Swedish crops for the use in production of tempeh

– Which raw materials grown in the Swedish crop-system can be applied in tempeh production?

Användning av svenska grödor i produktion av tempeh

- Vilka svenskodlade råmaterial kan appliceras i tempehproduktion?

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Master's Thesis • 30 hp
Swedish University of Agricultural Sciences, SLU
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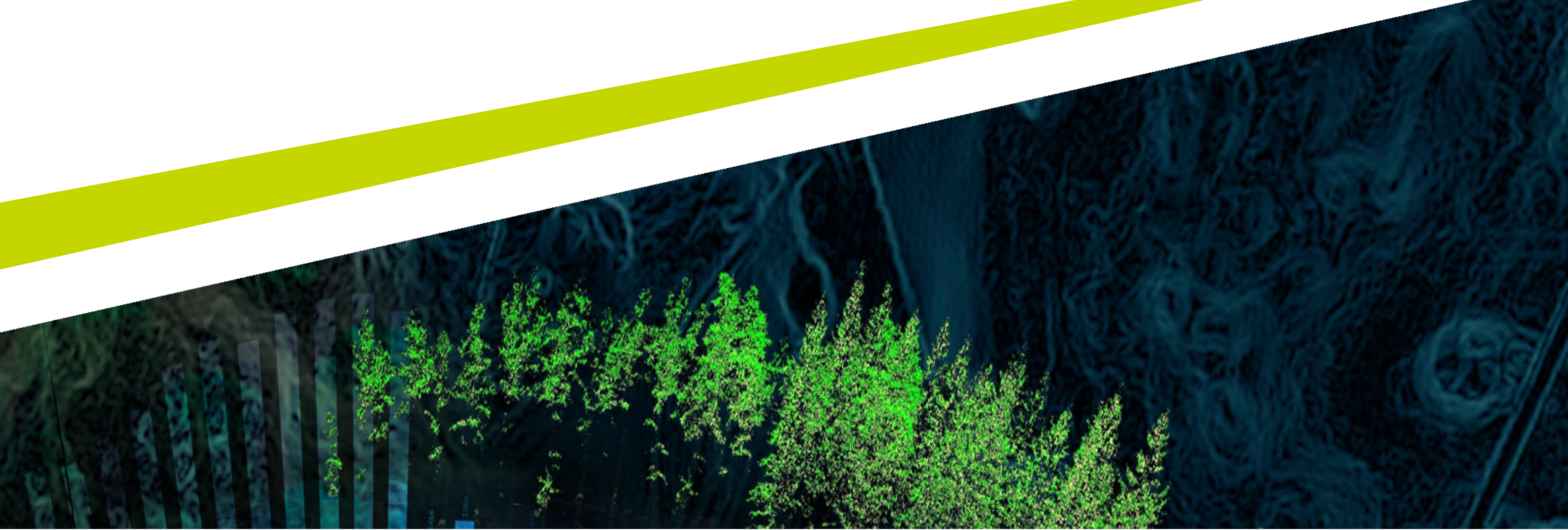
Molecular Sciences, 2020:11
Uppsala, 2020



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Användning av svenska grödor i produktion av tempeh

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Credits:	30 hp
Level:	Advanced (A2E)
Course title:	Independent project in Food Science – Master'sthesis
Course code:	EX0877
Programme/education:	Agronomist program in Food Science
Course coordinating dept:	Department of Molecular Sciences

Place of publication:	Uppsala
Year of publication:	2020
Title of series:	Molecular Sciences
Part number:	2020:11

Keywords: Antinutrients, cereals, fermentation, legumes, Sweden, tempeh

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Abstract

With a growing world population, the demands on the food industry are increasing. By reducing the intake of animal protein and replacing it with plant protein, the resource use and emission from the food industry would decrease. Tempeh is a fermented plant food, and acts as a substitute to meats. Tempeh is traditionally made by fermenting soybeans with the mould *Rhizopus oligosporus*. The objective of the fermentation in the tempeh manufacturing is primarily to alter the sensorial properties of the product and to change the nutritional content, for instance by increasing the bioavailability of nutrients. It is however possible to produce tempeh using other legumes, cereals, oilseeds etc.

The aim of the review was to present possible raw materials for tempeh manufacturing that are cultivated in Sweden. The review was limited to include legumes and cereals solely, with the focus on aspects like nutritional content, limiting circumstances with the raw material and the impact of tempeh manufacturing.

Four varieties of legumes and four varieties of cereals were included, selected due to their already established production in Sweden, some at larger and some at smaller scales. Limitations with legumes were foremost connected to the content of antinutrients and low yields. The human consumption of legumes in Sweden is limited. Cereals have low protein concentrations and there are already multiple applications of cereals in food products. The quantity of cereals produced in Sweden is much larger than that of legumes and the varieties are more familiar for the consumer.

The manufacturing steps decrease the antinutrients content and increase the bioavailability of nutrients in the raw material. The mixture of cereals and legumes in tempeh manufacturing was found to be a possible alternative to get a protein rich product were cereals can tempt the consumer, and the mixture of microorganisms as starter culture was found to have many benefits. Hopefully this review will act as a guideline for the food industry in producing plant-based substitutes to meats where the full potential of the raw material is utilized.

Keywords: Antinutrients, cereals, fermentation, legumes, Sweden, tempeh

Sammanfattning

Med en växande världsbefolkning så har kraven på livsmedelsindustrin ökat. Genom att minska intaget av animaliska proteiner och ersätta det med växtbaserat protein, så kan resursanvändningen och utsläppen från livsmedelsindustrin minska. Tempeh är ett fermenterat växtbaserat livsmedel och fungerar som ett substitut till kött. Tempeh görs traditionellt genom att fermentera sojabönor med mögelsvampen *Rhizopus oligosporus*. Syftet med fermenteringen i produktion av tempeh är främst att ändra de sensoriska egenskaperna hos produktion och att ändra näringsinnehållet, exempelvis genom att öka biotillgängligheten av näringsämnena. Det är emellertid möjligt att göra tempeh med andra baljväxter, spannmål, oljeväxter etcetera.

Syftet med granskningen var att presentera möjliga råmaterial för tempeh produktion som är odlade i Sverige. Studien begränsades till att endast inkludera baljväxter och spannmål, och focus lades på aspekter som näringsinnehåll, begränsande omständigheter hos råvara och den påverkan som tempeh produktion har på de nämnda aspekterna.

Fyra sorters baljväxter och fyra sorters spannmål inkluderades, valda till följd av en etablerad produktion i Sverige, i större eller mindre skalor. Begränsningarna hos baljväxterna var främst relaterade till innehållet av antinutrienter och låg avkastning. Konsumtionen av baljväxter är begränsad i Sverige. Spannmål har lågt proteininnehåll och det finns redan flertalet applikationer av spannmål i livsmedel. Mängden producerat spannmål är betydligt mycket högre än baljväxter och de olika sorterna är mer bekanta för konsumenten.

Tillagningsstegen sänker innehållet av antinutrienter och ökar biotillgängligheten av näringsämnen i råmaterialet. Att kombinera spannmål och baljväxter i tempeh produktion är ett möjlig alternativ för att få en proteinrik produkt där spannmål kan locka konsumenten, och att kombinera mikroorganismer i startkulturen har många fördelar. Förhoppningen är att granskningen ska fungera som ett hjälpmedel för livsmedelsindustrin för att producera växtbaserade substitut till kött där den fulla potentialen hos råmaterialet utnyttjas.

Nyckelord: Antinutrienter, baljväxter, fermentering, spannmål, Sverige, tempeh

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Abbreviations

CAP	Common Agriculture Policy, European Union
DM	Dry matter
EAAs	Essential amino acids
G6PD	Glucose-6-phosphate dehydrogenase
GAE	Gaelic acid equivalent
LAB	Lactic acid bacteria
PER	Protein efficiency ration
TCA	Trichloroacetic
TP	True protein
TPC	Total phenolic content

1. Introduction

With a growing world population, the pressure on global food production has increased in the form of increased yields and less land-use. The request for animal proteins is growing and it has turned the livestock sector into the primary user of agricultural land, a sector that has large emissions that are contributing to climate change. Climate changes and food security are two issues in the Sustainable Development Goals by the United Nations (2015).

By decreasing animal protein intake and increasing the intake of plant protein the climate impact of the livestock sector can be lowered (Pimentel & Pimentel, 2003). This would lower the emissions of global food production while continuously ensuring food security.

The European Union has been working towards becoming less dependent on imported soybeans through initiatives that promote the production of protein crops (European Union, 2017). The goal of the initiatives is to both increase the quantity, and also the varieties, of produced protein crops in Europe to meet the demand from the population within the union. With an increased interest in plant-based protein sources questions have been raised regarding the climate impact from the soybean production and transport (Lathuillière *et al.*, 2014). The initiatives from the European Union is an indication that the Union is taking action to meet the request from the public.

Tempeh, also spelled tempe, is a traditional Indonesian food product where whole soybeans are inoculated with starter culture from the *Rhizopus* spp (Eklund-Jonsson *et al.*, 2006). The fermentation initiated by *Rhizopus* spp will result in a firm cake that is bound together by the fungal mycelium. Tempeh can be, and is, made from alternatives to soybeans. The alternatives range from other legumes to grains of different sorts (Nout & Kiers, 2005). Lupins are one of the alternative legumes that can be used. Tempeh can act as a substitute for meats (Nout & Kiers, 2005), and can be cooked in multiple different ways that influence the flavour and texture of the final product.

