Development of Tempeh on Swedish Legumes

A process optimization, safety investigation and sensory analysis.

Nils Åström

Supervisors: PhD. Åsa Håkansson, M.Sc. Pamela Canaviri
Examinator: Prof. Marie Wahlgren

LUNDS UNIVERSITET
Lunds Tekniska Högskola

Abstract

The emerging market for meat substitutes has largely been filled with different types of soy products. Although soy usually has a lower environmental impact than meat, soy is not entirely unproblematic. Deforestation and global transportations are two negative effects of the increasing production of soybeans. One of these meat substitutes is tempeh: an Indonesian mold-fermented food usually made of soy. Tempeh can however be made with many types of legumes. In this thesis, the soybeans in tempeh have been replaced with Swedish grown yellow peas and brown beans. These Swedish legumes are not associated with the same environmental problems as soybeans.

The aim was to develop a good tasting tempeh on Swedish legumes which could become commercially viable. To ensure this, objective quality parameters were investigated as well as the safety of the product. A sensory evaluation with 21 volunteers was also performed.

To produce tempeh, eight process steps were established. The steps were: Washing, Soaking, Boiling, Drying, Peeling, Inoculation, Bag preparation, Incubation. A process optimization was executed, where the most important of those eight steps were optimized to observe the effect in the result. The idea was to optimize as many different parameters as possible and in the end combine the best ones to create the best possible tempeh regarding taste, looks and consistency.

Two investigations were conducted to ensure the safety of the tempeh. The microbiological investigation showed that the tempeh contains loads of microorganisms other than the obvious mold. The cfu/g on VRBD (E*nterobacteriaceae* or other bile-tolerant Gram-negative bacteria) was $>3*10^5$ for yellow pea tempeh and $3.35*10^4$ for brown bean tempeh. The bacteria have most probably originated from the starter culture. Those kinds of bacteria are generally indicators of bad food hygiene. When investigating a pea tempeh inoculated with *Lactobacillus plantarum* 299v, the cfu/g on VRBD was substantially smaller, indicating that the lactobacilli outcompeted the E*nterobacteriaceae*. The presence of lactobacilli could therefore be included in a safer, future version of the tempeh. The toxin investigation showed that the tempeh contained Ochratoxin A at levels of <1.0 µg/kg, Aflatoxin B1 at levels of <0.2µg/kg and Aflatoxin B2 at levels of <0.5µg/kg. These levels did not differ much from normal amounts found in legumes. Therefore, the tempeh made in this project do not contain dangerous amounts of these mycotoxins.

The sensory analysis showed that there is a significant difference in taste between tempeh made on yellow peas and brown beans respectively compared to soy. The analysis also showed that tempeh on brown beans is as favorable as soybean tempeh and that tempeh of all three legumes was appreciated by all volunteers.

Content

1 Introduction 3
2 Aim 5
3 Materials and Methods 5
3.1 Materials 5
3.2 Process optimization 6
3.3 Safety investigation 8
3.4 Sensory analysis 9
4 Results and discussion 9
4.1 Process optimization 9
4.2 Safety investigation 14
4.3 Sensory analysis 18
5 Conclusion 19
6 Future Outlook 20
7 Acknowledgements 21
8 References 22
9 Appendix 1- Process optimization trials 25
10 Appendix 2- Toxicological analysis 46
11 Appendix 3- Sensory analysis 48

1 Introduction

In western countries, substituting meat is a growing food trend. The demand for innovative replacement products are increasing by the day and the options are many. Soy based products hold a huge segment of this market with products such as tofu, tempeh, soy meat, soy bacon et cetera (Upadhyay, 2016).

Soy has a lot of great properties such as its nutritional value and a low price. However, soy has downsides as well. Two notable examples are rainforest devastation and transportations across the globe (WWF, 2018). Another downside with soy is the widespread soy allergy. Soy is one of the eighth most common food allergies in the U.S (AAFA, 2015).

Tempeh is a traditional soy-based food, native to Indonesia, where it has been consumed as a protein source for at least two hundred, but possibly a thousand years. As of now, tempeh is not widely spread in western countries except for the Netherlands (Shurtleff and Aoyagi, 2007).

Tempeh is made by fermenting boiled soybeans with the mold *Rhizopus oligosporus.* As can be seen in Figure 1 below, the result of the fermentation is a firm block of mold that makes the beans stick together. Traditionally, the boiled beans were inoculated with the mold from a previous batch and then wrapped with banana leaves to ferment in room temperature in Indonesian homes. (Shurtleff and Aoyagi, 2007). Nowadays, an industrially produced starter culture is usually used for inoculation and plastic or stainless-steel are often the materials of choice for fermentation. (Nout and Kiers, 2005)

Slices or cubes of tempeh are usually cut from the block and then cooked. Cooked tempeh can be eaten as it is, or incorporated in food dishes such as burgers, salads and stews. Recipes for cooking for tempeh involves frying, boiling, steaming, oven baking and grilling (Shurtleff and Aoyagi, 1979). The appearance of fried tempeh can be seen in Figure 2 below.

Tempeh is vegan, rich in protein, vitamins and has a higher amount of free amino acids than ordinary boiled soybeans (Murata et al., 1967). Tempeh fermentation also improves the digestibility of legumes by reducing the amounts of antinutrients (Dinesh et al., 2009).

There are risks when it comes to tempeh as well. It is possible for several different pathogenic microorganisms to grow during the fermentation of tempeh made from unacidified soybeans (Tanaka et al.1985). Hygienic practices and proper fermentation conditions are therefore of importance when producing tempeh.

As mentioned above, tempeh is fermented using *Rhizopus oligosporus* which belongs to Rhizopus microsporus group. In that group there are certain strains of mold capable of producing mycotoxins. However, no such capability has yet been found in *Rhizopus oligosporus* (Jennessen et al., 2005).

Tempeh is usually made with soybeans, but it is possible to make tempeh on all sorts of legumes (Neikell, 2016). This was what enabled the execution of this thesis.

The idea to develop a tempeh using Swedish legumes instead of soybeans was intriguing because it would benefit the environment, Swedish farmers and Swedish consumers.

Yellow peas have been cultivated and consumed in Sweden since the 13th century (Wikipedia, 2018) and brown beans since the 17th century (Blom, 2016). These Swedish legumes have properties similar to soy and grows well in Swedish soil. The legumes do not have to be imported from far away and does not increase deforestation. In contrast to soy, these Swedish legumes are not used in such a wide range of food applications, which provides a large potential to develop new products based on them. Although some people are allergic to peas and beans, these allergies are not as common as soy allergies. (Astma och Allergiförbundet, 2018)

This thesis will involve:

- A process optimization to create and improve tempeh on yellow peas and brown beans.
- A safety investigation on both a microbial and toxicological level because of the risks mentioned above
- A sensory analysis to evaluate the taste of the tempeh.

Figure 1. Appearance of raw soy tempeh.

Figure 2. Appearance of fried yellow pea tempeh.

2 Aim

The aim of this project is to develop a commercially viable tempeh where soy is replaced with Swedish yellow peas or brown beans. To accomplish this, safety, homogeneity, visual appearance and firmness was set as criteria. Other criteria were to make it at least as preferable as soybean tempeh.

3 Materials and Methods

3.1 Materials

Materials used in the different experiments are listed in table 1.

Table 1. List of materials used in the different parts of the project.

Name	**Producer, Country**
Yellow dried peas	Lantmännen ceralia, Sweden
Brown dried beans	Lantmännen ceralia, Sweden
Yellow dried peas, organic	Lantmännen ceralia, Sweden
Soybeans	Risenta, Sweden
White wine vinegar, 6% acid.	Ica, sweden
Tempeh starter cultures	Raprima ragi, Indonesia
“Probi mage” capsules	Probi, Sweden
Ethanol	Merck, Germany
VRBD Agar	Merck, Germany
Malt Agar	Merck, Germany
TSA Agar	Merck, Germany
Rogosa Agar	Merck, Germany
Rapeseed oil	Ica, Sweden

3.2 Process optimization

There were 8 steps in the production of this tempeh as illustrated in Figure 1 below.
To accomplish the desired results, a number of these steps were optimized and analyzed while keeping the rest constant. In each optimization trial, the parameter that gave the best results was the one which became the standard for the following trials. Firmness, homogeneity and visual appearance was judged according to the below mentioned criteria.

The reason why the process optimization took place before the microbial analysis was because it is irrelevant to investigate the safety of a product that no one wants to eat.

Firmness was based on the ability of sliced tempeh to hold together and not fall apart. Slices of tempeh were held up in one of the edges and jiggled around like a slice of bacon. If the slice held, it was judged as firm, if it held while holding but not while jiggling, it was judged as almost firm.

Homogeneity was judged as the percentage of mold covering the surface of the tempeh.

Visual appearance was judged as how many black areas of sporulated mold the tempeh contained, the fewer the better.

In total 15 trials of making tempeh was executed during this project, the layout and result of each trial can be found in Appendix 1.

The number of bags in the trials were always 10 or 12.
In between all trials, the incubator and all equipment were thoroughly washed by hand and disinfected by using ethanol.

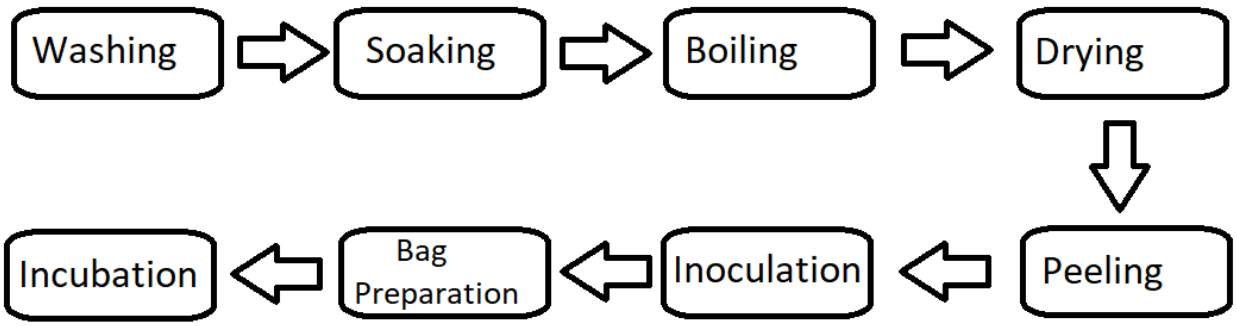


Figure 1. The different process steps in making the tempeh

Choice of legume
The legumes used in this project were yellow peas, organic yellow peas, soy beans and brown beans.

