Swedish “tofu” from faba beans – a textural and structural analysis

Svensk ”tofu” från fababönor – en analys av textur och struktur

Saga Preis

Bachelor’s degree project • 15 hp
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
Agricultural programme – Food Science
Molecular Sciences, 2023:07
Uppsala, 2023

Flowering *Vicia faba* L.
Photographer: Åsa Grimberg, SLU

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Credits:	15 hp
Level:	First cycle, G2E
Course title:	Independent project in Food Science
Course code:	EX0876
Programme/education:	Agricultural Programme – Food Science
Course coordinating dept:	Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2023
Title of series:	Molecular Sciences
Part number:	2023:07
Keywords:	Tofu, faba bean, *Vicia faba*, texture, microstructure.

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Abstract

Tofu, a traditional soybean product, is a popular plant-based alternative which could potentially be produced from Swedish-grown faba beans (*Vicia faba* L.). The aim of this project was to asses the viability of producing a tofu-like faba bean product using food grade gypsum (calcium sulphate) as coagulant. Bean milk from faba beans and soybeans, respectively, was prepared with a bean-water ratio of 1:6 and 1:9. The prepated milk was heated to a boiling temperature and then coagulated at 80°C using 0,4% (w/v) of calcium sulphate. Faba bean starch was removed from the milk prior to coagulation using enzymatic hydrolysis (amylase) and decantation, respectively. Solids content, moisture content, water-holding capacity, yield, texture and gel microstructural properties of faba bean tofu variants was analysed and compared to soybean tofu produced under the same processing conditions. Tofu texture was analysed using texture-profile analysis (TPA), and tofu microstructure using light microscopy (LM) and confocal laser-scanning microscopy (CLSM). Starch removal methods varied in efficiency. Compared to soybean tofu variants, the faba bean tofu variants exhibited a lower tofu yield, lower milk solids content, higher WHC, harder and less springy texture, and a densely aggregated and compacted gel structure. Nonetheless, the results indicate that there is potential for developing a tofu-like faba bean product, although further research and development is required to optimize textural and structural characteristics. Ultimately, industrial-scale testing and sensory panels will be decisive in determining the viability of commercial production.

Keywords: tofu, faba bean, *Vicia faba*, texture, microstructure

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1. Introduction

The current food system poses threats to both human health and planetary boundaries, driving climate change, biodiversity loss, freshwater consumption and land-use change. The Western diet, with a high meat consumption, is thus un sustainable. As the global population grows, transitioning to a plant-based diet will be essential to meet the increasing food demand while protecting the environment. This shift would also benefit human health (Godfray et al. 2018; Willett et al. 2019; Röös et al. 2020). Legumes and legume-based foods can come to play a key role in feeding and nourishing future generations (Semba et al. 2021) as they are nutritious, protein-rich crops cultivated around the world (Dhull et al. 2022). They are good sources of lysine-rich protein, carbohydrates, dietary fibers, minerals and vitamins with positive effects on human health (Röös et al. 2020).

In Sweden, there has been a noticeable increase in consumer demand for plant-based foods in recent years. As more consumers are reaching for plant-based alternatives, the market for these product has expanded dramatically. However, there is currently a lack of plant-based products made from Swedish raw materials, despite clear consumer demand (Maclean 2020; Växtbaserat Sverige 2020; Swedish Board of Agriculture 2022).

The current market for plant-based alternatives primarily consist of soybean-based products (Maclean 2020). Among these, tofu has gained popularity, especially among vegans and vegetarians, due to its high protein content and long-standing use in Asian cuisine (Chang & Liu 2012; Zheng et al. 2020). Although soybeans are rich in proteins and other nutrients (Zheng et al. 2020), their production faces challenges in the European climate, leading to heavy reliance on imports (Nendel et al. 2023). At present, Argentina and Brazil are the main exporters of soybean to countries of the EU (Boerema et al. 2016), but their rapid expansion of soybean cultivation has resulted in deforestation and human rights violation (Dreoni et al. 2022). Sweden alone imports approximately 250.000 tonnes of soy products every year (Swedish Board of Agriculture 2022). To address these issues, there is now a European-wide incentive to increase the domestic production of protein crops (European Commission 2020). Faba beans (*Vicia faba* L.) have attracted attention in Sweden, as they are well-adapted to Swedish climatic conditions and have a long history of cultivation in the region, primarily for animal feed (Swedish Board of

Agriculture 2022). As of now, faba bean cultivation accounts for only a few percent of the Swedish arable land with most of it is dedicated to animal feed. However, if there is an increase in consumer demand for Swedish-grown faba beans, this is likely to change (Röös et al. 2020). In this context, product development plays a key role in bringing attractive products based on faba beans to the market. Hence, this project aims to develop a tofu-like product made solely from Swedish-grown faba beans.

1.1 Tofu – a protein gel type food

Tofu is a traditional soybean product formed by gelation of soybean proteins (Zhang et al. 2018). Simplified, tofu production consists of soymilk preparation and soymilk coagulation. Soymilk is prepared by soaking and grinding soybeans in water followed by filtration to remove the bean solids (okara). The resulting milk is typically boiled, either before or after filtration. Soymilk coagulation is achieved by adding a coagulant. The coagulated curds are transferred to moulds and excess liquid is drained, often with the aid of pressing (Chang & Liu 2012). Based on the specific processing conditions and types of coagulant used, tofu products are commercially categorized into market types such as firm/soft and pressed/packed (Zhang et al. 2018).