Tempeh is highly nutritious and the fermentation process improves the digestibility of nutrients for monogastric animals, including humans (Nout & Kiers, 2005). When utilizing legumes for consumption antinutrients must be considered (Stoddard, 2017). Antinutrients can negatively affect factors including flavour and bioavailability of nutrients. There have been breeding programs within the

European Union that effectively have decreased the content of antinutrients in legumes (Stoddard, 2017). Additionally, cooking methods including soaking and boiling will lower the levels of antinutrients considerably, methods that are included in tempeh manufacturing. Fermentation is an important method when it comes to lowering the concentration of antinutrients, and can completely remove antinutrients like trypsin inhibitors and oligosaccharides (Ibrahim *et al.*, 2002). One issue is however the limited shelf-life of tempeh. That is due to the microbial activities of the *Rhizopus* spp (Nout & Kiers, 2005). Preservation methods of tempeh includes freezing and blanching.

Research have been made on alternative raw material that can be utilized for tempeh-fermentation (Lücke *et al.*, 2018; Abu-Salem *et al.*, 2014; Feng, 2006). The production of legumes is small in Sweden (Swedish Board of Agriculture, 2019) and soybeans have only been grown in smaller scale (Tarler, 2017). The production of lupin is also limited to smaller productions, and locally grown alternatives possible for tempeh-fermentation needs to be investigated.

The review aims to present alternative raw material that are cultivated in Sweden, acting as raw material in tempeh production. Aspects like nutritional content, the impact of tempeh manufacturing, limiting factors and cultivation will be included.

The research questions for the review are:

- Which crops within the Swedish farming system have the possibility to act as raw material in tempeh production?
    - What are to possibilities, and/or drawbacks, with the raw material?
- How will tempeh manufacturing affects the raw material, its nutritional content and anti-nutritional factors?

2. Background

In the section below, relevant background information for the project is presented regarding fermented food, tempeh, plant foods and Swedish agriculture.

2.1. Fermented plant foods

Fermented food products are foods where the nutritional status, digestibility, and/or the sensorial characteristics are modified biochemically by microorganisms (Khan *et al.*, 2018).

The fermentation of plant foods consist of a series of complex processes that are both dependent, and independent, of each other (Buckenhueskes, 2015). The processes are microbiological, chemical, biochemical, physical and enzymatic, and they will all play an important role for the end-result. In this review the focus will be on the microbiological processes and the commonly occurring microorganisms in tempeh production.

2.1.1. Microorganisms in food fermentation

In fermentation of plant materials there are a variety of different microorganisms that are involved (Buckenhueskes, 2015). Food fermentation can be divided depending on the microorganisms dominated. That will result in three different categories (Feng, 2006): Bacteria dominated fermentation, present in yogurt and sauerkraut, yeast dominated fermentation, present in wine and bread, and mould dominated fermentation, present in mould cheeses, tempeh and soy sauce.

In Europe and North America, the microorganisms associated with fermentation are mainly lactic acid bacteria and yeasts, while in Asian countries mould is used to a larger extent when fermenting food. Tempeh is an example of a fermented food with mould dominated fermentation that is from an Asian country.

The microorganisms in food fermentation can either be functional or non-functional (Tamang *et al.*, 2016). The functions include enhancing bioavailability of nutrients, even fortifying the food product with health-promoting bioactive compounds, improving the food safety of the food product and enhancing the sensorial properties. Many Asian food products that includes fermented soybeans have been seen to have antioxidative properties as well, tempeh being one of them (Nurrahman & Nurhidajah, 2019).

Moulds

Some of the microorganisms that take part in the fermentation of foods can degrade substances that are antinutritive, which results in substances that are consumable (Tamang *et al.*, 2016). In the case of tempeh the mould *Rhizopus oligosporus* will degrade indigestible oligosaccharides into disaccharides and monosaccharide that are digestible (Chinte-Sanchez, 2009). This will cause less discomfort for the consumer, including bloating in the digestive organs that can occur from consuming non-treated legumes.

R. oligosporus will alter the amino acid composition in the raw material used for tempeh as well (Handoyo & Morita, 2006). Enzymes produced by *R. oligosporus* will hydrolyse the available protein to get substrate that are used for the mould's metabolism. The enzyme activity will affect physical properties of the raw material also. The cell structure will be degraded, the colour will change, and the mycelia will continuously grow which affects the structure.

The benefits of *R. oligosporus* in tempeh-production is not only due to its positive effects on nutritional values. *R. oligosporus* do also have the properties to grow rapidly in high temperatures, and a high proteolytic and lipolytic activity (Cantabrana *et al.*, 2015).

R. oligosporus is responsible for producing beneficial antioxidative compounds including niacin, pyridoxine, folic acid, nicotinamide, and riboflavin in tempeh (Astuti, 2015). The antioxidative compounds produced in tempeh will have beneficial effects and can prevent oxidative stress. Oxidative stress can cause non-communicable diseases including diabetes, breast cancer, colon cancer and hyperlipidaemia.

Bacteria

Lactic acid bacteria (LAB) are associated with food fermentation and the commonly occurring bacteria includes *Lactobacillus, Enterococcus, Lactococcus* and *Pediococcus* among other (Tamang *et al.*, 2016). In the fermentation of legumes bacteria from *Bacillus* species are also occurring to large extent.

With an inoculation of LAB the risk of having pathogenic bacteria growing to harmful levels is low (Feng, 2006). Depending on which species that are inoculated, the growth of pathogenic microorganisms can be partly or even fully inhibited.

In tempeh manufacturing there are two main purposes with LAB. In the initial step, where the raw material for the production is soaked in water LAB plays an important role by acidifying the soaking water and by that reducing the potential growth of pathogenic bacteria (Feng, 2006). The second purpose of LAB is to limit the increment of pH that occurs naturally during tempeh fermentation. This lowering of the pH will inhibit the growth of pathogens. With a higher pH naturally occurring in the raw material there will be a need to have a larger inoculation of LAB, this to avoid the growth of pathogenic bacteria.

Yeasts

There are a great number of yeasts, and diversity of species, but the ones that are related to food and beverage fermentations are relatively a small group (Adams *et al.*, 2016). The most frequently used yeast species is *Saccharomyces cerevisiae*, a yeast with the nicknames baker's and brewer's yeast (Freimund *et al.*, 2003). Just as the nicknames suggests, *S. cerevisiae* is used to produce alcohol in beverages and to leaven bread.

There are positive effects associated with yeasts in food fermentation, including probiotic potential, a preventing effect on growth of moulds when in storage and a inhibitory effect on mycotoxin production (Feng, 2006).

Yeast have been found to occur in commercially produced tempeh, but there are few publications regarding the role yeasts have in tempeh production (Feng, 2006). Inoculation of yeasts in tempeh can have a positive effect on the content of ergosterol, but a high inoculation number of yeasts will inhibit the growth of funguses, and thereby preventing the mycelium development in tempeh.

2.2. Fermentations effect on nutritional quality

There are processes for improving the nutritional quality in foods. The parameters for evaluating the nutritional quality includes nutritional content, bioavailability and content of antinutrients. Fermentation is one of the processes.

2.2.1. Bioavailability

In plant foods phytochemicals occur in bound forms and therefor they are of low bioavailability (Yeo & Ewe, 2015).

Some of the microorganisms participating in the fermentation have the ability to modify plant constituents, e.g. the ability to releases compounds that are chemically bound. Through this action the bioavailability of phytochemicals will be higher in fermented food compared to non-fermented. In fermentation of soybeans the high concentration of the phytochemicals isoflavones that are non-bioavailable will be transformed to a bioactive form and the bioavailable counterpart during the fermentation (Ewe *et al.*, 2010).

The fermentation of cereals is mainly associated with bread and beer production. In beer and bread production enzymes and microorganisms that are present, naturally or added, change the bioavailability of compounds presents in the cereals, including phytochemicals. One example is sourdough fermentations. Sourdough fermentation has a positive effect on the nutritional properties of wheat, oat, and rye (Katina *et al.*, 2005). Yeast fermentation in bread production have been shown to increase the content of folate, both in wheat and rye breads (Yeo & Ewe, 2015). The antioxidant activity in cereals and legumes are increased through fermentation, reported for instance by Berghofer *et al.* (1998). Oat, fava beans and soybeans were fermented, and all showed an increased antioxidative potential (Berghofer *et al.*, 1998).

2.2.2. Nutritional content

The nutritional content and its alteration during a fermentation process is dependent on the composition of the raw material being fermented, and the microorganisms present at the fermentation (Yeo & Ewe, 2015). Studies have shown that fermentation of cereals with LAB will increase nutrients like soluble dietary fibre, digestible proteins, total phenolic compounds and folates (Hole *et al.*, 2012). Yeo and Ewe (2015) concludes that mixed fermentation, were both yeast and LAB are involved, have positive effects on nutritional values in cereals as well.