Washing

The legumes were placed in a sieve and washed carefully with cold water. The water used was food graded and all legumes were rinsed by the flow of water.

Soaking
The legumes were soaked with an excess of cold water for 12 hours each trial. The containers used were 10L stainless steel bowls.

Boiling
A hob with 1800 W was used on the highest effect in each trial. 8L water to 3kg legumes was used. When legumes were added to the boiling water the temperature always dropped to around 80 °C. It took between 8-10 minutes for the legumes to boil again after that.
The optimal boiling time was investigated by boiling soybeans for 30, 40, 50, 60, 70, 80 minutes and peas for 10, 20, 30, 40, 50, 60 minutes.

Drying
The effect of the wetness of the tempeh was investigated by comparing three different methods. In the first one, the legumes were rinsed with water right before the inoculation, in the second one the legumes had been out in the air for 30 minutes.
In the last one, the legumes were placed on perforated oven sheets and left to dry in the air for 30 minutes and then in the oven on 30 °C for 20 minutes.

The temperature was always controlled to be below 33 °C before moving on to the next step.

Peeling
Different ways of peeling and crushing the yellow peas were investigated. The peeling methods used were: unpeeled, hand-peeled and machine peeled. The crushing methods used were: food processor, chopping knife, hand blender, potato press and potato masher.

When using the machine peeling, 500 grams of yellow peas or soybeans were peeled at a time in a potato peeler, 30 seconds for each round. The hulls were still present but noticeably damaged by this method.
The brown beans were never peeled.

Inoculation
The legumes for one block of tempeh were put in a stainless-steel bowl. A ¼ teaspoon of starter culture were spread out over the legumes in the bowl. The legumes were mixed thoroughly until they all looked covered in starter. Then a second ¼ teaspoon was added and the procedure was repeated. After that, a tablespoon of vinegar was added and thoroughly mixed with the legumes in the bowl.

Three tempeh starter cultures from different retailers were investigated. The addition of *Lactobacillus Plantarum* 299v was also tried, as well as the addition of the Japanese mold Koji-Kin. Different amounts of both vinegar and starter culture were also tried.

Bag preparation
The bags were at first perforated with several holes. The bags were then filled with the inoculated legumes.

Different amounts of holes and sizes of holes were investigated. The holes were made by a large needle, with a diameter of 3 mm. A span of holes from 0 to 20 and the diameters of 1mm and 3mm were tried

The optimal amounts of legumes in the bags were investigated. A span of weights was tried, from 200g to 600 g.

Incubation
The bags were incubated at 30 °C for 48 hours. However, the time of 38 hours was also tried.

Three different incubators were tried, two of the same brand, and on older version. Two different air humidities were also tried by using the incubator in room humidity and by placing a bowl of water in the incubator.

3.3 Safety investigation

The microbiological quality of the starter cultures was investigated, as well as the microbial quality of the finished tempeh. Samples of finished tempeh were also sent to an accredited lab for a toxin analysis.

Starter cultures
The cultures were investigated by dissolving 1 g of culture into 9 ml of peptone water. A serial dilution was constructed and cultures of concentrations ranging from -1 to -6 were applied to four different agars. The amount of fluid applied was 0.1ml. The agars were: Rogosa (for lactobacilli), TSA (total count), VRBD (for *Enterobacteriaceae*) and Malt (for fungi)

Rogosa was incubated anaerobically in 37 °C for three days, TSA aerobically in 30 °C for 3 days, VRBD aerobically in 37 °C for 3 days and Malt aerobically in room temperature for a week.

Tempeh
Four different samples of tempeh were investigated microbiologically. The chosen ones were from a tempeh co-fermented with lactobacilli, a regular tempeh on yellow peas, a mold free spot from the same pea tempeh and a regular tempeh on brown beans.
Samples with 10 gram of tempeh and 90 gram of peptone water were decomposed in a stomacher. A similar serial dilution was used for the tempeh samples, but the concentration of -7 were the maximum this time.

Toxin analysis

300g each of the final versions of the yellow pea and brown bean tempeh were placed into plastic bags and sent to the accredited lab “Synlab” for toxin analysis. The toxins investigated were Ochratoxin A and Aflatoxins B1, B2, G1 and G2.

3.4 Sensory analysis

A sensory analysis was executed with 21 volunteers. The template used for the analysis can be found in Appendix 3. The first object was to investigate if the volunteers could taste the difference between tempeh made on soybeans, yellow peas, and brown beans. This was done using a triangle test. The volunteers were told to find the tempeh which tastes different from the others. The volunteers got three samples per plate and two plates in total. Two out of the three samples per plate was the reference tempeh- soy. The first plate handed to the volunteers contained pea tempeh and the and the second plate contained brown bean tempeh. To avoid the difference in colour between the tempeh, the volunteers had to sit in a dark room lit by IR light.

The triangle test was evaluated statistically to notice significant differences between the tastes of the three tempeh. The calculation can be seen in Appendix 3 and it is the standard data analysis for triangle tests. A chi-square distribution chart was used for evaluating the result extracted from the calculation. The chi-square value used was the one for 0.05, which means that the differences in taste could be proven with only a 5% risk for a type 1 error (Society of Sensory Professionals, 2018).

The second object was to tell which tempeh they favored out of the three, why, and to describe the taste of that one. They then got a plate with one sample of each tempeh.

The tempeh used in the sensory analysis was recently fried on a pan with lots of oil. 0.5cm thick pieces were cut to 1 cm in length and fried at 1800 W for four minutes in total (two on each side). No spices were added.

After the sensory analysis, the volunteers were asked if they liked the taste of all samples.

4 Results and discussion

4.1 Process optimization

The process optimization was the most time-consuming out of the three areas of this thesis. Many parameters that could have been investigated have not been so due to the time constraint. All trials done in the project and pictures of different batches of tempeh can be found in Appendix 1.

Choice of legume

In the beginning of the project, the idea was to only construct a tempeh with the yellow pea. The yellow pea was the primary choice due to its low cost.

Out of curiosity, the brown bean was tried as well. The results from the brown bean trial, which can be found in Table A9 were very good regarding the amount of mold and the firmness of the tempeh. Therefore, it was deemed better to construct two versions of Swedish tempeh. The good result obtained with the brown beans was possibly because the beans are missing an outer hull.
The inclusion of soy in the first and last trial was just to have a reference on how tempeh should taste and look. There was not a single shop selling soy tempeh to be found in Skåne county, (at least not visible on internet) and therefore the soy tempeh was made by hand.

In trial 11, organic peas were tried out after the suspicion that there was something in the regular peas peel that disrupted the growth of the mold. This did not work out well, possibly because the starter culture had been contaminated by *Lactobacillus plantarum* 299v from a previous trial. The organic peas cost much more but it could be interesting to investigate those again, that was not done due to time constraints.

Washing
The washing step was not optimized due to priorities and time constraint. What could be interesting is to investigate if there is something on the peels of the legumes that disrupts the fermentation, such as pesticides or toxins. There could also be different types of hardy bacteria that cause damage to the final product present in the peels. Being able to investigate if the washing could minimize the risks could be worth looking into.

Soaking
The step of soaking was not optimized. This was mainly because of the limited time period and because the difference in outcome was not perceived to be big depending on soaking time. This because the producer of the used legumes gave an approximal soaking time and not an exact one (Go Green, 2018) The time of 12 hours was chosen because both the producer and all recipes containing yellow peas and brown beans recommended 12 hours soaking. It would be possible to investigate the effect of a longer and shorter soaking process. The inclusion of vinegar and/or salt in this step could be investigated.

Boiling
The times were chosen to have the most common boiling times for each legume in the middle of the intervals. 30 minutes were found to be the optimal boiling time for peas because of the firmness and taste of the tempeh. 40 minutes were found to be the best boiling time for soybeans because of the same reasons. The results of the different boiling times can be seen in Table A1.

The boiling times were not entirely consistent because of the variation of time it took to get the legumes to the boiling point, it could differ with up to three minutes. The optimal time for the brown beans was not investigated because of the beans resulting in a great tempeh with the

first try of 30 minutes. This was also the most common boiling time to be found in various recipes found for brown beans.

Peeling

The effect of hand peeling seemed significant, the tempeh grew fast and had a perfect cake of mold. Only one handmade tempeh was however made during the project, so it is difficult to know if that was just a coincidence. The downside of hand peeling was that it was nearly impossible to peel large amount of peas by hand. That is why only one, completely hand peeled tempeh was made. The hand peeling method was therefore discarded after trial 3.
Because of the mold having difficulties to grow through the hull, the idea to damage the hulls arose (Tempeh Info, 2018). This is where the potato peeling machine came into the picture. The machine flung the peas around, creating visible scars on the hulls. The effect of the machine peeling was compared to that of unpeeled peas.

The idea of unpeeled peas was appealing because it would eliminate one step of the process line. In the end the machine peeling worked best, and it was the method of choice for the final product. In many trials it seemed like tempeh around 500 g was better with peeled peas. This can be seen in Table A6 and Table A8. With tightly packed tempeh around 600 g, the tempeh was much firmer with unpeeled peas. This might have to do with the peeled peas getting a bit mushy and the tempeh mold not preferring a too dense environment (Steinkraus, 1996).