The coagulation process is essential in tofu production as it is responsible for formation of curd (Hou et al. 1997). Firstly, boiling of the soybean milk leads tothe thermal denaturation of its proteins. Denaturation exposes hydrophobic and sylfhydryl groups on the surface of protein molecules, promoting protein aggregation (Zhang et al. 2018). With the addition of a suitable coagulant, such as a salt, acid or enzyme, the protein aggregates undergo cross-linking and polymerize, resulting in the formation of a stable three-dimensional protein gel network known as tofu (Zhang & Qin 2019; Zheng et al. 2020). As the protein gel network forms, non-protein components become entrapped within (Zhang & Qin 2019). Soybean lipids, released from the solid bean fraction during boiling, become inseparable from the tofu network through intense interaction with protein particles (Chang & Liu 2012). Water is also entrapped within the tofu gel, constituting approximately 80-90% of its total weight (Zhang et al. 2018).

1.2 Factors influencing tofu quality

Texture is an important quality parameter in tofu production and is closely associated with structural characteristics of the tofu protein gel (Zhang & Qin 2019). However, controlling and optimizing texture can be challenging for tofu manufacturers, as it is governed by complex interplay of various factors (Hou et al. 1997). This section will provide a brief overview of key factors influencing tofu quality.

Tofu quality is heavily influenced by protein content and composition of the raw material, as well as the concentration of the bean milk (Chang & Liu 2012). Of soybean proteins, β-conglycinin (7S) and glycinin (11S) play crucial roles as their relative contents impact the final tofu gel structure, texture and overall yield. A high content of 11S protein is often desired in tofu production (Zhang & Qin 2019; Zheng et al. 2020). Studies have found a positive correlation between soymilk concentration and tofu textural attributes such as fracturability, hardness, springiness, cohesiveness and chewiness. Soymilk concentration of solids and protein can be increased by lowering the bean-to-water ratio (BWR) during soymilk preparation (Rekha & Vijayalakshmi 2013). However, the optimal BWR varies depending on tofu market type, from 1:6 for soft tofu to 1:10 for firm tofu (Chang & Liu 2012).

Coagulant type and concentration are also important in terms of tofu quality, as they influence both tofu gel structure, texture (Kao et al. 2003) and yield (Prabhakaran et al. 2006). These effects can be attributed to variations in cross-linking behaviour and bond strength among different coagulants (Zhang & Qin 2019). The optimal coagulant concentration (OCC) may vary depending on the tofu raw material, coagulant type as well as the intended tofu market type (Chang & Liu 2012). Achieving complete coagulation of soymilk requires a sufficient concentration of coagulant, but an excessively high concentration may result in a hard, coarse and crumbly tofu texture (Kao et al. 2003; Chang & Liu 2012) and lead to increased water losses due to extensive internal bonding (Prabhakaran et al. 2006). Since tofu yield is positively correlated with moisture content, this also has economical implications for the manufacturer (Prabhakaran et al. 2006).

Non-protein components, such as lipids, starch and phytate, have an impact on tofu quality as well (Zhang et al. 2018). Bean lipids interact with proteins during bean milk coagulation and are incorporated into the protein gel network upon curd formation, acting as space-filling particles (Guo et al. 2002; Shin et al. 2015). When exposed to cooking temperatures, starch will gelatinise to produce a sticky or pasty gel-like substance, which may interfere with protein gel formation and affect tofu texture (Jiang et al. 2020). Phytate is a strong chelating agent that can form complexes with both bean proteins and coagulant salts. A high prevalence of phytic acid in soymilk has been found to retard gelation and increase the required amount of coagulant. In addition, phytate negatively impacts tofu hardness and fracturability (Chang & Liu 2012; Peng et al. 2016).

1.3 Soybeans and faba beans – a brief comparison of the tofu raw material

Soybeans and faba beans, despite belonging to the same botanical family, exhibit substantial differences in their chemical composition. The protein and lipid content of soybeans is high in relation to other legumes, ranging from 40-42% and 18-22%, respectively (Pagano & Miransari 2016). Glycinin (11S) and β-conglycinin (7S) are the main storage proteins in soybeans and constitute up to 70% of the total protein content. The soybean mass ratio of 11S/7S is found to be in the range of 1,5-2,5 (Chang & Liu 2012; Zheng et al. 2020). In comparison, faba beans contain approximately 30% protein of which 80% is comprised of legumin (11S) and vicilin/convicillin (7S). Legumin is the main faba bean storage protein and constitutes around 50% of total protein content. The suggested 11S/7S ratio in faba beans is 1-3 (Warsame et al. 2020). Faba beans, like most legumes, have a low lipid content (1-2%), which is considerably lower than the lipid content found in soybeans (Martineau-Côté et al. 2022). Another notable difference is starch content: faba beans contain ~40% starch on a dry weight basis (Jiang et al. 2020), while soybeans have a negligible amount of starch (Cai et al. 2002). The presence of starch in faba beans could have implications for tofu production, as heating of bean milk during the coagulation process can result in starch gelation. Fortunately, starch granules may be physically removed through decantation or filtration, or enzymatically degraded by amylases. It is worth noting that polysaccharides, the product of enzymatic starch hydrolysis, could possibly interact with proteins to alter their functional properties and consequently influence tofu gel formation (Jiang et al. 2020).

1.4 Project aims

The aim of this project is to assess the viability of producing a tofu-like faba bean product coagulated using food-grade gypsum, in comparison to traditional soybean tofu produced under the same processing conditions. The research will focus on evaluating the quality attributes of tofu in terms of solid content, moisture content, water-holding capacity, yield, texture and gel microstructural properties.

2. Materials and method

2.1 Materials

Faba beans (cv. "Tiffany") and amylase enzyme were supplied by Research Institute of Sweden (RISE) in Uppsala, Sweden. Soybeans and food-grade calcium sulphate ($CaSO_4 \cdot H_2O$) were purchased from the Yipin tofu factory (Nordic Green Food AB) in Vallentuna, Sweden. Other chemical reagents were supplied by the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden.

