation ratings for faba bean milk and faba bean yoghurt*

Product					FBM/FBY					
	Control	M	RB	CB	CC	S	HE90	HE75	HE80	HE85
Milk	1	2	4	4						
	1	2	4	4						
	1	2	4	3						
	1	2	4	4						
Average	1	2	4	3.75						
SD	0	0	0	0						
n	4	4	4	4						
Yoghurt	1	1	3	4	1	2	5	2	4	5
	1	1	3	4	2	4	5			
	1	1	3	3						
	1	2	3	4						
	1									
	1									
	1									
Average	1	1.25	3	3.75	1.5	3	5	2	4	5
SD	0	0.43	0	0.43	0.5	1	0	0	0	0
n	7	4	4	4	2	2	2	1	1	1