Alternative ways of affecting the peels was also investigated in the form of crushing.
A food processor, chopping knife, hand blender, potato press and potato masher were tried. They did not result in good tempeh because they had quite bad mold growth and unacceptably bad firmness as can be seen in Table A5. The best of the crushing methods was the chopping knife, possibly because that method was the mildest of the five. In the crushing methods the hulls were almost destroyed, but they were still present in the tempeh. It would have been interesting to compare the result with a similar trial but with the peels removed.

Drying

The different drying methods gave different results as can be seen in Table A6. The soaked tempeh resulted in almost 100% mold growth on the outside, but with a bad firmness. The solely air-dried tempeh had good mold growth but not an optimal firmness. The combined air and oven dried tempeh were best because of the favorable firmness. That one did however had the worst mold growth. Possibly, it is important to have a lot of moisture on the outside of the tempeh cake- but less so on the inside.

It is highly possible that a different moisture level than the one used in this project is the best for tempeh, and better optimization can result in an even better tempeh. The method of choice for the final product became the air and oven dried. It was the results of this trial that awoke the thought of increasing the air humidity to increase mold growth.

The temperature always had to be checked before inoculation because of the mold being killed by temperatures larger than 33 °C. When the drying procedure changed throughout this project,

a simple wetness test was performed. The test was to put a paper towel on the legumes and see if it was wetted.

Inoculation
The standard amount of 1/2 tsp starter culture and 1 tbsp of vinegar was used because it was the most occurring in recipes for tempeh.
This was investigated, and a bigger amount of culture was tried, but to little or no effect as is seen in the results in trial 10.
The cultures from different retailers did not differ in the results in trial 2 and were thus used interchangeably throughout the project. The different cultures could however have played a role in the amount of bacteria present in the final product.

In a study it was shown that the addition of a lactobacillus plantarum reduced amounts of other microbes (Ashenafi and Busse, 1988) In order to increase the microbial quality and safety of the tempeh, this was investigated during the project. The well-studied bacteria *Lactobacillus plantarum* 299v was used for these trials. When using one capsule of 299v (0.48g) per tempeh bag, the bacteria completely outcompeted the mold, resulting in no spots of mold at all in those bags of tempeh. Lesser concentrations with 0.04, 0.02 and 0.004 grams were tried instead. The bacteria were still too powerful, so that line of thought was put on hold.

Another way of trying to make a safe tempeh was to incorporate the thoroughly studied Japanese starter culture “Koji-Kin” used for making sake, soy sauce and miso (Machida et al., 2008). The tempeh cake from using Koji-Kin was not as good as when using regular tempeh starter. It smelled great when fried and tasted good but was not used more in the trials because of the inefficiency in making a firm, good looking tempeh.

The optimal amount of vinegar was investigated, and it was obvious that the mold could not grow when using three or more tbsps. of vinegar. This probably depends on the low ph. of the vinegar and not the level of moist which the mold could handle, see Figure A13. Vinegar is supposed to give a favorable environment for the starter culture (Cultures for health, 2017). but the tempeh with 0 tbsp of vinegar also worked out fine.

Some of the tempeh without vinegar smelled a bit strange. A reason as to why it worked out fine in trial 13 could be because the starter culture had been contaminated by *Lactobacillus plantarum* 299v by mistake from a previous trial. This was also what happened during trial 11 with organic peas where the tempeh smelled sour.

Bag preparation
One of the things noted in the beginning of the project was the impact of weight in the bags. It displayed a clear pattern with fuller bags resulting in better tempeh. After around 500g this effect stopped and more densely packed legumes than that risked breaking the bag and creating a crumbly tempeh with bad firmness. The best weight of the tempeh was around 500
for the peeled and around 600 for the unpeeled. Of those two, the peeled was chosen as the best due to its creamier taste, and more consistent quality. It could maybe be possible to create

tempeh with the small amount of peas tried in trial 2 as well, but then air flow would have to be optimized and fewer holes used.

The amount of air holes affected the tempeh in a direct way, as can be seen in Figure A3 The amount of oxygen seemed to be crucial to the development of black spots and on areas with no mold growth. These anomalies were always situated around the air holes. On the other hand, as can be seen in Table A3, reducing the amount of oxygen in the bags made the mold completely unable to grow. The trick was to find the fine line between too much air and too little. 12 holes with 1mm in diameter was the amount and size deemed to be best because of the relatively good and consistent results obtained. Also, the 12 holes version was chosen because it made it certain that not lack of oxygen was the source of less firm tempeh.

There is a possibility that the bags used were not sterile and thus could have been a source of contamination. Different wrapping materials for the tempeh could be investigated in the future. Traditionally the tempeh is wrapped in banana leaves (Shurtleff and Aoyagi, 2007), but the tempeh could very well be made in containers of plastic, glass and metal as well (Steinkraus, 1996).

Incubation

There were differences between the 38 hours and 48 hours incubation times for all legumes. When tempeh was incubated for a shorter time, the colour was more transparent and the tempeh looked a bit foamier. For the longer incubation time, the colour of the mold was clear white and the tempeh looked solid. The firmness was better in tempeh which have fermented for longer. The taste and smell were different depending on incubation time, a longer incubation time equaled more taste and odeur. The incubation time of tempeh could be compared to the ripening process of a cheese, where some prefer the stronger and more potent taste associated with longer fermentation times.

When doing observations in the incubator before 30 hours, black spots were rarely present and a good way to eliminate those could be to combine an optimal number of holes with a shorter incubation time. The reason why the time 48 was always used was to ensure that the time aspect was not what limited the mold growth and firmness.

The optimal incubation time for tempeh can vary from time to time (Neikell, 2016). For many trials in this project somewhere in between 38 hours and 48 hours could have been perfect. It is a possibility that longer fermentation times increases the risk of something unwanted growing.

The tempeh made in the three different incubators used in the project did not differ between each other. It is reasonable to assume that if the ventilation and temperature is the same in all incubators, it would not matter which incubator used for the tempeh fermentation.

When using bowls of water inside the incubator to increase humidity, no change in outcome was noted. The purpose was to try and eliminate the mold free spots on the tempeh which at one point was presumed to have dried out. That tempeh mold grows better in humid air was a

reasonable guess considering that tempeh is produced in houses in Indonesia with a relative humidity of 75% (Java Indonesia, 2011). Measuring and logging the humidity inside the incubator would also have been interesting to investigate.

A temperature and humidity log could have been incorporated to know exactly what temperature and humidity the incubators held throughout the incubation. This could also expose any differences between incubators. There are reasons to suspect that the temperature inside the incubators may have varied when the mold start growing, as the mold develops its own heat (Neikell, 2016). There could also be variations in air humidity in the experiment hall where the incubator was situated. This was thought of after the project was done and therefore these parameters were not logged.

4.2 Safety investigation

There is much historical evidence of the safety of tempeh, even under relatively unhygienic conditions. Like other traditional fermented foods, tempeh is proven to be safe, even though potentially pathogenic organisms exists in the food (Steinkraus 1996). As with meat: smell, looks and taste are crucial parameters when valuing the safety of tempeh. Even though tradition shows the relative safety of the product, tempeh is not immune to contamination, and fermented foods are always a risk (Tanaka et al., 1985).

Microbiology of the starter culture
The microbiological tests of the starter cultures can be seen in Table 2 below. The tests showed that *Enterobacteriaceae* was present in the starter cultures 1 and 3 in the dose of $2*10^2$ cfu/g and 10^2 cfu/g. These are not high values and a low amount of *Enterobacteriaceae* is generally tolerated in food (Motarjemi and Adams, 2006). However, because tempeh is fermented, even low amounts of bacteria can multiply and cause problems. There could have been *Enterobacteriaceae* present in starter culture 2 as well and it cannot be fully excluded that the detection limit of the used method is to low.

The tests showed that there were some lactobacilli in starter culture 2 and 3. They are not a safety risk (Bernardeau et al., 2006) but can rather act as a protection against the growth of other unwanted bacteria (Dembéle et al., 1998). As shown in Trial 7 however, lactobacilli can inhibit the growth of the mold and ruin the tempeh.

On Malt there is only mold growing in the plates with a high concentrations of starter culture. When the concentration drops, the bacteria or yeast becomes increasingly dominant and covers the whole plate on concentrations lower than 10^{-4}. The cfu/g of bacteria or yeast on Malt is $4.5*10^5$ for starter culture 1, $> 3*10^9$ for starter culture 2 and $1.4*10^5$ for starter culture 3 on Malt. It is harder to tell the cfu/ for the mold because the entire plates were covered, and because mold do not display clear colonies. There should not be bacteria growing at all on Malt. The suspicion arose when looking at the dry, scab-like appearance of the colonies who looked more like bacillus, and less like fungi.

For the high concentrations of starter culture on TSA, mold and different types of bacteria or yeast grew all over the plates making them impossible to count. The cfu/g was determined to be $1.5*10^7$ for starter 1, $1.5*10^6$ for starter 2 and $2*10^6$ for starter 3.

Table 2. The result from the plating of the different starter cultures.

Type	Starter 1(cfu/g)	Starter 2(cfu/g)	Starter 3 (cfu/g)
Rogosa	0	$8*10^2$	10^2
VRBD	$2*10^2$	0	10^2
Malt	$4.5*10^5$	$>3*10^9$	$1.4*10^5$
TSA	$1.5*10^7$	$1.5*10^6$	$2*10^6$

Microbiology of the tempeh

The tests on the microbiology of the tempeh can be seen in Table 3 below.
The 299v tempeh used was the one from trial 12 with 0.004 grams of Lactobacillus plantarum 299v. It was not a good tempeh and only had limited growth of mold on it.

It was obvious that the tempeh contained more microorganisms than the starter cultures. This was not surprising since the tempeh had been fermented. There were also big differences in the amount of microorganisms among the different tempeh.