2.2 Bean milk preparation

Dry beans (500 g) were soaked for 16-20 h in room temperature water until they reached approximately twice their original weight. Soaked beans were then strained and ground using a kitchen blender with an appropriate amount of water to reach a total weight of 2000 g. This resulted in a bean-water ratio (BWR) of 1:3 (w/w) on a dry bean weight basis. After grinding, a set amount of water (1.5 or 3.0 L) was added to the slurry to reach BWR of 1:6 and 1:9, respectively. The slurry was then filtered through a muslin cloth and manually pressed to separate the filtrate, which was referred to as bean milk. The same process was repeated using soybeans and faba beans to produce *soymilk* and *faba bean milk*, respectively.

The soymilk could be used immediately for tofu production, while the faba bean milk was treated for starch removal before further use. Two methods for faba bean starch removal were investigated: (1) enzymatic hydrolysis and (2) decantation.

For enzymatic hydrolysis, the filtered faba milk was heated to a temperature of 55-60°C using a water bath. Then, 1.0 ml of Ceremix-Flex (liquid maltogenic amylase; The AltGrain Co. Ltd., Southend-on-Sea, UK) was added to the milk. The milk was held at this temperature for 60 min before being used for tofu production.

For decantation, the filtered faba bean milk was left undisturbed at room temperature (~21°C) to allow starch granules to settle down. After two hours, the supernatant was carefully poured off to separate out the sedimented starch. The decanted supernatant was then used for tofu production.

Prior to tofu production, the pH of the treated milks were measured using a pH meter (VWR, Radnor, USA) that was calibrated using buffer solutions of pH 4,0 and 7,0. Bean milk samples were collected for dry matter content analysis.

2.3 Tofu production

Bean milk produced as previously described was heated in a stainless steel pot on a stove until it reached a boiling remperature (≥95°C). The milk was then held at this temperature for 5-10 min, with occasional stirring to prevent scorching, and subsequently cooled to 80°C using a water bath. At this stage, a suspension of calcium sulphate dihydrate (gypsum) in 50 mL of water was added to the milk. The amount of coagulant added corresponded to a concentration of 0,4% (w/v), or ~0,023M. The milk was stirred manually for ~10 seconds to distribute the coagulant evenly and then left for 30 min to allow coagulation, with intermittent stirring to prevent sedimentation of the coagulant. The curd was transferred to a stainless steel tofu mould ($16 \times 12 \times 8$ cm^3) clad with muslin cloth and pressed with a weight of 4 kg (20,83 g/cm^2) for 20 min, followed by 8 kg (41,67 g/cm^2) for an additional 10 min, with the tofu block being re-wrapped in-between pressings. After pressing, tofu blocks were allowed to cool and divided into halves. One half of each tofu block was used immediately for analysis of dry matter content and water-holding capacity, while the other was stored refrigerated (~6°C) in a plastic bag for up to 18 h prior to textural analysis.

In total, six variants of tofu were produced for testing. Four variants of faba tofu were manufactured using BWR of 1:6 and 1:9 with starch removal achieved through amylase treatment and decantation, respectively. In addition, two variants of soy tofu were manufactured using a BWR of 1:6 and 1:9 as reference samples. Each tofu variant was produced in duplicate, resulting in two tofu blocks per variant.

2.4 Dry matter content

The dry matter content of the bean milks and tofu products was analysed following the method described by Nielsen (1994). Briefly, 5.0 ml of bean milk or 5.0 g of tofu was placed in an aluminum container and subjected to air-drying in an oven at 105°C for 18-24 h. The difference in weight before and after heat-treatment was denoted as the sample moisture content (%). The dry matter content (%) was calculated by subtracting the sample moisture content from the original sample weight (100%). The dry matter content was measured thrice for each tofu duplicate.

2.5 Tofu water-holding capacity (WHC)

Water-holding capacity (WHC) is a parameter that reflects the ability of a material to retain water (Zhang & Qin 2019). The WHC of the three tofu variants with BWR of 1:9 was analysed as described by Jiang et al. (2020), with slight modification. 20 g of tofu were sampled into 50 mL centrifuge tubes and centrifuged at 2060 × g in room temperature (~21°C) for 30 min. The supernatant was carefully collected and subjected to air-drying in an oven at 105°C for 18-24 h. The weight of the evaporated water was denoted as the released water from the sample (Wr). The total amount of water in the tofu sample (Wt) was calculated based on its average dry matter content. Finally, the water-holding capacity (WHC) was calculated as the ratio (%) of the remaining water in the centrifuged sample (Wt-Wr) to the total amount of water in the sample (Wt). Centrifugation and supernatant collection was performed within a maximum of 72 h after tofu production. WHC measurements were performed three times for each tofu duplicate.

2.6 Tofu yield and textural analysis

The wet weight of each tofu replicate was measured immediately after pressing. Tofu yield was defined as the wet weight (g) of fresh tofu obtained per 100 g of dry beans. Tofu texture was analyzed by uniaxial compression using a texture analyzer (model TA-HDi; Stable Micro Systems Ltd., Surrey, UK) fitted with a 50 kg load cell. Six cylindrical samples (2.0 cm diameter and 2.0 cm in height) were excised from the center portions of each tofu block and compressed twice to 75% of their original height at a speed of 1.5 mm/s using a cylindrical probe with a diameter of 50 mm (P50).