Fermentation also have positive effects on antioxidative activity, which may have positive effects on health. The effects include preventing and treating atherosclerosis and cancer. The antioxidative properties of foods based on legumes, seeds and nuts have shown to increase during fermentation (Yeo & Ewe, 2015; Berghofer *et al.*, 1998). The increased antioxidative properties are both due to an increment of phytochemicals with antioxidative properties and an increment of antioxidant activity in the present phytochemicals. Phytochemicals with antioxidative properties include isoflavones, saponins, phytosterols and tocopherols.

Fermentation can also alternate the amino acid composition and thereby affect the availability of proteins (Ewe *et al.*, 2010). Some strains of LAB have proteolytic activity, and the ability to produce amino acids and short peptide chains which are needed for the continuous growth of the bacteria strain. Other strains of LAB produce proteinases that have the ability to hydrolyse longer oligopeptides. This will increase the nutritional content of peptides and amino acids that are bioavailable.

The content of vitamin B_{12} is not commonly sufficient in vegetarian diets for humans. There are bacteria that can produce vitamin B_{12} during tempeh fermentation, and therefor make the vegetarian food tempeh a source of this vitamin (Feng, 2006). *Klebsiella pneumoniae* is foremost related to B_{12} production in tempeh and it does not interfere with the growth of *R. oligosporus* (Okada *et al.*, 1985).

2.2.3. Antinutrients

Legumes and other plant sources uses a variety of secondary compounds to protect themselves from herbivores and oxidative stress (Stoddard, 2017). These compounds are called antinutrients and compounds that are included in this group are alkaloids, isoflavonoids and saponins. Antinutrients can limit the nutrient digestibility of legumes.

Although the sensitivity among humans towards antinutrients differs greatly, it is recommended to use appropriate food processing methods on plant sources aimed for human consumption that contain antinutrients (Popova & Mihaylova, 2019). The methods include soaking, heat treatments and fermentation. What the safe levels of antinutrients for human consumption are has however not been found in the research for this review.

In animal feed the antinutrients trypsin inhibitors can cause problems. Trypsin inhibitors are present to protect the crop from insects but will negatively affect the feed conversion efficiency in animals and can cause stress in animal´s pancreas. Therefor feed containing trypsin inhibitors, as many grain legumes do, must be heat treated in order to denature the antinutrient (Stoddard, 2017). Breeding programs have been able to reduce the levels in grain legumes (Gresta *et al.*, 2017; Stoddard, 2017).

There are significant levels of antinutrients in soybeans, including trypsin inhibitors and phytic acid (Nout & Kiers, 2005). Phytic acid forms insoluble mineral-phytate complexes and lowering the content of phytic acid is important for the bioavailability of minerals (Hallberg *et al.*, 1989). When making tempeh the content and activity of antinutrients will be decreased due to the manufacturing steps of tempeh. The *Rhizopus* spp. produces enzymes that have a significant effect on lowering the phytic acid content (Feng, 2006).

In a study by Kasaoka *et al.* (1997) iron-deficient rats were feed tempeh and compared to iron-deficient rats feed with cooked soybeans. The rats feed with tempeh achieved higher levels of liver iron compared to rats fed with cooked soybeans, indicating that the fermented soybeans had more bioavailable iron.

Cereal grains have multiple antinutrients as well (Feng, 2006). These include phytic acid, tannins, and polyphenols.

The removal of undesirable compounds in food, including antinutrients, is essential for raw material aimed to be for human consumption (Abu-Salem *et al.*, 2014). It can improve the nutritional quality and flavour, which will simplify the acceptance from the consumer. The fermentation by *R. oligosporus* can reduce or even eliminate the content of antinutrients (Hachmeister & Fung, 1993).

2.3. Tempeh

Tempeh is the joint name for beans, cereals or by-products from food processing, bound together into a sliceable mass by mycelium (Nout & Kiers, 2005). The spores inoculated usually come from the *Rhizopus* ssp, most commonly *Rhizopus oligosporus. R. oligosporus* has a secondary metabolism that benefits tempeh production; the fungi produces a high number of compounds with sensorial and nutritional properties (Denter *et al.*, 1998).

There are also other microorganisms present in the fermentation, including yeast and bacteria (Cantabrana *et al.*, 2015). The microorganisms use the raw material as a substrate for their metabolism.

Tempeh is a traditional Indonesian food that has spread in Asia mainly and has become a part of the food industry. When transforming the production from smaller scales to a more modern food production there has been a need for more standardized and pure starter strains for consistent results.

The prime objective for fermenting legumes, cereals and other plant materials to tempeh is not to affect the shelf-life as a form of preservation, but rather to change the nutritional and sensorial properties (Nout & Kiers, 2005). Tempeh is highly nutritious; it is easily digestible, and the product is known to have good sensorial properties. In the western gastronomy the fermentation of food products using mould is unusual, but the implementation of moulds has many possibilities when it comes to creating foods with desirable textures and flavours.

Tempeh made from soybeans is praised for its high-quality protein content (Shurtleff & Aoyagi, 1979). During the fermentation of the seeds, grains or other plant substrates, an enzymatic conversion and degradation will take place that will result in more bioavailable substances, including proteins (Nout & Kiers, 2005).

2.3.1. Tempeh manufacturing

Tempeh evolved in Indonesia and have traditionally been made at home. With an industrialization of tempeh manufacturing the process has gone through a standardization to get an uniform end product.

In tempeh manufacturing there is a division between productions were the hull is removed during a wet process or were the hull is removed in a dry process (Nout & Kiers, 2005).

In small scale productions the removal of hull in a wet process has the benefits on not needing advanced equipment (Nout & Kiers, 2005). The dehulling process is labour intensive and in smaller productions people specialized in the technique can be utilized for this task.

In larger production, dehulling through mechanical processes in dry state will be much more economically viable due to labour costs. The drawbacks with mechanical dehulling is the loss of material other than the hull.

In the production of tempeh, the raw material is soaked after dehulling and the time for soaking differs depending on the raw material. The purpose of soaking is to increase the moisture content of the raw material, to enable the microbial activity of naturally occurring microorganisms, and to extract antinutrients from the raw material, including bitter compounds and antimicrobial substances. Before proceeding to the next step, boiling, the soaking water must be replaced with fresh water in order to get rid of the antinutrients.

The boiling time will as well be dependent on the substrate and the time needed to produce a satisfactory texture.

After boiling the raw material must be cooled while the superfluous water can be steamed-off. If too much extendable water is available there is a risk that spoilage microorganisms will start to grow.

The raw material is inoculated with starter culture after cooling. The starter culture can contain moulds from the *Rhizopus* spp and possibly other microorganisms, usually LAB. Common within larger production are pure cultures of freeze-dried spores. Also, spores from a readily made tempeh can be inoculated to provide the culture.

In order to get the proper texture and result there is a need to inoculate a sufficient amount of starter culture. According to Nout and Kiers (2005) the optimum amount is around 10^4 colony-forming units/gram of substrate. If the inoculated level of starter culture is too low it will result in a fungal growth that is irregular, the fermentation time will increase, and the risk of high levels of spoilage bacteria will rise. High levels of starter culture on the other will result in a fermentation that is unmanageable as the temperature in the tempeh will rise, resulting in a premature death of the mould and a loss of mycelia.

When the raw material has been inoculated the mixture is packed in suitable packing materials, such as banana leaves or perforated plastic, where limited amounts of air is available. By limiting the air supply the mould will not, or at a small amount, produce fungal spores. The pigmented sporangiospores would cause discoloration of the tempeh through black or grey spots. The low air supply will instead give an attractive white tempeh cake. The incubation will proceed for 1-2 days at a temperature around 25 to 30°C.

Tempeh is cooked before consumed and common cooking approaches include frying, Tempeh can also be added in stews or even dried, grinded into a flour and eventually added in food products.

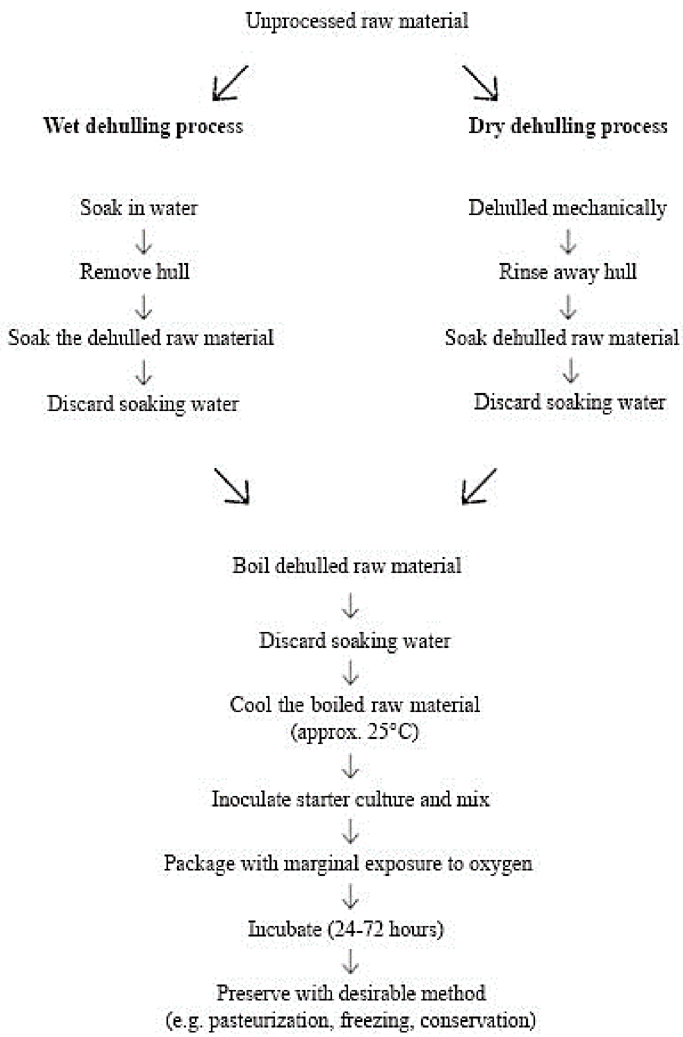

Figure 1. The tempeh manufacturing process. Illustration by Lovisa Nilsson, with inspiration from Nout and Kiers (2005)

2.3.2. Raw material in tempeh manufacturing

Although yellow-seeded soybeans are the most common raw material in tempeh manufacturing there are multiple raw materials that can be used (Nout & Kiers, 2005). The raw materials include other types of legumes, cereals, plant material, and by-products from the food industry.