All tempeh contained much higher amounts of lactobacilli than the starter cultures did. The tempeh with the most lactobacilli was not surprisingly the 299v tempeh with a cfu/g of $3*10^8$. The second most was from the mold free spots on the pea tempeh with a cfu/g of $7*10^7$. This matches the fact that those kinds of spots were missing mold and smelled sour. The pea tempeh contained $8*10^6$ cfu/g and the brown bean tempeh contained the least with a cfu/g of $2*10^4$.

It is possible that yellow peas provide a better substrate for the lactobacilli, and therefore more mold-free spots were present on the pea tempeh compared to the brown bean tempeh. It is however also possible that the mold grows worse on the peas and therefore enables growth of bacteria. A combination of the two is also possible.

Out of the four tempeh, yellow pea tempeh contained the most growth on VRBD, followed by the brown beans. Due to an insufficient serial dilution, the exact cfu/g could not be established for the yellow pea tempeh, but the value was bigger than $>3*10^5$.

There are no established limits for *Enterobacteriaceae* in tempeh, but low amounts are always preferred in any food. The values from the tempeh could however be compared to the guideline amounts of *Enterobacteriaceae* in ready-to-eat food from the Swedish and Irish food safety authorities. SLV classifies amounts $<10^4$ as satisfactory (SLV, 2009). FSAI classifies amounts of

$<10^2$ as satisfactory, amounts of $<10^2$ to $\leq 10^4$ as borderline and amounts of $> 10^4$ as unsatisfactory (FSAI, 2016). According to these criteria, the spot tempeh and 299v tempeh would classify as satisfactory in Sweden and borderline in Ireland. The brown bean tempeh and would be categorized as unsatisfactory in both countries, but only by a slight margin. The yellow pea would be classified as unsatisfactory in both countries as well.
Does this mean that the amount of *Enterobacteriaceae* in the tempeh is hazardous? No, it does not. Ready-to-eat food are supposed to be eaten straight away, sometimes without pre-heating. Tempeh should always be cooked properly before eating. Even if the tempeh would be eaten raw, it is not certain that the amount of *Enterobacteriaceae* would pose a risk due to it being only slightly higher than the ready-to-eat foods (except in the case of the yellow peas).

Enterobacteriaceae die when heated up, so it is important that the tempeh reaches at least 60 °C when cooking (Denis et al., 2006). Some producers however claim that tempeh should at least be heated to 80 °C (Tempeh Info, 2018). This could be because there are many different types microorganisms present in the tempeh. The heat treatment is what completely disarms the eventual hazard of *Enterobacteriaceae*. Lower levels of that kind of bacteria should however always be strived for when developing food products.

When comparing the results of the Rogosa plates with those of the VRBD it seems like the lactobacilli outcompeted *Enterobacteriaceae* in the tempeh. The suspicion arose because the yellow pea and brown bean tempeh contained much more *Enterobacteriaceae* with $>3*10^5$ and $3*10^4$ cfu/g respectively. The 299v tempeh and the spot tempeh contained only $3.5*10^2$ and $2.5*10^2$ cfu/g respectively.

The lactobacilli did not seem to influence the growth on the TSA plates which contained similar amounts of bacteria for all four tempeh, if anything, the lactobacilli rich tempeh contained more cfu/g on the TSA plates.

The Malt showed no growth of mold at all in the samples from tempeh. This is strange considering the vast amount of mold present in the tempeh. This could be because the mold had lost its potency, but more probably because there was such a big growth of other microorganisms. This result differed from the investigation of the starter cultures, where the mold was growing on Malt in the high concentrations of the culture.

Table 3. The result from the plating of the different types of tempeh.

Type	299v Tempeh (cfu/g)	Pea tempeh (cfu/g)	Brown bean tempeh(cfu/g)	Mold free spot from pea tempeh (cfu/g)
Rogosa	$2.95*10^8$	$8.8*10^6$	$2*10^4$	$6.5*10^7$
VRBD	$3.5*10^2$	$>3*10^5$	$3.35*10^4$	$2.5*10^2$
TSA	$5.35*10^8$	$1.9*10^8$	$2.75*10^8$	$6.2*10^8$

Malt	-	$2*10^8$	$1.045*10^9$	$5.8*10^8$

Toxicological investigation

The results of the toxin investigation can be found in Appendix 2. The chosen toxins were the mold toxins most common in the Swedish legumes. For the moment, ochratoxin and aflatoxin in legumes do not have European limits (Johnsson and Thim, 2006). Therefore, the toxin analysis in the tempeh was compared to the naturally occurring amount in legumes to see if the amount increased during fermentation. These values were taken from a report made by the Swedish food agency (Fredlund et al., 2009).

As can be seen in Table 4 below, the levels of ochratoxin in the bean tempeh were roughly three times as high as the normal amount of toxin. The levels in the pea tempeh were also a bit higher than normal.
Aflatoxin B1 in both sorts of tempeh was a bit lower than the normal amounts found in legumes. For aflatoxin B2, the amount found in pea tempeh was double the normal amount in peas. For beans, there was no normal value present, but the amount found in the bean tempeh was < 0.5 (µg/kg).

To know if the amount of ochratoxins in the tempeh is high, the normal amount found in brown beans and yellow peas must be known, and not just “peas” and “beans” clumped together. Also, it would have been interesting to send the peas and beans in by themselves, without having been fermented, just for reference. This was not done due to the limited budget of the project. Rhizopus oligosporus is known to inhibit the growth of at aflatoxin B1 (Dinesh et al., 2009). That could be a possible explanation as to why it was only aflatoxin B1 out of the toxins that was less prevalent in the tempeh compared to the legumes.

It might be incorrect to compare the amount of toxin in the tempeh to that of the result from regular legumes. This because all the values of toxin in the tempeh appears to be below the detection limits of the lab. What can be concluded is however that these are good results and that there is no risk in consuming this tempeh regarding the examined types of mycotoxins.

Table 4. Comparison between the normal amounts of ochratoxin and aflatoxin found in legumes and the amounts of the same toxins found in tempeh on yellow peas and brown beans.

Legume	Normal amount of ochratoxin A (µg/kg)	Amount of ochratoxin A found in tempeh (µg/kg)	Normal amount of aflatoxin B1 (µg/kg)	Amount of aflatoxin B1 found in tempeh(µg/kg)	Normal amount of aflatoxin B2 (µg/kg)	Amount of aflatoxin B1 found in tempeh(µg/kg)
Peas	<0,3- 0,6	<1.0	< 0.3-0.5 µg/kg	< 0.2	< 0.3	< 0.5

Beans	< 0.3	<1.0	< 0.3 µg	< 0.2	-	< 0.5

4.3 Sensory analysis

As can be seen in Table 18, the X^2 was 11.57 for the peas and 9.14 for the brown beans. Both are bigger than the chi distribution chart X^2,1,0.05 of 3.84. This implies that there were significant differences in taste between the pea and soy tempeh and between brown bean and soy tempeh. This might to some degree depend on the looks of the different tempeh despite the IR light. It would however probably still be correct to assume, that there is a real difference in taste between tempeh made on different legumes. If the test would have been made to include the taste of the different boiled legumes as they are, the panelists would probably discriminate between them easier than they would the tempeh.

Regarding the personal preferences, out of the 21 people in this test, the same amount of people found brown bean tempeh as likeable as soy tempeh with 8 persons preferring each. With the yellow pea tempeh, 5 people found that one best. Even though more volunteers would be appreciated, the study gives a hint on the likeability of the different tempeh. Even though the pea tempeh was the least favorable, that one was still ranked the best by 5 of the 21 volunteers. Also, there could be a tendency to favor the soy tempeh as it was the most eaten tempeh in the preceding triangle test.

When asked, all 21 volunteers in the sensory analysis answered that every type of tempeh tried in the analysis tasted good. All comments from the last question of the sensory analysis can be seen in Table 19.

5 Conclusion

The best tempeh created in this project was using the following method with either brown beans or yellow peas.

Wash the legumes thoroughly with cold water. Soak the legumes for 12 hours. Place 3000 grams of legumes in 8 litre of boiling water, give the water 10 minutes to rise to the boiling point, boil the legumes for 30 minutes. Let the legumes air dry for 30 minutes and oven dry on 30 °C for 20 minutes. Make sure the legumes are dry enough to not wet a paper towel. There is no need to peel the brown beans, but if using yellow peas with hull, damage the hull by using a potato peeler for 30 seconds per 500 grams of peas. Mix 500g of legumes with at least ½ a tsp of starter culture and with 1 tbsp of white wine vinegar. Perforate zip-lock plastic bags of 0.5 L with 12 holes of 1mm in diameter. Fill the bags with the inoculated legumes. Incubate the bags at 30 °C for 38 to 48 hours. In general, the mold grows faster on the brown beans and those could be taken out earlier than the peas.

It could be concluded that the homogeneity, visual appearance and firmness was satisfactory in some tempeh made in certain batches. Due to the inconsistency however, not all tempeh in a batch, even with the best method applied accomplished that aim.

The microbial investigation showed that the microbial quality needs to be improved and further characterized to ensure a safe product. There were many different microorganisms growing inside the tempeh. In some tempeh, a lot of *Enterobacteriaceae* was present, which is an indicator of unhygienic food. When having a big growth of Lactobacilli, the amount of *Enterobacteriaceae* was reduced substantially. It could be concluded that the aim of establishing a microbiologically safe tempeh was not accomplished due to missing facts about the microorganisms.

The toxin analysis on the other hand, showed that the tempeh does not contain dangerous amounts of either ochratoxins or aflatoxins.

The sensory analysis concluded that there were notable differences in taste between the tempeh on different legumes. It also showed that brown bean tempeh was as favorable as the soybean tempeh.

It could be concluded that the aim to make the tempeh on Swedish legumes at least as favorable as the soy tempeh was accomplished in the case of brown beans, and almost accomplished regarding yellow peas.

6 Future Outlook

More work is needed to ensure continuity in the outcome of the product. Tempeh is a hard food to master and even with exact routines, there will always be a certain randomness in the outcome. As of now, the successful tempeh in each batch is nearly perfect whereas the unsuccessful is much less so. It would not be accepted by the modern consumer to have that amount of variety in quality.