The texture analysis data obtained from the compression tests was used to calculated various textural parameters using the texture-profile analysis (TPA) curve, following the methods described by Bourne (2002). Fracturability, representing the brittleness of the tofu sample, was defined as the force (N) required to produce a distinct break in the curve of the 1st compression cycle. Hardness was defined as the maximum force recorded during the 1st compression cycle, indicating the firmness of the tofu sample. Springiness, or elasticity was expressed as the horizontal difference (millimeters) between the starting point of and peak of the 2nd compression cycle. It represents the ability of the tofu to regain its original shape after deformation. Cohesiveness was defined as the ratio in positive force area during the 2nd compression cycle to that of the 1st compression cycle, reflecting the degree of internal bonding within the tofu sample. Chewiness was calculated as the product of hardness, springiness and cohesiveness. By analyzing these parameters, we can gain insights into the texture characteristics of the tofu samples and assess their sensory attributes related to firmness, brittleness, elasticity, cohesiveness, and overall chewiness.

2.7 Microstructural analysis by CLSM and LM

Microscopy sample preparation and imaging was executed by supervisor Jing Lu.

Microstructural analysis was performed using a confocal laser-scanning microscope (CLSM) supplemented with a supersensitive GaASp detector (model LSM780; Zeiss, Jena, Germany). The microscope was built on an inverted Zeiss Axio Observer and modified from a previous approach (Lu et al. 2018).

For CLSM analysis, samples of unfiltered bean milk (slurry), treated bean milk and fresh tofu were prepared as follows. For slurry and bean milks, 10 μL of liquid sample were distributed onto a concave slide and stained for 2 min with a mixture of 0.01% (v/v) calcofluor white (CAL) for fiber detection, 0.25% (w/v) fluorescein 5-isothiocyanate (FITC) for starch detection, and 0.025% (w/v) rhodamine B (RHO) for protein detection. The fresh tofu samples were cut into 2 mm^3 cubes and embedded in freeze medium, after which they were sliced into 30 μm sections. Sliced samples were stained with the same mixture of staining dye as described above and then washed with deionized water.

The dyes were detected through excitation and recording of emission wavelengths. An Argon laser with an excitation wavelength of 488 nm was used to excite FITC, and emission wavelengths between 520 nm and 550 nm were detected. A He-Ne laser with an excitation wavelength of 543 nm was used to detect RHO, and emission wavelengths from 565 nm to 660 nm were detected. The excitation and emission wavelengths for CAL were around 355 nm and 400-460 nm, respectively. All images were acquired using a C-Apochromat 40x oil immersion objective with a resolution of 1024 x 1024 pixels.

The structure of the protein network and starch granules present in the tofu samples were also observed using light microscopy (LM). Double-staining with light green (LG) and iodine (IOD) was employed to differentiate between protein and starch, respectively. Cryostats from Leica CM1860 in Austria were used to obtain 30 μm thick sections, which were examined using a Nikon Eclipse Ni-U microscope. To capture the images, a Nikon Digital Sight DS-Fi2 camera was utilized, and ImageJ software (Fiji.se/Fiji) was employed for image processing.

3. Results

3.1 pH, yield, dry matter content and WHC

Tofu yield was expressed as grams of tofu obtained per 100 grams of dry beans (Table 1). Soybean tofu variants (SOY) produced a substantially higher yield than faba bean variants. However, SOY tofu variants exhibited a relatively high degree of syneresis during refrigerated storage which would result in a lower yield over time. Amylase-treated (AM) faba bean tofu variants had a higher yield than decantation (DEC) variants. The pH of the bean milks ranged from 6,04-6,23, with the DEC variants at the higher end of the range.

Table 1. Mean milk pH, tofu weight and tofu yield.

Tofu variant	**Mean pH of prepared bean milk**	**Mean tofu weight after pressing (g)**	**Mean tofu yield (g tofu per 100 g dry beans)**
AM 1:6	6,04	694,9	139,7
DEC 1:6	6,23	567,3	114,1
SOY 1:6	6,04	1000,9	201,3
AM 1:9	6,08	687,3	137,9
DEC 1:9	6,22	588,6	118,1
SOY 1:9	6,12	1052,8	211,3

AM (amylase) and DEC (decantation) indicate faba bean starch removal method. Numbers indicate bean-to-water ratio.

Dry matter content was determined for both bean milks and tofus (Table 2). For each of the six tofu variants, uncertainty is given as the standard deviation of the replicate mean value. The soymilk had a higher dry matter content compared to the faba bean milks of the same BWR. In addition, amylase treatment resulted in a higher milk dry matter content compared to decantation for faba bean milks. Mean WHC was estimated for 1:9 BWR tofu variants due to technical error and time constraints (Table 3). The soy tofu variants displayed a noticeably lower WHC compared to the AM and DEC faba bean tofu of the same BWR.

Table 2. Mean dry matter content and moisture content.

Tofu variant	Mean milk dry matter content (%)	Mean tofu dry matter content (%)	Mean tofu moisture content (%)
AM 1:6	6,710 ± 0,755	16,410 ± 0,767	83,590 ± 0,767
DEC 1:6	4,982 ± 0,647	18,930 ± 2,370	81,070 ± 2,370
SOY 1:6	8,635 ± 0,632	18,754 ± 0,675	81,246 ± 0,675
AM 1:9	4,430 ± 0,331	19,750 ± 0,555	80,250 ± 0,555
DEC 1:9	3,506 ± 0,636	20,704 ± 1,079	79,296 ± 1,079
SOY 1:9	5,181 ± 0,407	20,876 ± 1,210	79,124 ± 1,210

Table 3. Mean tofu WHC.

Tofu variant	Mean tofu WHC (%)
AM 1:9	85,635 ± 4,333
DEC 1:9	88,189 ± 2,953
SOY 1:9	75,982 ± 2,542

3.2 Textural analysis

TPA testing produced twelve texture-profile curves per tofu variant. Each curve was individually analysed to quantify the TPA parameters of interest. Based on these results, the mean fracturability, hardness, springiness, cohesiveness and chewiness was calculated for each of the six tofu variants (Table 4). In general, the soy tofu varints exhibited softer and springier textures compared to the faba bean tofu variants. Cohesiveness, however, was similar across all tofu variants. DEC tofu variants showed the highest values for fracturability, hardness and chewiness among all variants. The DEC 1:6 variant was the least springy, while the SOY 1:9 variant was the softest and most springy of all variants tested. AM variants had the lowest mean fracturability compared to other variants of the same BWR.