Other legumes then soybeans that have been used in tempeh production include sweet lupins (Signorini *et al.*, 2018), peas of different varieties (Ashenafi, 1991), common bean (Reyes-Bastidas *et al.*, 2010), cowpea (Annor *et al.*, 2009), kidney bean and mung bean (Abu-Salem *et al.*, 2014).

Cereals in tempeh manufacturing, some solely and other in mixtures with other raw material, include barley (Feng, 2006), finger millet (Reddy *et al.*, 2008), maize (Cuevas-RodríGuez *et al.*, 2004), and rice (Cempaka *et al.*, 2018)

Other plant materials, and bi-products from the food industry used in tempeh manufacturing are apricot seeds (Tunçel *et al.*, 1990), quinoa (Pilco *et al.*, 2019), sunflower seeds (Berghofer *et al.*, 1998), okara (Ko Swan & Hesseltine, 1979), and rapeseed meal (Rozan *et al.*, 1996).

The above mentioned alternative raw materials are not the only alternatives, and many of these raw materials have not been so far scientifically investigated.

2.4. Legumes

Legumes are a part of the family Fabaceae. Legumes serve as break crops for cereals in crop rotations (Biddle, 2017a). Benefits of growing legumes are not only due to their nutritional values but also due to their ability to fixate atmospheric nitrogen to soil.

Grain legumes is a term used for legumes that are grown for its seeds, seeds that are edible. Pulses is another term used for legumes. Pulses include grain legumes but exclude those grain legumes that are primarily used for oil extraction.

Grain legumes acts as a good source of nutrients for humans. They have high content of protein, carbohydrates, vitamins and minerals, with variations depending on type, species, growing conditions and cultivar (Biddle, 2017a). Compared to animal products, legumes have a high amount of dietary fibre and unsaturated fats, positive compounds for the human health. In 2013 grain legumes, excluding soybeans, stood for 5 % of the dietary protein supply in human diet around the world, a number that is comparable to the one for fish and seafood.

Antinutrients are a concern when it comes to legumes. Antinutrients can affect the bioavailability of nutrients negatively, but there are ways of decreasing antinutrients, including soaking and boiling.

The growing conditions in Sweden is not suitable for all types of legumes, and only 1,7% of the total cropland in Sweden was used for grain legumes in 2019 (Swedish Board of Agriculture, 2019). Today there are mainly two legumes grown in Sweden, fava beans and yellow peas. Most of the legume harvest is used as animal feed.

2.5. Cereals

Cereals are grains or edible seeds from the grass family, *Gramineae* (McKevith, 2004). Cereals are cultivated for their high nutritional content and are used both for human consumption and as livestock feed. Cereals are considered to be the most important source of food (Alexandratos & Bruinsma, 2012). Cereals are a major source of protein, energy, minerals and B vitamins, and are in many cases easily stored, transported and applicate in foods.

The major cereals in the world are wheat, rice, maize, barley, oats, rye, millet, sorghum and triticale (McKevith, 2004). The cereals that are dominating the Swedish production is wheat, specifically winter wheat. More than half of the cereals harvest in Sweden 2019 came from winter wheat (Swedish Board of Agriculture, 2019). Other cereals that are cultivated in large quantities in Sweden are spring wheat, rye, winter and spring barley, oats and triticale.

Cereals must typically go through processing before applicated in products for human consumption (McKevith, 2004). Milling is commonly applied followed by changes in the cereal grains that occur. These changes include an increased enzymatic activity, an important change that have positive effects for bread making.

Fermentation is traditionally applied to cereals when produce products like bread and alcoholic products, including beer, whiskey and vodka. The microorganisms used are then yeasts and LAB. Cereals have also been applied in tempeh manufacturing published material regarding fermented barley, wheat and oats in tempeh is available (Feng, 2006).

2.6. The Swedish crop-system

Sweden is a member state in the European Union and must work within the EU Common Agricultural Policy – CAP (Agriculture and Rural Development, 2019). CAP has goals for the European agriculture, goals that the Swedish Agricultural Board, Jordbruksverket, work towards within the Swedish agriculture. The goals are the following:

- Increase agricultural productivity
- Ensure farmers a reasonable standard of living
- Stabilise markets in agricultural products, i.e. reduce the effects of variation in supply and demand
- Ensure supply of agricultural products
- Guarantee consumer access to agricultural products at a reasonable price

The area of Sweden is large, and half of the area is covered by forest (Swedish Board of Agriculture, n.d.). About 6,5 percent of the area is devoted to agricultural land, which consists of 2,7 million hectares. The climate in Sweden is favourable for cultivation, but the variations are big depending on where in the country you are situated. One example is the growing season. It is around 100 days longer in the south of Sweden, then in the north. The farms are getting fewer, but the size and production is increasing on the existing farms.

The trade of agro-foods is increasing, and cereals and cereal foods are the largest export products (Swedish Board of Agriculture, n.d.). Most of the trade is made with other European countries, and the highest percentage of trade is made with the Nordic countries, Denmark, Norway and Finland.

The crop production is mainly focused on cereals, where barley, oats and wheat are dominating. The yields are higher in the south of Sweden; and lower in the north. Oilseed production is focused on the south and central parts of the country, while sugar beets are solely grown in the south.

In Sweden the protein contribution from grain legumes was 1 percent in the average human diet in 2017 (Röös *et al.*, 2018). This can be compared to 21 percent from cereals and 28 percent from animal proteins the same year. The consumption patterns of grain legumes within the population in Sweden is divided, with 50 percent of women and 44 percent of men that are including legumes in their diet.

3. Materials and methods

The research questions guiding the review are:

- Which raw materials cultured in Sweden have the possibility to act as substrate for tempeh manufacturing.
- What type of nutritional quality can be expected from the raw material after being processed into tempeh?
- What are the possibilities and/or drawbacks with the raw material?

The review will include legumes and cereals as possible raw materials for tempeh. Oilseeds and raw materials from other sources will be excluded due to the delimitations of the thesis.

This type of extensive compilation has not been made earlier and it has the possibility to act as inspiration for novel food development with plant materials that are underutilized today. By increasing the possible applications of plant protein sources, the animal protein intake can be reduced, lowering the climate impact of food production.

Material is collected through available databases; Web of Science, Scopus, PubMed, and Google Scholar, and through publications from authorities with required data, authorities including the European Union, the United Nations and the Swedish Board of Agriculture. Many books were also used in the process, books collected from the library at the Swedish University of Agricultural Sciences or found online in the earlier mentioned databases.

Keywords for the material collection were antinutrients, antioxidants, cereals, Europe, fermentation, food, germination, grain legumes, lactic acid bacteria, legumes, nutrition, *Rhizopus oligosporus*, Sweden and tempeh.

4. Result and discussion

Potential raw materials from the Swedish crop system were identified. The identified raw materials were selected due to their established production in Sweden and if literature regarding the raw material in tempeh manufacturing is available.

They are presented below and divided into two categories dependent on origin. The categories are legumes and cereals and they are presented in relation to the Swedish crop-system.

4.1. Legumes as raw material

Four varieties of legumes have been identified as possible raw materials for tempeh; common beans, fava beans, lupins and peas. The identified legumes are cultivated in Sweden, both at a small and large scale. The raw materials are presented below with the focus on their nutritional content, the limitations connected to the crop, and how the tempeh manufacturing affects the end product. The chapter is completed with a table that compiles the result for legumes, followed by a discussion of the chapter.

4.1.1. Common bean

Phaseolus vulgaris, or common beans, is a division made to distinguish between varieties of beans (Biddle, 2017b). White, navy, pinto, black, kidney, pink, yellow, black and brown are some of the bean varieties included.

Common beans were originally domesticated independently in areas that today are a part of Mexico, Colombia and Bolivia, and were brought to Europe in the 15th century (Stoddard, 2017). From Europe it was spread to Africa and Asia. Common beans are mainly used for human consumption, and not for animal feed.