Making tempeh on other Swedish grown legumes such as broad beans and green peas could be tried out in the future. To incorporate seeds, cereals or to mix different legumes are also ways of creating new types of tempeh.

The possibility to develop a Koji-Kin based Swedish legume product could be investigated, even though that product would technically not be a tempeh.

The safety of the tempeh is of great concern. There is still much work to be done to establish a controlled, known fermentation process. As of now, the complex microflora of this tempeh is relatively unknown. To create a fermentation chart with all dominating microorganisms throughout the life cycle of the tempeh would be useful, especially if it could be controlled with some key parameters. Finding suitable lactobacilli to co-ferment with the mold would not only increase the safety of the product, it would also enable the chance of calling the product probiotic.

Even though a sensory analysis test was performed, a bigger and more general survey on what consumers like about the product, and how they would use it would be informative.

If this tempeh becomes commercialized, it will be impossible to produce it manually with cheap labour as is custom in Indonesia. Therefore, a smart industrial production method will need to be developed for this product if it is to have any chance in the western markets.

There are also other aspects to consider before commercializing, will the tempeh be sold raw with related risks? Will it be heat treated? In that case, how?

Hopefully, this thesis will provide some first steps in creating a commercial product combining an old fermentation technique from Indonesia with legumes from Sweden. If the product would become popular, it would bring great benefits for the producer, the customers, the farmers, the environment and the public health. That could be a goal worth striving for.

7 Acknowledgements

I would like to express my gratitude towards my supervisor Åsa Håkansson, my examinator Marie Wahlgren and M.Sc. Pamela Canaviri for all the help and support I have received during this project.

Thank you, Richard Clerselius and Carl-Johan Frelander at Orkla foods Sweden for the idea behind the project and for the collaboration during it.

Thank you, Prof. em Göran Molin, M.Sc Elin Oscarsson and fellow students Hanna Bergström and Lucas Lissner for all the valuable inputs and ideas.

Lastly, big thanks to my girlfriend, friends and family for all the support and positive energy given to me throughout this project.

8 References

Ashenafi, M., Busse, M. 1989. Inhibitory Effect of Lactobacillus plantarum on Salmonella infantis, Enterobacter aerogenes and Escherichia coli during Tempeh Fermentation. *Journal of Food Protection,* Vol. 52, No. 3, pp 169-172. Available: http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-52.3.169?code=fopr-site [Accessed 9th of June 2018]

Asthma and Allergy Foundation of America. 2018. *What is a food allergy?* AAFA. Available: http://www.aafa.org/page/food-allergies.aspx [Accessed 7th of July 2018]

Astma och Allergiförbundet. 2018. *Matallergi.* Astma och Allergiförbundet Available: https://astmaoallergiforbundet.se/information-rad/matallergi/ [Accessed 7th of July 2018]

Bernardeau, M., Guguen, M., Vernoux, J, P. 2006. Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiology Reviews*: Volume 30, No. 4, pp. 487–513.

Blom, E. 2016 [radio]. *Bruna bönornas historia med Edward Blom*. P4 Västernorrland.19th of April 2016 10.15. Available: https://sverigesradio.se/sida/artikel.aspx?programid=110&artikel=6414246 [Accessed 9th of June 2018].

Cultures for health. 2017. *Tempeh FAQ*. Cultures for health. Available: https://www.culturesforhealth.com/learn/soy/tempeh-faqs/ [Accessed 9th of June 2018].

Dembéle, T., Obdrálek, V., Votava, M. 1998. Inhibition of bacterial pathogens by lactobacilli. *Zentralblatt für Bakteriologie:* Volume 288, No. 3, pp. 395-401.

Denis, C., Cadot, P., Leguerinel, I., Thuault, D,. Sohier, D. 2006. Heat resistance of coliform species isolated from cooked ham, snail flesh, and 'bouchées á la reine'. *Letters in Applied Microbiology:* No. 42, pp. 160-164.

Dinesh Babu, P., Bhakyaraj R., Vidhyalakshmi R. 2009. A Low Cost Nutritious Food "Tempeh"-A Review. World Journal of Dairy & Food Sciences No. 4, pp. 22-27. Available: https://pdfs.semanticscholar.org/d65b/b37af9717c98c21dd17e630d1f3751b9392d.pdf [Accessed 9th of June 2018].

Fredlund, E., Abramsson-Zetterberg, L., Thim, A, M, Olsen, M. 2009. *Mögel och mykotoxiner i livsmedel.* Livsmedelsverket R 2009:4. Uppsala: Livsmedelsverket. Available: https://www.livsmedelsverket.se/globalassets/rapporter/2009/riskprofil-mogel-och-mykotoxiner-i-livsmedel.pdf [Accessed 9th of June 2018].

FSAI. 2016. *Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market*. Food Safety Authority of Ireland Guidance Note No 3. Dublin: Food Safety Authority of Ireland. Available: [Accessed 9th of June 2018].

Go Green. 2018. *Svenskodlade Gula ärter.* Go green. Available: http://www.gogreen.se/produkter/arter/svenskoldade-gula-arter/#product-info [Accessed 3rd of July 2018].

Java Indonesia. 2011. *Climate, Weather, and Temperature on Java, Indonesia.* Java Indonesia.org. Available: http://www.javaindonesia.org/general/climate-weather-temperature-java-indonesia/ [Accessed 9th of June 2018].

Jennessen J., Nielsen, KF., Houbraken, J., Lyhne, EK., Schnürer, J., Frisvad, JC., Samson, RA. 2005. Secondary metabolite and mycotoxin production by the Rhizopus microsporus group. *Journal of agricultural and food chemistry.* No. 53(5), pp. 1833-40. Available: https://www.ncbi.nlm.nih.gov/pubmed/15740082 [Accessed 9th of June 2018].

Johnsson, P., Thim, A, M. 2006. *Mögel och mykotoxiner.* Livsmedelsverket R 2007:13. Uppsala: Livsmedelsverket. Available: https://www.livsmedelsverket.se/globalassets/rapporter/2007/2007_livsmedelsverket_13_riksprojekt2006.pdf [Accessed 9th of June 2018].

Motarjemi, Y., Adams, M. 2006. *Emerging foodborne Pathogens*. Cambridge: Woodhead Publishing

Machida, M., Yamada, O., Gomi, K. 2008. Genomics of Aspergillus oryzae: Learning from the History of Koji Mold and Exploration of Its Future*. DNA research.* Vol 15, Issue 4, pp. 173-183 Available: https://academic.oup.com/dnaresearch/article/15/4/173/376904 [Accessed 3rd of July 2018].

Murata, K., Ikehata, H., Miyamoto, T. 1967. Studies on the Nutritional Value of Tempeh. *Journal of food science,* journal paper no 32, 520. Available: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1967.tb00837.x [Accessed 9th of June 2018].

Neikell, J. 2016. *Fermentera, mat med jäst mögel och bakterier.* Stockholm: Norstedts.

Nout, M., Kiers, J. 2005. Tempe fermentation, innovation and functionality: update into the third millenium. *Journal of applied microbiology.* No 98, pp 789-805. Available: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2672.2004.02471.x [Accessed 3rd of July 2018].

Society of Sensory Professionals. 2018. *Triangle test.* Sensorysociety. Available: https://www.sensorysociety.org/knowledge/sspwiki/pages/triangle%20test.aspx

[Accessed 7th of July 2018].

Shurtleff, W., Aoyagi, A. 1979. *The book of tempeh.* Brooklyn: Ballantine Books.

Shurtleff, W., Aoyagi, A. 2007. *History of tempeh, A Special Report on The History of Traditional Fermented Soyfoods*. Lafayette: Soy info centre. Available: https://luk.staff.ugm.ac.id/itd/artikel/Shurtleff-Aoyagi-HistoryOfTempeh.pdf [Accessed 9th of June 2018].

SLV. 2009. *Ätfärdiga livsmedel.* Norrbottens län: Livsmedelsverket. Available: https://www.livsmedelsverket.se/globalassets/kommunala-projektrapporter/2010/atfardiga_livsmedel_norrbotten_2010.pdf?_t_id=1B2M2Y8AsgTpgAmY7PhCfg%3D%3D&_t_q=&_t_tags=language%3Asv%2Csiteid%3A67f9c486-281d-4765-ba72-ba3914739e3b&_t_ip=66.249.76.37&_t_hit.id=Livs_Common_Model_MediaTypes_DocumentFile/_1398ada1-0d3f-47a4-affe-32d46fdff8d3&_t_hit.pos=61 [Accessed 9th of June 2018].

Steinkraus, K, H.,1996. *Handbook of Indigenous foods.* New York: Marcel Dekker inc.

Tanaka, N., Kovats, S, K., Guggisberg, J, A., Meske, L, M., DOYLE, M, P. 1985. Evaluation of the Microbiological Safety of Tempeh Made from Unacidified Soybeans. *Journal of Food Protection*: Vol. 48, No. 5, pp. 438-441. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-48.5.438?code=fopr-site

Tempeh info. 2018. *Frequently Asked Questions*. Tempeh info. Available: https://www.tempeh.info/faq/faq.php [Accessed 9th of June 2018].

Upadhyay, S. 2016. *Meat Substitute Market By Product Type (Tofu, Tempeh, Textured vegetable protein, Quorn, Seitan), Source (Soy, Wheat, Mycoprotein), Category (Frozen, Refrigerated, Shelf-stable) - Global Opportunity Analysis and Industry Forecast, 2014 - 2020*. Allied market research. Available: https://www.alliedmarketresearch.com/meat-substitute-market [Accessed 9th of June 2018].

Wijaya, H., Gunawan-Puteri, M, D. 2015. ”Tempe Semangit”, the Overripe Tempe with Natural Umami Taste. *UMAMI INDONESIA, Vol 3 2015.* Available:http://channywijaya.staff.ipb.ac.id/2015/06/05/tempe-semangit-overripe-tempe-natural-umami-taste/ [Accessed 9th of June 2018].