Table 2. TPA parameters of tofu variants.

Tofu variant	Mean fractur-ability (N)	Mean hardness (N)	Mean springiness (mm)	Mean cohesiveness (unitless)	Mean chewiness (Nm)
AM 1:6	5,639 ± 0,514	16,929 ± 1,366	9,420 ± 1,369	0,213 ± 0,004	0,0339
DEC 1:6	6,454 ± 0,934	20,402 ± 2,270	8,682 ± 1,017	0,227 ± 0,011	0,0403
SOY 1:6	6,154 ± 0,983	12,900 ± 1,520	10,884 ± 1,281	0,226 ± 0,008	0,0318
AM 1:9	4,451 ± 0,443	15,849 ± 1,554	9,354 ± 0,670	0,216 ± 0,011	0,0321

DEC 1:9	7,369 ± 0,665	23,893 ± 1,857	9,539 ± 0,927	0,213 ± 0,009	0,0485
SOY 1:9	5,612 ± 0,933	13,146 ± 1,660	11,344 ± 1,063	0,245 ± 0,017	0,0365

All mean values are reported ± SD, except for chewiness.

Average force-time curves were generated from the TPA data using the Python-based programme *Thonny* (version 4.0.2, available at https://thonny.org/) (Figure 1). Tofu variant names were shortened to AM/DEC/SOY followed by a single number corresponding to BWR (6 for 1:6, or 9 for 1:9). In the force-time curves, all tofu samples showed a distinct fracture point characterized by two peaks during the 1st compression cycle. During the 2nd compression cycle, a single peak was generally produced, although it can be noted that there was considerable noise in this region for all samples analyzed. These force-time curves provide a visual representation of the tofu's mechanical properties and illustrate the differences in texture between the variants.

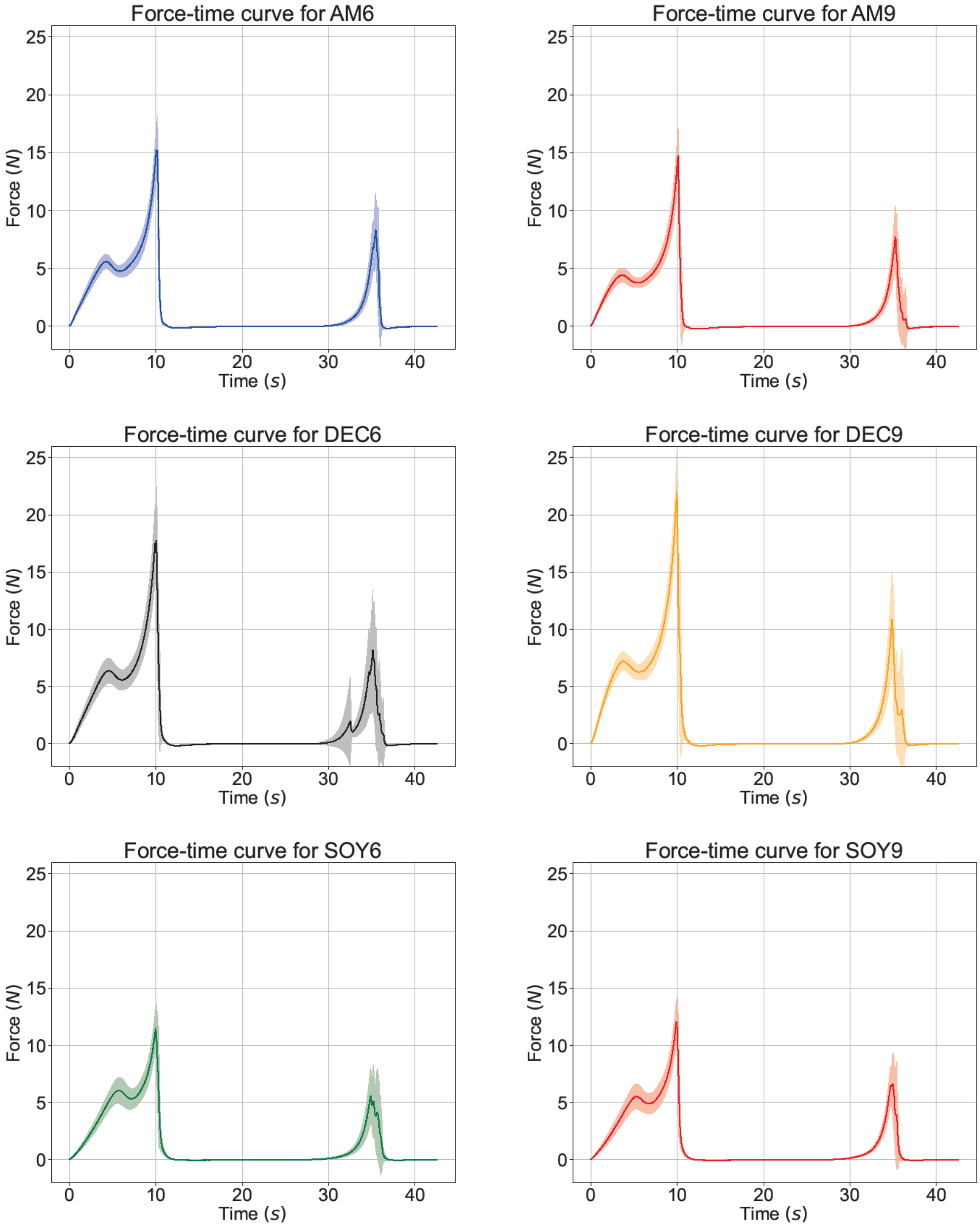

Figure 1. Average force-time curves for each of the six tofu variants. Each data point is reported ± its standard deviation, shown as an opaque area above and below the main curve.