Common beans are frost sensitive and require a specific climate with a warm and dry autumn (De Ron *et al.*, 2015). Therefore the cultivation in Sweden of common beans has mainly been restricted to the islands south-east of Sweden, Gotland and Öland, and some areas in southern Sweden, where the climate is suitable (Fogelberg, 2008). Brown beans, a common bean variety, is the only variety included in the statistics from the Swedish Board of Agriculture. In 2019 678 hectares where used for the production of brown beans, less than 0,03 percent of the total farming areal (Swedish Board of Agriculture, 2019). The interest for Swedish cultivated common beans is however increasing and more varieties are produced today, including black beans, white beans, brown beans and kidney beans (Säfström, 2016). These four varieties are the possible raw materials for the manufacturing of tempeh, however, studies made on varieties of common beans will be included in this review.

Possibilities and drawbacks with common beans

The protein content in common beans is low compared to other legumes, 20 percent, (Watson & Stoddard, 2017) and the quality of the protein is not optimal. This is due to the low concentrations of cysteine and methionine, two sulphur-containing amino acids (De Ron *et al.*, 2015). Cysteine and methionine are considered to be relevant parameters when assessing nutritional content and protein quality according to the FAO (2013). There are however breeding programs that have been working with increasing the total protein content in the seeds and improving the composition of the essential amino acids present (De Ron *et al.*, 2015).

Common beans give rise to large seeds and the flowers are self-pollinating, an important development of the legumes since it gives the crop a uniform maturation and makes it suitable for mechanical harvest.

Antinutrients are in focus when it comes to common beans as well (De Ron *et al.*, 2015). Phytic acid is an example of an antinutrient that occurs in common beans.

One possibility is to use genetic modification to handle the limitations connected to common beans, including the composition of amino acids or the content of antinutrients. Genetically modified organisms are however a controversial topic in Europe and Sweden, and regulations are prohibiting the use (Fischer *et al.*, 2019).

The effect of tempeh manufacturing on common beans

Abu-Salem *et al.* (2014) studied how the levels of antinutrients in common beans are affected by tempeh fermentation. The factors investigated were phytic acid, total phenols and trypsin inhibitors.

The beans were inoculated solely with the mould *R. oligosporus.* which is not representable for the traditional tempeh fermentation but can occur in a modern industrialised tempeh production (Nout & Kiers, 2005). Traditional tempeh fermentation will include multiple different microorganisms, including multiple strains of LAB.

The result showed that all stages in the tempeh production; soaking, cooking and fermentation, had an impact on the content of trypsin inhibitor and that the content was fully reduced in tempeh made from common beans, see table 1 (Abu-Salem *et al.*, 2014). The content of phytic acid was also reduced in common bean tempeh, with 34.6 % in the end product. The total phenolic content was reduced in the process and soaking had the largest impact on reducing the levels. In the study it was shown that it is possible to produce tempeh with satisfactorily texture from common beans.

Table 1. Effects of tempeh fermentation of kidney beans on the antinutrients trypsin inhibitors, phytic acid and phenolic acid. Reconstructed from Abu-Salem et al. (2014)

Common beans	**Trypsin inhibitor (mg/g)**	**Reduction (%)**	**Phytic acid (mg/g)**	**Reduction (%)**	**Total phenolic acid (mg/g)**	**Reduction (%)**
Raw seeds	13.7		5.4		225.3	
Soaked 12 h	11.4	16.7	4.6	15.0	170.1	24.5
Soaked 12 h & dehulled	10.0	27.0	4.2	22.0	146.7	35.0
Cooking of dehulled and soaked beans	1.8	87.0	3.9	27.8	139.1	38.3
Tempeh fermented beans	0.0	100.0	3.4	34.6	128.9	42.8

In a study by Reyes-Bastidas *et al.* (2010) tempeh was also made from common beans. The tempeh was dried and milled to a flour after fermentation and the physicochemical, nutritional and antioxidant properties were studied, and the result was compared to a flour made from unfermented common beans. Table 2 displays the nutritional content of the flours from the study. The content of protein, both crude and true, increased in the study. True protein (TP) is the amount of total nitrogen minus the content of non-protein nitrogen (Reyes-Bastidas *et al.*, 2010). In the study the tempeh process increases the amount of bioavailable protein and the content of protein in common beans, see table 2 and 3.

Table 2. Proximate composition of flour made from fermented and unfermented common beans. Reconstructed from Reyes-Bastidas et al. (2010)

Composition (%)	**Unfermented**	**Fermented**
Crude protein	23.06	28.06
True protein	19.61	21.4
Lipid	1.64	1.01
Carbohydrates	70.83	68.37

The study by Reyes-Bastidas *et al.* (2010) further investigated the content of essential amino acids, EAAs. In table 3 the result of the amino acid composition is displayed, as well as the *in vitro* protein digestibility and protein efficiency ratio (PER). PER is measurement used for evaluating the protein quality in foods and is based on the weight gain in relation to the intake of protein. The composition of EAAs was limited in the unfermented bean-flour due to low content of methionine and cysteine. In the fermented bean flour the level of methionine and cysteine has reached over 2.5 g/100g of protein which is the recommended level by FAO/WHO (1991).

Table 3. Content of essential amino acids (EAAs) in flour made from fermented and unfermented common beans. Reconstructed from Reyes-Bastidas et al. (2010)

EAAs g/100g of protein	**Unfermented**	**Fermented**
Histidine	2.45	2.62
Isoleucine	3.09	3.33
Leucine	7.21	7.18
Lysine	6.52	6.31
Methionine + Cysteine	2.28	2.51
Phenylalanine + Tyrosine	8.55	9.39
Threonine	3.52	3.78
Tryptophan	1.23	1.14
Valine	3.53	3.69
Total	38.38	39.97
Limiting EAAs	Methionine + Cysteine	-
EAAs score	91	100
In vitro protein digestibility (%)	69.25	75.14
Calculated protein efficiency ratio	1.62	2.51

4.1.2. Fava beans

Fava beans, *Vicia faba*, also called faba beans, horse bean, field bean or broad beans, is a legume that is rich in protein (Duc *et al.*, 2015). Fava beans are also a high source of fibres and secondary metabolites that are beneficial for human health (Aune *et al.*, 2011).

The cultivation of fava beans has a long tradition in the northern temperate parts of the world, including Sweden (Duc *et al.*, 2015). Statistics from the Swedish Board of Agriculture keeps fava beans and peas in the same category, therefor no statistics were found regarding the amount of produced fava beans alone. Peas and fava beans does however make up for 89 percent of areal used for legumes, and 1,5 percent of the total farming areal in Sweden (Swedish Board of Agriculture, 2019). The harvest is primarily made on dry seeds, seeds that are utilized for human consumption or as feed. Fava beans can also be harvested when still fresh and sold as a vegetable.

There are goals to increase the amount of cultivated protein crops in Europe, and include grain legumes in the crop rotation (European Union, 2017). The ability of fava bean to fixate atmospheric nitrogen, grow in temperate areas and provide plant protein for human consumption makes it a highly suitable protein crop for the Nordic countries. Although breeding programs for fava beans are rare, they are mainly focused on increasing the yield. An increased and stable yield will make fava beans competitive towards other protein crops.

Possibilities and drawbacks with fava beans

The fava bean is highly adaptive and can be grown in different climates (Duc *et al.*, 2015). However, the bean is sensitive to drought, and the yields are low and unstable. This is a limitation in the competitiveness for fava bean as a crop. One example is the 2019 harvest of fava beans in Sweden was 60 percent higher than the year before (Swedish Board of Agriculture, 2019). This was due to the 2018 drought in Europe. Fava beans are however the second most produced legume in Sweden, only peas are produced in higher quantities (Swedish Board of Agriculture, 2019). Many efforts have been made to reduce the levels of antinutrients in fava beans (Stoddard, 2017).

Fava beans contain antinutrients including protease inhibitors, phytic acid, saponins and α-galactosidase (Multari *et al.*, 2015). The concentration of antinutrients can however be reduced considerably using simple methods, including soaking and cooking. The content of antinutrients can through cooking methods be lowered up to 100 percent.

Favism is a genetic condition where a severe hemolysis can occur in humans after ingestion of fava beans (Rizzello *et al.*, 2016). The compounds in fava beans causing the condition are two pyrimidine glycosides, vicine and convicine. They are the precursors of aglycones divicine and isouramil, which are the main factors causing favism. Favism occurs in humans with a glucose-6-phosphate dehydrogenase (G6PD) deficiency (Odièvre *et al.*, 2011). The deficiency of G6PD is asymptomatic at steady state, and it is therefore possible to have the affliction undiagnosed. This causes an uncertainty regarding human consumption of fava beans.

Vicine and convicine does however serve a propose in fava beans. They are a part of the plant resistance mechanism to fight attacks from fungi and insects (Multari *et al.*, 2015). With a low content of vicine and convicine the yield might be more unstable, and the crop less prone to attacks prom pests.

The effect of tempeh manufacturing on fava beans

A study by Rizzello *et al.* (2016) it was investigated how fermentation effects the levels of vicine and convicine, the precursors to compounds causing favism. In the article they point out that there are earlier studies that have been investigated the same topic, and that a reduced level could increase the amount of produced and consumed fava beans.