Wikipedia. 2018. *Ärtsoppa.* Wikipedia. Available: https://sv.wikipedia.org/wiki/%C3%84rtsoppa#cite_note-1 [Accessed 9th of June 2018].

WWF. 2018. *Environmental & social impacts of soy.* [Online]. WWF webpage: WWF. Available: http://wwf.panda.org/our_work/food/agriculture/soy/impacts/ [Accessed 9th of June 2018].

9 Appendix 1- Process optimization trials

Trial 1:

Purpose: Comparison between soy and peas, and the effect of cooking time.
Soaking: 12 hours.
Boiling: 8L of water was brought to boil. When the legumes were added, the temperature dropped to 80 °C. After 8 minutes the legumes boiled and after this point the boiling timer was set. The different times can be seen Table A1 below.
Drying: Paper towels were used together with perforated oven sheets. The legumes were dried for 40 minutes until they no longer soaked touching paper towels.
Peeling: Hand peeling was tried, it was extensive work and only about 10% could be peeled.
Inoculation: The legumes for each bag were mixed in a stainless-steel bowl together with ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Bag preparation: 12 holes with 3mm in diameter were used for all the bags. The weight is displayed in Table A1 below.
Incubation: The soy bags were incubated for 38 hours and the peas for 48 hours.
Results:
Table A1. The raw data and result of each tempeh in trial 1.

Legume	Boiling time (minutes)	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Soy	30	257	90	No	15
Soy	40	272	90	Yes	10
Soy	50	322	90	Yes	2
Soy	60	284	30	No	6
Soy	70	344	90	No	3
Soy	80	281	20	No	2
Pea	10	311	80	No	9
Pea	20	349	30	Almost	10
Pea	30	312	90	Yes	4
Pea	40	301	10	No	1
Pea	50	246	5	No	5
Pea	60	350	20	No	1

Remarks: The tempeh was way blacker around the holes. The taste was strongest when the tempeh was black. More content in the bags resulted in better tempeh. The tempeh on both legumes tasted very similar. A creamy, crunchy and nutty taste.

Figure A1. The result of the pea tempeh.

Figure A2. Close up of the soy tempeh.

Trial 2:

Purpose: Investigate different starter cultures and the amount of peas in the bag.
Soaking:12 hours.
Boiling: 8 L of water was brought to boil. When the legumes were added, the temperature dropped to 80 °C. After 9 minutes the legumes boiled and after this point the boiling timer was set. The peas were boiled for 30 minutes.
Drying: The peas were dried for two hours in perforated oven sheets and ten minutes in a well-ventilated oven on 30 °C.
Peeling: Some of the pees were peeled by hand. All the peas went into a manual potato peeler.
Bag preparation: 12 holes with 3mm in diameter were used for all the bags. The weight is displayed in Table A2 below.
Inoculation: The legumes for each bag were mixed in a stainless-steel bowl together with ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation: 48 Hours
Result:

Table A2. The raw data and result of each tempeh in trial 2.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)

Starter 3	450	95	Yes	5
Starter 3	390	90	Yes	9
Starter 3	360	70	No	7
Starter 3	303	80	Almost	6
Starter 3	250	60	No	16
Starter 3	196	40	No	18
Starter 1	455	90	Yes	8
Starter 1	453	90	Yes	3
Starter 2	450	90	Yes	4
Starter 2	451	90	Yes	5
Starter 3	450	90	Yes	4
Starter 3	452	90	Yes	5

Figure A3. Oil fried pea tempeh.

Figure A4. Pea tempeh of different weights.

Remarks: It was an obvious difference between the results depending on how well they were packed. More peas resulted in a firmer and whiter tempeh. This might depend on the amount of oxygen present. No difference was noted between the three starter cultures. In all blocks of tempeh, black spots were present, this will try to be minimized.

Trial 3:

Purpose: Investigate the effect of oxygen on the tempeh.
Soaking: 12 hours.
Boiling: 8 L of water. 8 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Electric potato peeler for 30 seconds per bag of peas.
Bag preparation: Different number of holes, all with 3mm.
Inoculation: ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation:48 hours.
Result:

Table A3. The raw data and result of each tempeh in trial 3.

Type	Weight (gram)	Holes	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Skalad	410	0	10	No	0
Skalad	407	1	20	No	0
Skalad	409	2	50	Almost	0
Skalad	408	3	80	Almost	1
Skalad	412	4	90	Almost	1
Skalad	409	6	90	Almost	5
Skalad	407	8	90	Yes	6
Skalad	409	10	95	Yes	12
Skalad	408	12	95	Almost	8
Skalad	409	20	95	Almost	9

Figure A5. Tempeh made with different amount of air holes.

Remarks: Acceptable mold growth occurred when the number of holes exceeded four. In the bags with few holes the mold barely grew. With many holes the tempeh became blacker. Dry spots occur in the tempeh where there is a lot of oxygen. These spots will try to be eliminated in future trials. The black spots disappeared when there were few holes.
Almost means that the slices can be held but not be jiggled.

Trial 4:

Purpose: Investigate the effect of peeling and number of and sizes of holes.
Soaking: 12 hours.
Boiling: 8 L of water. 10 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Hand-peeled, machine peeled (potato peeler) and unpeeled.
Bag preparation: Different number of holes, with 1mm and 3mm diameter in sizes.
Inoculation: ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation: 48 hours
Result:
Table A4. The raw data and result of each tempeh in trial 4.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Handpeeled	451	99	Yes	0
Machinepeeled	456	95	Yes	7
Machinepeeled	457	95	Yes	7
Unpeeled	455	95	Almost	8
Unpeeled	452	95	Almost	7
13 Holes	461	90	Yes	7
32 Holes	456	90	Yes	26
4 Holes	455	95	Almost	6
12 Big holes	462	95	Yes	9
12 Small holes	458	95	Yes	0

Figure A6. Difference between 12 small holes and 12 big holes.
Figure A7. Close up of hand peeled tempeh.

Remarks: The small holes were better than the big ones because of the lesser amount of black spots. The hand-peeled had an excellent growth with little black spots. However, it tasted very much like yeast and alcohol. The machine-peeled and the unpeeled tasted similar but the unpeeled was a lot tougher. The small holes had small circular black dots around the holes, in contrast to the big holes which had bigger areas of blackness. The small-hole tempeh had a lesser amount of dried out peas. A weight over 500 will be investigated.

Trial 5

Purpose: Investigate different types of crushing.
Soaking: 12 hours.
Boiling: 8 L of water. 9 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: None.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation:48h.
Result:

Table A5. The raw data and result of each tempeh in trial 5.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Potato masher	477	90	No	3
Potato masher	501	90	No	1
Potato press	479	80	No	1
Potato press	488	90	No	2
Hacking knife	493	95	No	0
Hacking knife	483	90	No	1
Hand blender	468	95	No	0
Hand blender	501	95	No	0
Food processor	495	80	No	0
Food processor	491	90	No	1

Figure A8. The different crushing methods
Figure A9. The result from the different crushing methods.

Remarks: All were practically white after 48 hours, only tiny spots of black appeared. The hand blender tempeh tasted nice but felt more like a pea beef than a tempeh. The hacking knife were best. All fell apart.

Trial 6

Purpose: Investigate the effect of dryness, try the maximum amount of peas per bag.
Soaking: 12 hours.
Boiling: 8 L of water. 12 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Rinse the peas before inoculation, Airdried, Air + oven tried.
Peeling: Unpeeled and machine peeled.
Bag preparation: 12 holes with 1mm diameter.

Inoculation: ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation: 48 h.
Result:
Table A6. The raw data and result of each tempeh in trial 6.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Air Dried	510	95	Almost	0
Air Dried	518	95	Almost	0
Rinsed	504	100	No	0
Rinsed	598	100	No	0
Regular drying	526	80	Yes	0
Regular drying	518	90	Yes	0
Peeled max	618	95	Almost	1
Peeled max	624	95	Almost	2
Unpeeled max	614	95	Yes	3
Unpeeled max	610	90	Yes	2

Remarks: The oven+ air tempeh looks the worst on the outside but is the firmest one (except in the non-moldy areas). The rinsed tempeh looks perfect on the outside but crumbles apart when cut. The unpeeled max tempeh had better firmness than the machine peeled max tempeh as can be seen in Figure 10 and Figure 11.
Maybe it is the lack of moist that causes the dry spots. A different air humidity must be tried.

Figure A10. The unpeeled maximally filled tempeh.
Figure A11. The peeled maximally tempeh.

Trial 7

Purpose: Investigate co-fermentation with *Lactobacillus plantarum* 299v
Soaking: 12 hours
Boiling: 8 L of water. 12 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Unpeeled and machine peeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: ½ a tsp of starter culture and 1 tbsp of white wine vinegar. For the ones with 299V, a capsule of "Probi Mage" (10^9 CFU/capsule) was mixed into the legumes.
Incubation: 48 hours.
Result:

Table A7. The raw data and result of each tempeh in trial 7.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Unpeeled 299	524	0	No	0
Unpeeled 299	522	0	No	0
Peeled 299	518	0	No	0
Peeled 299	524	0	No	0
Unpeeled	519	70	Almost	0
Unpeeled	520	70	Almost	2

Peeled	524	70	Almost	12
Peeled	522	90	Almost	0
Unpeeled 299 Max	602	0	No	0
Unpeeled Max	607	80	Almost	0

Figure A12. The result of the co-fermentation with 299v.

Remarks: The mold was unable to grow together with 299v as visible in *Figure 12*. The bags with 299v smelled very sour. The regular bags were not as good as usual and was possibly affected by being in the same incubator as the bags with 299v. This might depend on chance, or on the fact that the bags were touching slightly.

Trial 8

Purpose: Try to get a mold growth of 100% with no black spots.
Soaking: 12 hours.
Peeling: Unpeeled and machine peeled.
Boiling: 8 L of water. 11 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Unpeeled and machine peeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: 1 tsp of starter culture and 1 tbsp of white wine vinegar.