3.3 Microstructural analysis

3.3.1 CLSM of faba bean slurry and milk

The microstructural properties of fiber, starch, and protein network in the 1:6 bean-water ratio faba bean slurry, milk, and tofu were analyzed using confocal laser-scanning microscopy (CLSM) and staining techniques.

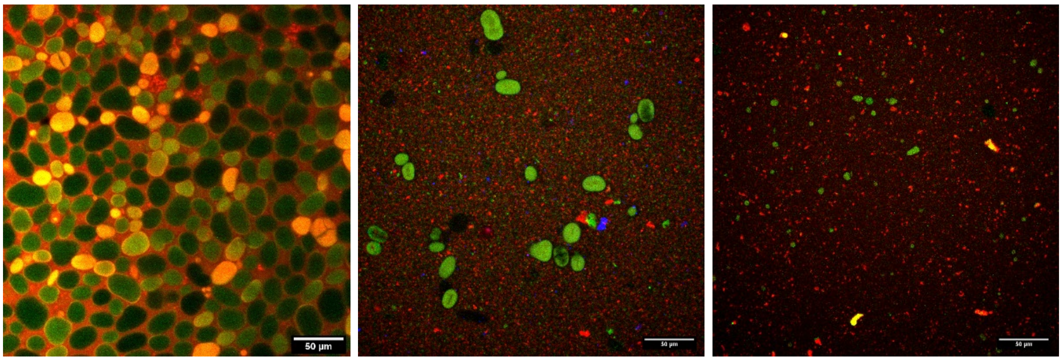

Figure 2. CLSM images of 1:6 BWR slurry (left), amylase-treated faba bean milk (middle) and decanted faba bean milk (right). Fiber is visualized in blue, starch in green, and protein in red.

Figure 2 shows CLSM images of faba bean slurry, amylase-treated faba bean milk and decanted faba bean milk. The faba bean slurry exhibits densely packed starch granules (green) suspended in a protein-rich liquid phase (red). There are also small pieces of fiber (blue) suspended in the liquid phase, although somewhat difficult to see. In the amylase-treated faba bean milk, relatively large starch granules are visible, while the decanted milk displays a reduced presence of intact starch granules. There is also a scattering of small green speckles in the background of the amylase-treated milk, which is not visible in the decanted milk.

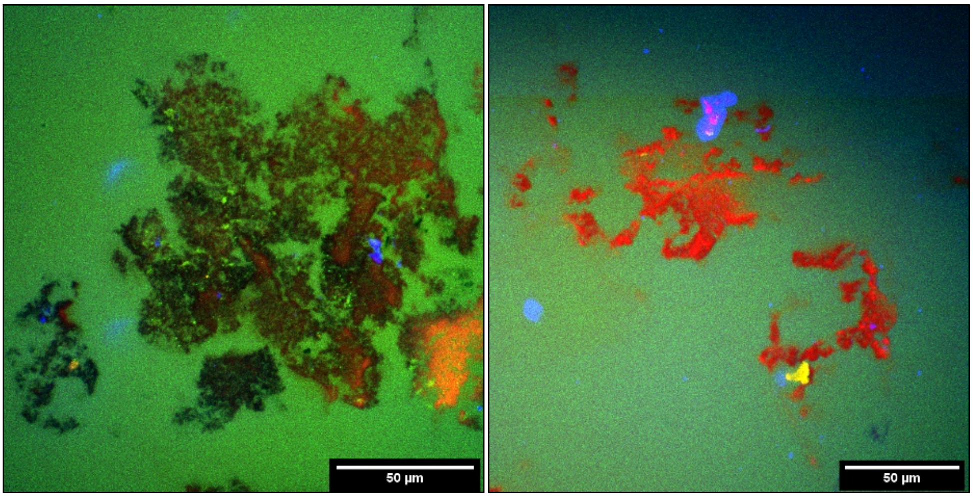

Figure 3. CLSM images of AM 1:6 (left) and DEC 1:6 (right) faba bean tofu, respectively.

Figure 3 shows CLSM images of AM 1:6 and DEC 1:6 faba bean tofu, respectively. Staining reveals that the predominant component in the tofu gel structure is protein, as indicated by the red coloration. However, small speckles of starch can be observed, depicted in green in the CLSM image of the AM tofu (left).

3.3.2 LM of slurry and tofu

To visualise the tofu gel network and starch distribution, light microscopy (LM) and staining was performed on 1:6 BWR tofu variants. Figure 4 shows LM images of faba bean tofu variants, namely AM 1:6, DEC 1:6 and SOY 1:6, respectively. The tofu samples were double-stained with LG and IOD. All images are captured at the same resolution (3x10).

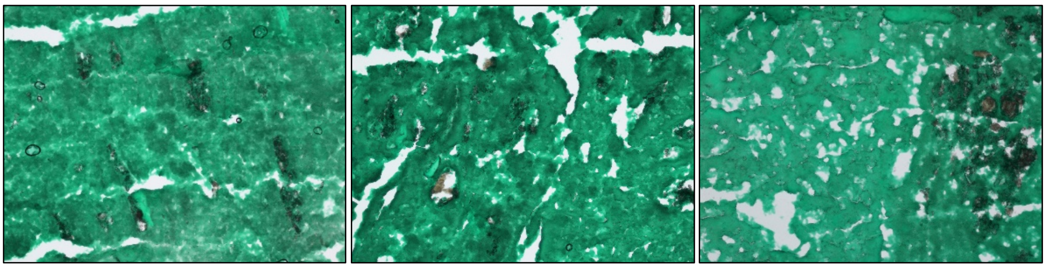

Figure 4. Light microscopy staining images of AM 1:6 (left), DEC 1:6 (middle) and SOY 1:6 (right). Green, protein; dark blue to reddish-brown, starch.