The fermentation was however not made with the mould *R. oligosporus*, the LAB *Lactobacillus plantarum* was the fermenting microorganism (Rizzello *et al.*, 2016). This makes the result from the study difficult to be compared to tempeh fermentation where *R. oligosporus* commonly is the main fermenting microorganism. The occurrence of LAB in tempeh manufacturing is however common, and LAB is usually fermenting the raw material during the soaking stage (Nout & Kiers, 2005).

In the study by Rizzello *et al.* (2016) three replicates of dough were made from fava bean flour. One was inoculated with *L. plantarum*, one was left as a control without any treatment, and one was chemically acidified, and antibiotics were added. The levels of vicine and convicine was measured at the initiation moment (0 hour), after 12 hours, after 24 hours and after 48 hours. The measurements showed a decrease in all doughs initially but at 48 hours the levels of vicine and convicine decreased by 90 and 95 percent respectively in the dough inoculated with *L. plantarum*.

The levels were clearly decreased in the control, most distinctly the level of vicine. The authors mean that this is due to the control having a spontaneous fermentation occurring with LAB. The degradation in the inoculated dough was however intense and faster. The study by Rizzello *et al.* (2016) showed that *L. plantarum* had a 2.7-times higher β-glucosidase activity than the spontaneously fermented dough. This was expected by Rizzello *et al.* (2016) that mean that earlier studies have shown that *L. plantarum* has a very high β-glucosidase activity.

There are other methods for decreasing the levels of vicine and convicine. The levels can be completely eliminated through continuous flow soaking at water with temperatures from 50-60°C (Multari *et al.*, 2015). Adding acetic acid enhances the decrease further and other cooking techniques, like roasting and boiling, will also lower the levels. Frying will decrease the levels by approximately 40 percent. Many of the mentioned cooking techniques can be, or are, applied in tempeh manufacturing.

In a study by Berghofer *et al.* (1998) satisfactorily tempeh in terms of sensorial aspects was produced with fava beans as substrate. Tempeh made from fava beans can be found in Swedish supermarkets today.

4.1.3. Lupins

The genus *Lupinus* consist of about 170 different species (Gresta *et al.*, 2017). There are three lupin varieties that are native to Europe and possible raw material for tempeh, white lupin (*Lupinus albus* L.), yellow lupin (*L. luteus* L.) and narrow-leafed lupin (*L. angustifolius* L.) (Lucas *et al.*, 2015). All the mentioned lupins produce seeds for consumption with a protein content of up to 44 percent and the protein is of a high nutritional quality. Narrow-leafed lupin, also known as Australian sweet lupin, is known to be a great source of essential amino acids and bioactive compounds (Khan *et al.*, 2018).

The protein ferritin is abundant in lupins, a property it shares with some other legumes. Ferritin is rich in iron, and iron deficiency is one of the most common nutritional disorder in the world (Lucas *et al.*, 2015). Lupins can therefor act as an iron source for humans consuming the legume.

Just like many legumes, lupins have the possibility to fixate atmospheric nitrogen to soil, which will lower the requirements of fertilizing (Lucas *et al.*, 2015). The European countries that are producing the largest amount of lupins are Italy, France and Spain (Gresta *et al.*, 2017). In Europe 234 297 hectares were utilized for cultivate lupins in 2018 and the harvest was 341 970 tonnes, compared to Australia in 2018 that utilized 612 014 hectares and the harvest was 714 254 tonnes (FAOSTAT, 2020). No information regarding the quantity lupins produced in Sweden was found.

Possibilities and drawbacks with lupins

The greatest obstacles in spreading lupins as a crop is the low yields, its bad tolerance to alkaline soils, the bitter and toxic alkaloids that are present, and its bad resistance to the fungal disease anthracnose (Gresta *et al.*, 2017).

In Australia lupin is successfully grown as a protein crop and lupins are applicated in various forms, one of them being protein fractions. The production of lupins in Europe is not considered to be sufficient yet for the feed and food industry since the cultivation land used for this purpose are still too small and the yields can vary greatly (Lucas *et al.*, 2015). There are however differences between the different variants. White lupin is considered to have the highest yield among the lupins that are cultivated in Europe (Święcicki *et al.*, 2015). White lupins have a low alkaline content (>0.02%) and it is resistant to the fungal disease anthracnose.

The cultivation of lupin became more widespread when it was realised that the bitter taste of lupines, due to the high alkaloid content, could be reduced by soaking the seeds in water (Gresta *et al.*, 2017). There is however an interest to breed crops of lupins with a high content of alkaloids in the green mass, but not in the seeds (Lucas *et al.*, 2015). That is a good protection against wild animals, including deer and rabbits, that are causing problems and destroying lupin fields.

There are difficulties in processing lupins due to the off-flavours that are related to the alkaloid content (Lucas *et al.*, 2015). When milling lupin flour, off-flavours will occur during storage and when used, complicating the flour application. The lupin flour will however bring properties as a higher protein content, technological possibilities and health benefits, and previous research has investigated the use of lupin flour as a substitute to cereals for increasing the protein concentration of various food products (Kohajdova *et al.*, 2011).

The effect of tempeh manufacturing on lupins

In a study by Khan *et al.* (2018) lupins where used as the raw material when making tempeh and the nutritional content was investigated. An extra treatment was added to the manufacturing: the seeds where germinated at 25°C as a pre-treatment. The change in antinutrients, and the increment of phytic acid and antioxidant potential was measured.

The result showed that the total phenolic content (TPC) in the seeds increased as the germination duration increased, see table 4. Fermentation alone also increased the TPC but not to the same extent as when the seeds had been pre-treated through germination. The increase of the TPC positively effects the bioavailability of nutrients and the antioxidant potential (Khan *et al.*, 2018).

Table 4. Total phenolic content of fermented lupins. Prepared either without germination or with germination with different length as a pre-treatment. Measured as gaelic acid equivalent (GAE) per 100 g of dry matter. Reconstructed from Khan et al. (2018)

Germination time (hr)	**Germinated lupin (mg GAE/100 g DM)**	**Fermented lupin (mg GAE/100 g DM)**
0	4.8	24.8
3	6.2	32.1
6	7.1	37.7
9	8.5	44.2
12	10.9	47.7

The levels of antinutrients were studied by Khan *et al.* (2018). The changes in the antinutrient content were dependend on the treatment the lupins were subjected to. Phytates and tannins were the measured antinutrients and the changes can be seen in table 5 and table 6.

The result indicates that solely a fermentation of lupins will lower the levels of tannins and phytate between 17 percent (tannins) and 29 percent (phytate), while adding germination as a pre-treatment for 12 hours will lead to a further reduction of tannins and phytate, yielding a total reduction of 92 and 67 percent respectively. The authors Khan *et al.* (2018) recommend that germination is utilized when applying lupins as the raw material for tempeh. This increases the antioxidant potential and bioavailability of nutrients while at the same time the levels of antinutrients are reduced.

Table 5. Mean values of condensed tannins (mg/g) in fermented lupins, prepared from either germinated or non-germinated seeds. Reconstructed from Khan et al. (2018)

Germination time (hr)	**Germinated lupin**	**Fermented lupin**
0	0.88	0.73
3	0.81	0.63
6	0.81	0.37
9	0.74	0.19
12	0.53	0.07

Table 6. Mean values of phytate content (mg/g dry matter) in fermented lupins, prepared from either germinated or non-germinated seeds. Reconstructed from Khan et al. (2018)

Germination time (hr)	**Germinated lupin**	**Fermented lupin**
0	14.2	10.1
3	10.7	6.1
6	10.3	5.4
9	9.8	5.4
12	9.3	4.7

In the study Khan *et al.* (2018) it was not consider how the tempeh is affected sensorially by the germination. This needs to be considered in the production of tempeh, even though the content of anti-nutrients is lowered the texture must still be accepted by the consumer.

In a study by Fudiyansyah *et al.* (1995) the protein quality of lupins was investigated, in both cooked lupins and tempeh made from this legume. The result was compared to soybeans, both cooked and in tempeh.

The result showed that the protein quality was considered low in lupin, compared to soybeans. The protein quality change was negatable when fermented, and the same result was seen in soybeans. Sensorial tests however showed that the acceptability of the lupin tempeh was the same as for the soybean tempeh. Fudiyansyah *et al.* (1995) claims that the low protein content will not limit the use of lupin tempeh. The authors suggest to combine lupin tempeh with food containing sulphur amino acids in order to improve the protein quality. Possible sources of sulphur amino acids are cereals grains, including rice, wheat and oats. Tempeh is often consumed in combination with cereal grains, and the tempeh could be manufactured with a mixture of cereals and legumes. Tempeh made from Swedish cultivated lupins are today available (Lupinta, 2020).

The levels of anti-nutritional factors will however be lowered in the tempeh manufacturing, resulting in an increased bioavailability of protein and minerals (Khan *et al.*, 2018).

4.1.4. Peas

Pea, *Pisum sativum*, is one of the crops that were domesticated earliest by humans (Warkentin *et al.*, 2015). Peas are today primarily cultured in temperate areas and there are multiple different varieties within the species.

The production of dried peas is most common, and the country producing the largest quantity of dried peas is Canada (Biddle, 2017a). Breeding programs working with peas are mainly focused on improving the yields and increasing the tolerance to diseases (Biddle, 2017b), and modern varieties are much more tolerant and have higher yields than their precursors. Customers today require peas with a sweet taste, without off-flavours.