Incubation: 48 hours.
Result:
Table A8. The raw data and result of each tempeh in trial 8.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Unpeeled max	615	70	No	0
Unpeeled max	611	90	Almost	2
Peeled max	618	80	No	4
Peeled max	622	80	No	1
Unpeeled	520	85	Almost	1
Unpeeled	515	80	No	0
Peeled	522	90	Yes	3
Peeled	517	90	Yes	5

Remarks: The unpeeled maximum filled tempeh and the peeled “normally” filled tempeh were by far the best ones. To use double the amount did not affect the result noticeably.

Trial 9

Purpose: Investigate the possibilities of brown beans and to find the optimal amount of vinegar.
Soaking: 12 hours.
Boiling: 8 L of water. 11 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Yellow peas peeled in the machine, brown beans unpeeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: ½ a tsp of starter culture and 0 to 3 tbsp of white wine vinegar.
Incubation: 48 hours.
Result:
Table A9. The raw data and result of each tempeh in trial 9.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
BB 0	514	100	Yes	0
BB 1	523	100	Yes	0

BB 3	508	0	No	0
YP 0	518	100	Yes	2
YP 1	520	80	Almost	0
YP 3	500	0	No	0

Remarks: YP 1 had some spots with no mold growth. That YP1 was relatively bad was surprising considering the exact same recipe had been tried before with better results.

Figure A13. The results from trial 9.

Trial 10

Purpose: Investigate the mold Koji kin, the bacteria 299v and a mixture of all.
Soaking: 12 hours.
Boiling: 8 L of water. 9 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Machine peeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: 1 tsp Koji-Kin för all bags labeled KK. 1 tsp Starter culture for all bags labeled RO and 1/10 of a capsule of 299v for all bags labeled 299.1 tbsp white wine vinegar for all.
Incubation: 47 h
Result:

Table A10. The raw data and result of each tempeh in trial 10.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held	Black spots (counts)
KK	500	95	Almost	0, green spots.
KK	513	90	Almost	0, green spots.
KK Ro	524	90	Almost	0
KK Ro	518	90	Almost	0
299 KK	509	0	No	0
299 KK	520	5	No	0
299 Ro	504	5	No	2
299 Ro	515	20	No	0
299 KK Ro	509	20	No	0
299 KK Ro	517	10	No	0

Remarks: KK tempeh smelled a bit like a mixture of sweet, meet and seaweed when fried. KK RO did not smell like regular tempeh. 299KK grew better than 299 RO, but there are visible colonies of bacteria or mold on those peas. KK RO did grow quite well

Figure A14. The results from trial 10.
Figure A15. Peas fermented with Koji-Kin

Trial 11

Purpose: Investigate the possibilities of organic peas and the effect of higher air humidity.
Soaking: 12 hours.
Boiling: 8 L of water. 12 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Unpeeled and machine peeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: ½ a tsp of starter culture and 0 to 3 tbsp of white wine vinegar.
Incubation: 48 hours, with a large bowl of water inside the incubator.
Result:
Table A11. The raw data and result of each tempeh in trial 11.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
Unpeeled max	618	85	No	0
Unpeeled max	606	90	No	0
Peeled max	618	95	Almost	4
Peeled max	622	95	Yes	3
Unpeeled	523	90	Almost	1
Unpeeled	520	90	Almost	1
Peeled	521	100	Yes	5
Peeled	524	95	Yes	1

Remarks: All tempeh smelled and tasted very sour. Otherwise no notable difference from having a humid environment or using organic peas. It was also obvious in this trial that it works best to peel the peas in the machine instead of using unpeeled peas.

Trial 12

Purpose: Investigate the optimal dose for co-fermenting with 299v.
Soaking: 12 hours.
Boiling: 8 L of water. 9 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Machine peeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: ½ a tsp of starter culture and 0.004-0.04 g of 299v and0- 1tbsp of white wine vinegar.
Incubation: 48 hours.
Result:
Table A12. The raw data and result of each tempeh in trial 12.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
0.04 299v	523	5	No	1
0.04 299v	514	20	No	3
0.02 299v	519	15	No	1

0.02 299v	519	20	No	4
0.004 299v	516	50	No	5
0.004 299v	517	50	No	9
0 vinegar	523	100	Almost	4
0 vinegar	520	95	Almost	5
1 vinegar	520	95	Almost	7
1 vinegar	518	90	Almost	5

Remarks: Even with tiny amounts of 299v the mold was hindered from growing good. The tempeh without 299v now smelled sour as well and were not firm. The suspicion that the starter culture might have been contaminated by 299v arose.

Trial 13

Purpose: Investigate the amount of vinegar and try different incubators.
Soaking: 12 hours.
Boiling: 8 L of water. 8 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Yellow peas peeled in the machine, brown beans unpeeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: ½ a tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation: 38 hours. ⅓ of the bags were put in the usual incubator ⅓ in a identical and ⅓ in an oven put on 30 °C
Result:

Table A13. The raw data and result of each tempeh in trial 13.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
BB Inc 1	512	90	Almost	1
BB Inc 2	516	85	Almost	0
BB Inc 3	510	90	No	4
YP Inc 1	511	100	Almost	3
YP Inc 2	513	95	No	5
YP Inc 3	513	80	Almost	9
BB 0 vinegar	508	100	No	0

BB 0 vinegar	515	100	No	1
BB 1 vinegar	511	40	Yes	0
BB 1 vinegar	514	90	No	2
YP 0 vinegar	505	95	No	0
YP 0 vinegar	512	70	No	0
YP 1 vinegar	515	100	Almost	1
YP 1 vinegar	509	95	No	3

Remarks: The tempeh looked very good on the outside. However, all tempeh more of less fell apart and smelled very sour. It was concluded that the starter culture had been contaminated by 299 V and that particular package was not used anymore. The firmness might have been affected by the shorter incubation time. The black spots have most probably been affected by the time.

Figure A16. Tempeh being fermented in the incubator.

Trial 14

Purpose: Combine all optimized parameters to make the best possible tempeh, compare two different incubation-times, try a mixture of peas and beans.
Soaking: 12 hours.
Boiling: 8 L of water. 8 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Yellow peas peeled in the machine, brown beans unpeeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: 1 tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation: 38 and 48 hours
Result:

Table A14. The raw data and result of each tempeh in trial 14.

Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
BB 30	510	100	Yes	0
BB 30	512	90	Almost	0
BB 48	510	85	Yes	2
BB 48	509	100	Yes	3
YP 30	511	85	No	0
YP 30	510	90	Almost	0
YP 48	514	100	Yes	4
YP 48	509	95	Yes	3
YPBB 48	513	95	Yes	0
YPBB 48	512	95	Yes	1

Remarks: The longer they were incubated, the whiter and firmer the mold became. After 30 hours it looked a bit foamy and transparent and after 48 a much more dense and thick white colour. 48 hours resulted in a stronger both taste and smell, and more black spots.

Trial 15

Purpose: Combine all optimized parameters to make the best possible tempeh. This tempeh will be used in a sensory analysis.
Soaking: 12 hours.
Boiling: 4 L of water. 8 minutes to boil after addition of legumes. Boiling for 30 minutes.
Drying: Air dried for 30 minutes and oven dried on 30 °C for 20 minutes.
Peeling: Yellow peas peeled in the machine, brown beans unpeeled.
Bag preparation: 12 holes with 1mm diameter.
Inoculation: 1 tsp of starter culture and 1 tbsp of white wine vinegar.
Incubation: 48 hours.
Result:

Table A15. The raw data and result of each tempeh in trial 15.

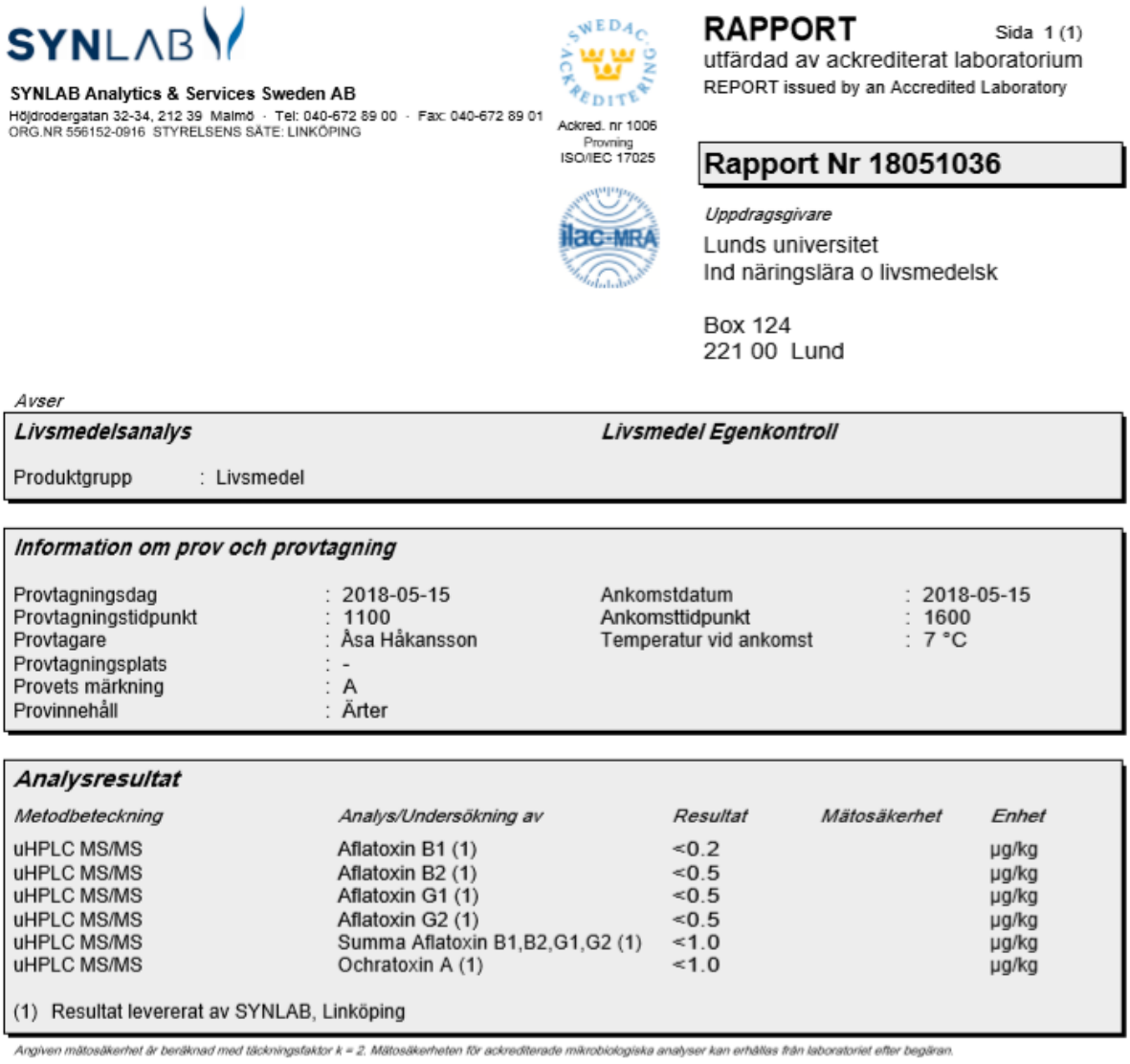
Type	Weight (gram)	Amount of mold (%)	Firmness (Holds together when held)	Black spots (counts)
BB	510	90	Yes	4