In the LM images, protein appears as a continuous phase, forming a network-like structure. This protein network provides texture and cohesion to the tofu matrix. The starch component, however, appears as dispersed granules within the protein network. The distribution and arrangement of starch granules is non-uniform, hence producing a discontinuous phase. In the decanted tofu, the protein network displays a higher degree of aggregation and compactness, with protein strands densely arranged throughout the matrix. In the amylase-treated tofu, on the other hand, the protein network displays a more fragmented arrangement of components, producing a looser and more dispersed structure. In contrast, the soy protein network appears the least compacted among the tofu variants, exhibiting a low degree of protein aggregation and round pores evenly distributed throughout the matrix. These differences in protein network structure contribute to contrasting qualities and textures between the tofu variants.

4. Discussion

Contrary to the initial hypothesis, it was observed that a lower bean-water ratio (BWR) was not required to achieve coagulation of the faba bean milk. Thus, a minimum solids content of 3,5% (± 0,6%) in the bean milk proved sufficient for lab-scale tofu production. While reducing BWR did result in an increased solids content in the bean milk, it did not have a major influence on the yield nor solids content of the resulting tofu. The moisture content in tofu was consistent across all variants, averageing around 80-84%.

Faba bean tofu variants produced a substantially lower yield compared to soybean (SOY) variants.The average fresh yield of faba bean tofu variants, AM and DEC, were 67,3% and 56,3%, respectively, in relation to the average fresh soy tofu yield. In addition, the soymilk exhibited a relatively higher solids content when compared to faba bean milk at the same BWR. These differences may be attributed to the dissimilarity in protein content between the two raw materials.

Among the faba bean variants, it was observed that amylase-treated faba bean milk had a relatively higher solids content than decanted faba bean milk of the same BWR. This observation can be explained by the presence of starch hydrolysates, i.e. sugars, in the AM milk. Interestingly, AM milk resulted a higher tofu yield than DEC milk. Considering that the moisture content was similar across all tofu variants, these results suggest that water content is not the sole factor influencing tofu yield. Indeed, the increased yield of AM tofu variants could possibly be the result of incorporation of faba bean starch or its hydrolysates into the tofu gel network. These findings highlight the impact of protein content and starch hydrolysates on the yield and composition of tofu variants derived from soybeans and faba beans.

An interesting finding was the discrepancy in water-holding capacity (WHC) between different tofu variants. Specifically, the AM and DEC tofu variants had a higher WHC of 86% and 88%, respectively, compared to the soybean tofu variant with a WHC of 75%. In addition, soybean tofu variants exhibited a relatively high degree of syneresis during the first 24 h of refrigerated storage when compared to faba bean tofu variants. These observations suggest that water is more extensively bound to the protein network in faba bean tofu compared to soy tofu. One possible explanation for this phenomenon is the presence of faba bean starch, which may contribute to water retention within the tofu gel. Additionally, faba beans may contain some other type of water-binding compound, such as a chelating agent or surfactant, which further increases water retention. Protein content, composition, subunit profile are other factors that may influence tofu WHC. These factors determine the type and number of bonds formed within the protein network, thus influencing water-holding capacity. Lastly, WHC of legume proteins is influenced by their surface hydrophobicity and solubility (Ma et al. 2022). Thus, differences in hydrophobicity and solubility of faba bean and soybean proteins may also contribute to the observed dissimilarity in tofu WHC.

Tofu texture was found to be influenced by multiple factors, including the raw material used, BWR, and the method for starch removal in faba bean milk. Overall, the soybean tofu exhibited a softer and springier texture compared to faba bean tofu. Cohesiveness, however, was similar across all tofu variants. The DEC variants produced the highest values for fracturability, hardness and chewiness, resulting in a more challenging eating experience. The increased chewiness in DEC variants can be primarily attributed to their higher hardness values, as springiness and cohesiveness did not differ substantially from other tofu variants. In addition, DEC 1:6 exhibited the lowest springiness of all tofu variants. In contrast, the SOY 1:9 variant was the softest and most springy. Although a reduced BWR did not consistently correlate with increased tofu fracturability, hardness, springiness, cohesiveness and chewiness in all tofu variants, the DEC 1:9 and SOY 1:9 variant scored higher in hardness and springiness compared to their respective 1:6 variants. AM variants exhibited the lowest mean fracturability compared to other variants at the same BWR. In addition, while handling the AM tofu samples, a slightly paste-like texture was observed, which is an attribute commonly associated with starch gels. This suggested that the residual faba bean starch granules in the AM faba bean milk may have gelatinised during the coagulation process, resulting in a more starch-gel-like texture in the tofu gel.

Hardness in faba bean tofu is an issue that needs to be addressed, as it was substantially higher compared to soybean tofu. Reducing coagulant concentration could be of interest, as this is correlated to decreased hardness in soybean tofu. In addition, the effects of varying other processing parameters, including type of coagulant, should be investigated for optimization of faba bean tofu texture. As previously mentioned, tofu hardness is closely correlated to factors such as total protein content and the relative content of 11S/7S proteins, although no such measurements were performed in this project. One possible explanation for faba bean tofu hardness is the high degree of aggregation and compactness exhibited by the protein gel matrix, in which the low lipid content in faba beans could play a role. As mentioned, lipids are incorporated into the tofu matrix by interaction with bean proteins and act as space-filling particles. Hence, the lack of lipids thus could be responsible for the heterogenous and compacted structure in faba bean tofu. Shin et al. (2015) found that reducing the lipid content in soymilk lead to increased hardness in the resulting tofu. In addition, the protein gel in low-fat tofu exhibited a more disorganized, coarse and heterogenous structure. Further research is needed to investigate the factors contributing to faba bean tofu hardness and optimize product texture.