For this study there are three varieties of interest. Those are split peas, both green and yellow, and field pea. They are cultivated in Sweden today.

Green peas can be harvested fresh and early in the growing season, or dry at the end of the season (Biddle, 2017b). Green peas are sold as snack food, canned or dried, and also utilized for animal feed.

Yellow peas are combined with fava beans the dominating grain legume produced in Sweden (Swedish Board of Agriculture, 2019). The majority of produced yellow peas are however used for animal feed. Yellow peas can be grown in most soil types and gives a higher yield than most other peas (Biddle, 2017b). Yellow peas are used for canning, milled into flour or prepared into meals aimed for human consumption (Biddle, 2017b).

Field peas are the oldest form of pea cultivated in Sweden (Leino *et al.*, 2013). Until the early 20th century most cultivars produced were domestic for Sweden and close to no foreign cultivars were introduced in Swedish soils. Field peas have been grown extensively in Sweden both as human food and feed.

In the statistics from the Swedish Board of Agriculture (2019) peas are divided into two categories, one of them being a combined group with fava beans and the other category being named canned peas that includes green peas. The category consisting of peas and fava beans uses 89 percent of areal used for legumes, 39 390 hectares, and 1,5 percent of the total farming areal in Sweden, as mentioned earlier in the chapter regarding fava beans. Canned peas on the other hand utilize 4 096 hectares, which is 0,16 percent of the total farming area.

Possibilities and drawbacks with peas

Earlier studies have concluded that peas are an alternative to meats with less emission of greenhouse gases, while at the same time providing a satisfactory nutritional content (Röös *et al.*, 2018; Davis *et al.*, 2010). Peas were the most produced legume in Sweden in 2019 (Swedish Board of Agriculture, 2019).

Peas are a great protein source, but the digestibility of protein is limited before being processed (Habiba, 2002). The utilization of peas for human consumption is limited due to the presence of antinutrients, including tannins, phytic acid and protease inhibitors (Habiba, 2002; Bishnoi & Khetarpaul, 1994). Ordinary cooking methods, including boiling, pressure cooking or microwave heating can lower the amount of antinutrients in peas (Habiba, 2002). There are however variations in how the cooking method affects the antinutrients.

The effect of tempeh manufacturing on peas

The information regarding peas as a substrate for tempeh manufacturing is limited. Therefor studies investigating other varieties than the ones included in this study are presented in the result. In a study by Stodolak and Starzyńska-Janiszewska (2008) the variety grass-pea was utilized as the raw material for tempeh manufacturing. The antinutrients trypsin inhibitors and phytates were decreased by 99 and 22 percent respectively, which positively affected the bioavailability in the tempeh. Also, the bioavailability of protein was increased by nearly 25 percent.

In a study by Nowak and Szebiotko (1992) yellow peas and soy beans where separately fermented using *R. oligosporus* to tempeh. The goal of the study was to investigate the biochemical changes in the raw material before, during and after the tempeh manufacturing, including nitrogen. The aim was to study how the distribution of nitrogen occurs during the manufacturing steps, as well as the bioavailability.

The dehulling of the peas lowered the content of protein nitrogen while the percentage of trichloroacetic acid (TCA) soluble nitrogen increased (Nowak & Szebiotko, 1992). In contrast to fermentation the boiling (autoclavation) of the peas did not considerably change the distribution of nitrogen. The fermentation significantly decreased the levels of protein nitrogen, the TCA, whereas soluble ammoniacal and amino nitrogen increased. After 48 hours of fermentation, the distribution changes of nitrogen were the largest.

The changes in what form the nitrogen was present are due to the proteolytic activity of *R. oligosporus* (Nowak & Szebiotko, 1992). Even though the total amount of nitrogen is stable the amount of water-soluble nitrogen is increased in the raw material, giving more bioavailable nitrogen.

Table 7. Biochemical changes in yellow peas before, during and after tempeh manufacturing. Reconstructed from Nowak and Szebiotko (1992)

	Total N	**Protein N**		**TCA soluble N**		**Ammoniacal N**		**Amino N**	
Substrate	(mg/g DM)	(mg N/g DM)	% Total N	(mg N/g DM)	% Total N	(mg N/g DM)	% Total N	(mg N/g DM)	% Total N
Dry peas	47.2	39	82.8	8.1	17	1	2	0.2	0.4
Soaked and dehulled	56.9	43.9	77.1	12.8	22.5	1.9	3.4	3.1	5.4
Soaked, dehulled and autoclaved	54.2	41.3	76.1	12.5	23.1	2.4	4.5	3.5	6.4
Fermented 24 h at 37°C	50.1	38	75.9	12.1	24.1	2	5	5	9.9
Fermented 48 h at 37°C	48.9	32	65.4	16	32.7	4.3	8.7	6.6	13.5

Peas have been used in tempeh manufacturing and given rise to tempeh with a desirable texture (Rizwan *et al.*, 2016). Tempeh made with peas as the raw material is today available in Swedish supermarkets (Kung Markatta, n.d. b).

4.1.5. General discussion regarding legumes as raw material for tempeh manufacturing

The consumption of legumes is limited by the presence of antinutrients (Shi *et al.*, 2017). There are however methods for lowering or eliminating the concentration of the antinutrients.

In table 8 limitations associated with legumes for human consumption are presented and possible actions for controlling the limitations, and what effect they have, are presented with results from earlier studies.

The concentration of antinutrients can in many legumes be decreased through a tempeh manufacturing, and thereby the limitation can be addressed. Off-flavours, low bioavailability and harmful compounds can be reduced and legume-based products with positive properties can be served to consumers.

Germination is a pre-treatment used by Khan *et al.* (2018). The pre-treatment has positive effects on the levels of tannins and phytate but the effect on the end product is however not discussed. Germination could be applied on all legumes mentioned, but a concern is raised regarding how the raw material is altered, mainly physically and how the end product, tempeh, possibly is affected. There is a need to further explore the effects of germination in a tempeh production and what possibilities and drawbacks there are before establishing germination in the manufacturing process.

Table 8. An overview of limitations associated with legumes and the effect of the manufacturing steps

Limitation	**Method**	**Course of action**	**Raw material**	**Effect**	**Reference**
Antinutrients	Soaking	Removal of bitter alkaloids	Lupins	A prolonged soaking will remove the alkaloids and improve the flavour of lupins	(Gresta *et al.*, 2017)
	Soaking, boiling and/or fermentation	Lowering the levels of α-galactosidase that causes gas production in the human intestine	Soybeans	The cooking methods soaking, boiling and fermentation will all lower the levels of α-galactosidase	(Nout & Kiers, 2005)
	Boiling	Lowering the levels of tannins, trypsin inhibitors and phytic acid	Peas	Boiling will reduce the levels of tannins, trypsin inhibitors and phytic acid with will improve the protein digestibility	(Habiba, 2002)
	Fermentation	Lowering the levels of α-galactosidase that causes gas production in the human intestine	Fava beans	LAB fermentation will lower the levels of α-galactosidase	(Verni *et al.*, 2017)
	Fermentation & germination	Lowering the levels of tannins and phytate	Lupins	A 12-hour germination as a pre-treatment to fermentation reduced the levels of tannins with 92% and phytate with 67%	(Khan *et al.*, 2018)

Antinutrients Concentration of vicine and convicine	Soaking	Reducing the levels of vicine and convicine	Fava beans	Continuous flow soaking in water, 72 h at 50°C, 60 h at 55°C, and 48 h at 60°C, completely removed the concentrations of vicine and convicine	(Jamalian & Ghorbani, 2005)
	Fermentation	Reducing the levels of vicine and convicine	Fava bean flour	After 48 hours of fermentation with the LAB strain *L. plantarum* 95% of vicine and 90% of convicine were eliminated	(Rizzello *et al.*, 2016)
	Fermentation	Reducing the levels of vicine and convicine	Fava bean flour	Through spontaneous fermentation for 48 hours the levels of vicine and convicine were reduced	(Rizzello *et al.*, 2016)
Bioavailability and quality of protein and nitrogen	Dehulling	Increasing the crude protein content	Field pea	The removal of the seed coat increased the crude protein content by 5,4-10,4%	(Wang *et al.*, 2008)
	Fermentation	Increasing the low protein quality	Lupins	No significant difference	(Fudiyansyah *et al.*, 1995)
	Fermentation	Increasing the low bioavailability of nitrogen	Peas	The proteolytic activity of *R. oligosporus* increases the amount of water-soluble nitrogen, hence the bioavailable nitrogen	(Nowak & Szebiotko, 1992)
	Fermentation	Increasing the limiting levels of methionine and cysteine, a level affecting the essential amino acid composition	Common beans	Fermentation by *R. oligosporus* increased the levels of methionine and cysteine from 2,28 to 2,51 which is above the recommended level by the FAO/WHO	(Reyes-Bastidas *et al.*, 2010)

Allergies are not a part of the table but that is however an aspect when it comes to the utilization of legumes for human consumption (Cabanillas et al., 2018). Peanut is the legume that have been investigated most closely regarding the content of allergens. An increased interest for the allergens in legumes including peas, lupins and green beans is rising according to Cabanillas et al. (2018), and in the Mediterranean countries and India the allergens in legumes have been categorized. Since the human consumption of grain legumes is limited in Sweden (Röös *et al.*, 2018) the concerns regarding allergens in grains legumes might not be as widespread as it is in the Mediterranean countries or India. There are processing methods that can change the allergenic features of legumes (Cabanillas *et al.*, 2018). The methods include thermal treatment but the outcome differs between the treatments. Boiling has been proven to be effective in lowering the allergenicity in peanuts. Since the boiling is applied in tempeh manufacturing it could lower the potential for allergic reactions to legume-based tempeh. Further research is however needed to observe what the effects of tempeh manufacturing are in the case of allergens.