BB	510	95	Yes	2
BB	512	100	Yes	4
BB	511	95	Yes	3
YP	509	90	Yes	2
YP	512	100	Yes	1
YP	510	95	Yes	8
YP	511	90	Almost	3
SOY	513	100	Yes	6
SOY	513	95	Almost	3
SOY	511	90	Almost	4
SOY	510	95	Yes	5

Remarks: The soy did not have as good firmness as the other two, this might have depended on the bigger need for drying soybeans due to their ability to soak big amounts of water.

Figure A17. Appearance of the finished, final version of the tempeh.

10 Appendix 2- Toxicological analysis



SYNLAB

SYNLAB Analytics & Services Sweden AB
Höjdrodergatan 32-34, 212 39 Malmö · Tel: 040-672 89 00 · Fax: 040-672 89 01
ORG.NR 556152-0916 STYRELSENS SÄTE: LINKÖPING

Ackred. nr 1006
Provning
ISO/IEC 17025

RAPPORT Sida 1 (1)
utfärdad av ackrediterat laboratorium
REPORT issued by an Accredited Laboratory

Rapport Nr 18051036

Uppdragsgivare
Lunds universitet
Ind näringslära o livsmedelsk

Box 124
221 00 Lund

Avser

Livsmedelsanalys ***Livsmedel Egenkontroll***

Produktgrupp : Livsmedel

Information om prov och provtagning

Provtagningsdag	: 2018-05-15	Ankomstdatum	: 2018-05-15
Provtagningstidpunkt	: 1100	Ankomsttidpunkt	: 1600
Provtagare	: Åsa Håkansson	Temperatur vid ankomst	: 7 °C
Provtagningsplats	: -		
Provets märkning	: A		
Provinnehåll	: Ärter		

Analysresultat

Metodbeteckning	*Analys/Undersökning av*	*Resultat*	*Mätosäkerhet*	*Enhet*
uHPLC MS/MS	Aflatoxin B1 (1)	<0.2		µg/kg
uHPLC MS/MS	Aflatoxin B2 (1)	<0.5		µg/kg
uHPLC MS/MS	Aflatoxin G1 (1)	<0.5		µg/kg
uHPLC MS/MS	Aflatoxin G2 (1)	<0.5		µg/kg
uHPLC MS/MS	Summa Aflatoxin B1,B2,G1,G2 (1)	<1.0		µg/kg
uHPLC MS/MS	Ochratoxin A (1)	<1.0		µg/kg

(1) Resultat levererat av SYNLAB, Linköping

Angiven mätosäkerhet är beräknad med täckningsfaktor k = 2. Mätosäkerheten för ackrediterade mikrobiologiska analyser kan erhållas från laboratoriet efter begäran.

Figure A18. Toxin analysis of the yellow pea tempeh

SYNLAB

SYNLAB Analytics & Services Sweden AB
Höjdrodergatan 32-34, 212 39 Malmö · Tel: 040-672 89 00 · Fax: 040-672 89 01
ORG.NR 556152-0916 STYRELSENS SÄTE: LINKÖPING

SWEDAC ACKREDITERING
Ackred. nr 1006
Provning
ISO/IEC 17025

ilac-MRA

RAPPORT Sida 1 (1)
utfärdad av ackrediterat laboratorium
REPORT issued by an Accredited Laboratory

Rapport Nr 18051037

Uppdragsgivare
Lunds universitet
Ind näringslära o livsmedelsk

Box 124
221 00 Lund

Avser

Livsmedelsanalys ***Livsmedel Egenkontroll***

Produktgrupp : Livsmedel

Information om prov och provtagning

Provtagningsdag	: 2018-05-15	Ankomstdatum	: 2018-05-15
Provtagningstidpunkt	: 1100	Ankomsttidpunkt	: 1600
Provtagare	: Åsa Håkansson	Temperatur vid ankomst	: 7 °C
Provtagningsplats	: -		
Provets märkning	: B		
Provinnehåll	: Böner		

Analysresultat

Metodbeteckning	*Analys/Undersökning av*	*Resultat*	*Mätosäkerhet*	*Enhet*
uHPLC MS/MS	Aflatoxin B1 (1)	< 0.2		µg/kg
uHPLC MS/MS	Aflatoxin B2 (1)	< 0.5		µg/kg
uHPLC MS/MS	Aflatoxin G1 (1)	< 0.5		µg/kg
uHPLC MS/MS	Aflatoxin G2 (1)	< 0.5		µg/kg
uHPLC MS/MS	Summa Aflatoxin B1,B2,G1,G2 (1)	< 1.0		µg/kg
uHPLC MS/MS	Ochratoxin A (1)	< 1.0		µg/kg

(1) Resultat levererat av SYNLAB, Linköping

Angiven mätosäkerhet är beräknad med täckningsfaktor k = 2. Mätosäkerheten för ackrediterade mikrobiologiska analyser kan erhållas från laboratoriet efter begäran.

Figure A19. Toxin analysis of the brown bean tempeh

11 Appendix 3- Sensory analysis

Sensory test- tempeh

Mark with an X, the tempeh which taste the most different from the others.

Trial 1	A	B	C
Trial 2	D	E	F

Out of the three tempeh: Å Ä Ö. Which one taste the best?

_____

Could you describe the taste of the best tempeh?

_____

Could you describe the difference between the three tempeh?

_____

Thank you!

Figure A20. The questionnaire handed to the volunteers of the sensory analysis

Table A 18. The statistical analysis of the triangle test.

Yellow peas	n =	21
	Oc: number of observed correct answers =	16
	Ec: number of expected correct answers: n(1/3) =	7
	$X2 = (Oc-Ec)^2/Ec =$	11,57143
Brown beans	n =	21
	Oc: number of observed correct answers =	15
	Ec: number of expected correct answers: n(1/3) =	7
	$X2 = (Oc-Ec)^2/Ec =$	9,142857
	Degrees of freedom =	1
	Chi-square from chart at p=0,05	3,84

Table A 19. The descriptive part of the sensory analysis, BB=Brown beans, S= Soybeans, YP= Yellow peas.

Best tasting	Taste description of the best	Difference between the three
BB	Bean tempeh	Bean tempeh vs normal tempeh
BB	The crispness enhances the taste, also were “oily”	BB feels more cooked and crispier, S feels less and YP in between. Possibly oil content from S->YP->BB
BB	More nutty and more crispy	Cooked at different temperatures or length. Difference in crispyness aswell
BB	I’ts softer and a little bit nutty	The colour, the bite of the beans-> more softer. Taste not like a raw bean
BB	A bit butter grilled sandwich taste	BB= Fatty YP= more like potato
BB	Crunchy surface and creamy	S tasted more nutty, the others were more soft

	inside, soft, not so distinguishable taste	and beany
BB	More of a cooked flavour	Difference in texture(more or less crunchy) in flavour(some seemed more cooked than others
BB	Softest beanest tasting	BB most soft, YP most crunchy and S in the middle
S	All of them were neutral in taste: not fungal, thats good	Mainly the texture. I like it more firm. Maybe boil the beans shorter time? Or mix the beans
S	Round, fine taste but still with character	YP was finest in consistency but with a bit bitter aftertaste. BB was quite anonymous, S was a good mix of them both
S	Mild, nutty taste	BB= mild flavor and soft consistency= Softest. YP= Slightly more pungent taste. S= Mild nutty taste and good crunchy texture. BB and S tasted the same= Better taste YP and S had same texute= Better texture
S	Crunchy, somewhat sweet	BB= too soft, YP= soft, S=more firm
S, then BB, then YP	Nutty, creamy, roasted	Texture in YP brought down evaluation. If BB had a longer crunch then it would have been best
S	Full of taste, toasted flavour, crunchy outside, soft inside	The level of oil, the texture, the size of the beans
S	Least “bean tasting”	Most good
S	Nutty taste	BB was overcooked, YP had better texture but was very oily, S had the right cooking conditions
YP	Richer in flavour, lack of salt and very oly. Beany	BB more bitter, the others is the bean
YP	A bit nutty, good texture	BB is much dryer, YP and S I found very similar
YP	Nutty	No
YP	Crispyness, mouthfeel better	S had a nutty good flavour, almost as good as YP
YP	More taste	More taste in YP, the other quite alike