CLSM analysis illustrated the efficiency of starch removal and tofu gel composition. Regarding the faba bean milk, both enzymatic hydrolysis and decantation appeared most efficient in reducing the number and size of starch granules in the liquid, albeit to varying extents. In the amylase-treated faba bean milk, residual starch granules appear to be present, although reduced in number and size compared to the initial faba bean slurry. These starch residues may become incorporated into the faba bean tofu gel upon coagulation. In the decanted faba bean milk, however, larger starch granules seem to have been effectively removed by decantation. Consequenty, the resulting tofu matrix is expected to exhibit a noticably lower presence of visible starch granules compared to the amylase-treated variant. Nonetheless, small granular fragments are still visible in the decanted milk. This is in accordance with expectations, as larger particles experience greater gravitational pull and are thus more effectively removed by decantation. In the final faba bean tofu products, small speckles of starch were visible only in the AM tofu variant and not in the DEC variant. To summarize, these results suggest that decantation was more efficient at removing faba bean starch than amylase-treatment. However, it is worth exploring the use of other hydrolysis conditions or different amylase enzymes, as they may result in higher hydrolytic efficacy.

Based on LM imageing, both faba bean tofu and soybean tofu consists of a continuous phase of protein (green) with a discontinuous phase of starch (brown). Nonetheless, they display slight observable differences in microstructure. Soybean tofu exhibits a homogenous and loose protein network structure with an even distribution of round pores. In comparison, faba bean tofu gel structure appears irregular with densely aggregated protein strands and large slits interrupting the matrix. The many round pores could be a contributing factor to soy tofu springiness, as they may allow for more movement within the structure. Thus, the lack of pores in the faba bean tofu variants may contribute to rigidness within the gel structure. To conclude, the microstructural differences between faba bean and soybean tofu gels are likely the result of variation in several factors, including the size and organization of starch granules within the protein matrix, as well as the organization of the protein network itself. Further microstructural analysis by scanning electron microscopy (SEM) is revelant to obtain more detailed information about the textural attributes and internal framework of tofu. Hence, this would allow for a deeper understanding of microstructural characteristics of faba bean tofu.

As previously mentioned, the bean milk boiling step can be performed either before or after the removal of okara by filtration. However, during the initial trials of this project, it was not possible to obtain a stable tofu gel from faba bean milk that had been boiled prior to okara removal. A solid coagulum did not form as a result of coagulant addition, neither using calcium sulphate nor magnesium chloride (nigari). This suggests that boiling the bean milk in presence of okara may release some component that may hinder the process of protein gelation and curd formation. For example, soybean okara has been shown by Toda et al. (2007) to affect the physicochemical properties of soymilk and soy tofu texture. Conducting a similar analysis on faba bean milk and tofu is relevant to further explore the potential implications of okara components in faba bean tofu production.

This project consisted of a small-scale laboratory production of faba bean tofu with several manual steps, such as stirring and pressing, which is very different from industrial processing. Thus, the methods used here are not directly applicable to industrial tofu production. In addition, industrial processing methods are standardized to yield a more consistent result. Summarized, large-scale faba bean tofu production required adaptation to industrial processing conditions.

Stirring was found to be of major importance for proper coagulation of both soybean and faba bean tofu. For some tofu variants, such as the amylase-treated 1:6 faba bean tofu and the 1:9 soy tofu, only a slim layer of curd had formed at the bottom of the pot after 30 min of coagulation and there was no clear separation from the whey. However, in these cases, satisfactory coagulation could be achieved by additional stirring at the 30 min mark followed by another 10 min of coagulation. In the 1:6 soymilk curd, small speckles of gypsum could be seen in the bottom layers of the pot, however coagulation was successful after 30 min nonetheless. Based on these observations, it is unprobable that bean milk concentration was the issue. As previously mentioned, there are many interdependent factors that influence bean milk coagulation, and in this small-scale project, it is therefore difficult to determine causal relationships. Nonetheless, it seems that sufficient stirring is required to evenly disperse the coagulant. Therefore, instead of sporadic stirring during the first 15 min after coagulant addition, stirring should be continuous. In addition, increasing the water volume from 50 ml to 100 ml when preparing the coagulant suspension could aid in proper coagulant dispersal.

4.1 Final conclusions and ideas for further research

The results indicate that there is potential for developing a tofu-like faba bean product. Indeed, faba bean milk can be coagulated using calcium sulphate to produce a curd (protein gel) with textural characteristics similar to traditional soybean tofu. Nonetheless, it is essential to modify the starch removal method to increase efficiency. For this purpose, it is worth exploring alternative amylase enzymes and varying hydrolysis conditions to achieve satisfactory faba bean starch hydrolysis. Other physical starch removal methods, such as ultrafiltration, may also be of interest. In addition, other processing parameters in faba bean tofu production, such as coagulant types and concentration, temperature, and stirring method, should be further investigated to optimize textural and structural properties of faba bean tofu. Moreover, further analysis of tofu microstructure should be undertaken to gain deeper insights into the gel formation behaviour of faba bean proteins. Ultimately, conducting industrial-scale testing with sensory panels will be decisive in determining the feasibility of commercially producing this type of product with high quality.

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Acknowledgements

I could not have finished this project without the support and encouragement from my supervisors, peers and family. I owe a special thank you to my supervisors, Jing Lu and Fredrik Fogelberg, who grounded my initial ambitions and guided me through the highs and lows of tofu production. Last but not least, I owe a thank you to Erik Karpelin, who helped me compile my force-time curves by programming and empathised in my struggles.

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