Results from multiple articles show the positive effects of fermenting legumes, and the microorganisms comes from different origins. It includes LAB (Rizzello *et al.*, 2016; Coda *et al.*, 2015; Ashenafi, 1991), mould (Khan *et al.*, 2018; Annor *et al.*, 2009; Berghofer *et al.*, 1998) and yeast (Suwanto *et al.*, 2013). In a study by Starzyńska-Janiszewska *et al.* (2012) a comparison was made between grass peas fermented to tempeh with a pure culture *R. oligosporus* or fermented with two inoculated moulds, *R. oligosporus* being one of them. The study showed that the tempeh fermented with a mixed culture had a higher content of minerals and compounds with antioxidative potential.

There is a potential for using mixed cultures in the fermentation of tempeh and results from earlier studies supports the case (Starzyńska-Janiszewska *et al.*, 2012; Moreno *et al.*, 2002). The presence of LAB and yeast in the soaking stage of tempeh manufacturing is earlier clarified but Nurdini *et al.* (2015) mean that the effect of microorganisms other than moulds in the tempeh manufacturing needs to be further investigated.

When fermenting food, the risk of contamination must be considered. In the case of tempeh there have been an outbreak of gastroenteritis connected to unpasteurized tempeh (Griese *et al.*, 2013). In this particular case *Salmonella enterica* had contaminated the fermentation by *R. oligosporus*, causing the outbreak. The contamination of *Aspergillus flavus* and *Aspergillus parasiticus* in tempeh manufacturing must also be considered, since they produce aflatoxins. There have however been reports that *R. oligosporus* produces antibacterial compounds that will inhibit the growth of the mentioned moulds (Babu *et al.*, 2009).

The low intake and protein contribution from grain legumes in the Swedish diet (Röös *et al.*, 2018) can be managed by producing, promoting and establish legume-based tempeh in the Swedish market. Another approach could be to combine legumes and cereals as the raw material for tempeh. Through that action the Swedish customer would be more familiar with parts of the content, and that would hopefully make it easier to establish the products.

Tempeh can be dried and milled into flour (Reyes-Bastidas *et al.*, 2010). By adding tempeh flour to products, it is possible to add nutritional values including protein digestibility, bioavailability of minerals, vitamins, and antioxidants.

The European consumers have a positive attitude towards a higher intake of plant protein. The main trends in the European food market are natural, healthy and environment friendly food and vegetarian substitutes for animal product (Lucas *et al.*, 2015). Plant protein sources applicated in novel food products to fit into these trends attract the European consumer. There are a variety of plant-based protein sources available today that meets these requirements, including tempeh products.

Most of the legumes in the study are produced on a small scale today in Sweden. The legumes that are produced on a larger scale are peas and fava beans (Swedish Board of Agriculture, 2019). This could be an advantage for fava beans and peas. Crops that are produced on a larger scale usually have a lower kg price, making the production cost for the tempeh manufacturer lower. This circumstance speaks in favour of fava beans and peas, and today there are manufactures selling tempeh made from these legumes in Swedish supermarkets (Kung Markatta, n.d. b; Kung Markatta, n.d. a).

The concerns regarding antinutrients and low bioavailability of nutrients limits the consumption of legumes. Tempeh manufacturing decreases the antinutrients, and many studies have shown an increases bioavailability (Reyes-Bastidas *et al.*, 2010; Habiba, 2002; Nowak & Szebiotko, 1992). The low yield limits the production of legumes, and the low production of legumes today could be due to the yield combined with a low demand. The protein content in legumes is however high compared to that of cereals and ranges from 20 percent in common beans and peas to over 40 percent in lupins (Murphy-Bokern *et al.*, 2017). The low human consumption of legumes in Sweden provides a possible application for the legumes included in the review, and a space for tempeh in the food consumption in Sweden.

4.2. Cereals as raw material

Four species cereals have been identified as possible raw materials for tempeh, wheat, oat, barley and rye. All the cereals are today cultivated in Sweden and the production and harvest are large enough to be published in statistics from the Swedish Board of Agriculture (2019). Triticale is not included in the review. This is due to earlier research being limiting, and the research founds shows that triticale is not suitable as raw material from tempeh production (Hachmeister & Fung, 1993).

The raw materials are presented below with a focus on their nutritional content, the limitations connected to the crop, and how the tempeh manufacturing affects the end product. The chapter is completed with a table that compiles the result for cereals, followed by a discussion of the chapter.

4.2.1. Wheat

Wheat, *Triticum*, is sowed in both autumn and spring in Sweden, which gives two classifications of wheat: winter wheat and spring wheat (Swedish Board of Agriculture, 2019). In Sweden winter wheat is dominating the production, and in 2019 the distribution of land between the categories was 423 434 hectares used for winter wheat and 48 750 hectares used for spring wheat.

The classification of wheat is generally not based on the variety but rather on the growing period, the milling the cereal undergoes and the baking quality the flour provides when used (McKevith, 2004). There are multiple varieties of wheat that provides the same properties.

Wheat is mainly used in human consumption and there are multiple different application areas, from flour to bulgur, couscous and wheat starch (McKevith, 2004). The properties of wheat are unique, and it makes in applicable in many different foods as a functional ingredient. The gluten storage proteins and the high content of it in wheat provides viscoelastic properties to doughs.

The starch content ranges between 60-70 percent of the whole kernel and 65-75 percent of white wheat flour (Shewry, 2009). The protein content is low in wheat and it commonly ranges between 8-15 percent.

Possibilities and drawbacks with wheat

High yields, a large genetic diversity and the unique properties of wheat doughs have been the key success factors for wheat. The gluten protein fraction is responsible for the viscoelastic properties but is also an allergen.

Celiac disease, nonceliac gluten sensitivity and wheat allergy are all different forms of diseases causing reaction to wheat and gluten. Celiac disease has a prevalence of nearly 1 percent in Western nations (Green *et al.*, 2015), with the percentage varying between the countries. Other cereals containing gluten proteins are barley and rye. The content of gluten can limit the spread of wheat tempeh.

Another limitation is the already large utilization and application of wheat in food products, including bread, pasta and pastries. Wheat is not in the need of another application area, there are already a variety of food products where it is utilized.

The effect of tempeh manufacturing on wheat

Earlier studies where wheat has been applied as the raw material for tempeh production have found that wheat tempeh had a desirable aroma and flavour (Hachmeister & Fung, 1993). The texture was however unacceptable, and a tempeh patty produced by wheat crumbled when sliced.

In a study by Shekib (1994) the crude protein, true protein and non-protein nitrogen was measured in naturally fermented wheat. The wheat was milled, and no starter culture was added, the naturally occurring microorganisms accounted for the fermentation. The result from the study is therefore not comparable to wheat fermented with *R. oligosporus* but the study can however act as an interesting guideline in how fermentation affects protein and nitrogen in wheat. Since other microorganisms than *R. oligosporus* often are present when fermenting tempeh, the result could therefore be applicable on wheat used for tempeh manufacturing.

The crude protein content was not affected significantly, in raw wheat the crude protein content was 12.54% and in fermented wheat the crude protein content was 13.92% (Shekib, 1994). The *in vitro* protein digestibility did however increase, in raw wheat it was 82.60% and in fermented wheat the digestibility had increased to 87.12%. The authors concluded that the natural fermentation of cereals can provide a higher digestibility of protein.

4.2.2. Oat

The most important cultivate of oat is *Avena sativa* L, also called common oat (Butt *et al.*, 2008). Oat is an annual crop and is used both for human consumption and for animal feed. The harvest of oat in 2019 was 659 700 tonnes and made up for nearly 11 percent of the total production of cereals in Sweden (Swedish Board of Agriculture, 2019).

The applications of oats are however not as extensive as the applications of wheat (Butt *et al.*, 2008). The lack of gluten makes oat unsuitable for application in bread. There are other applications of oat, like in porridge, flakes or production of breakfast cereals. Oat flour can also be combined with wheat flour and added in baked goods.

Possibilities and drawbacks with oat

Oats contain high amounts of desirable nutrients, like fibres, minerals, unsaturated fatty acids, vitamins and phytochemicals (Butt *et al.*, 2008). The protein content is commonly around 13 percent in oat grains, the lipid content around 7,5 percent.

Oat has been recognised as a healthy cereal mainly due to its high content of the dietary fibre β-glucan. β-glucan has impressive nutritive properties and helps prevent heart diseases (Butt *et al.*, 2008). The functional properties of β-glucan are also impressive, it has the possibility to form a gel at low concentration.

The content of dietary fibre in oat is however associated with some drawbacks. Antinutrients are associated with dietary fibres and in the case of oat phytic acid and oxalic acid must be considered (Butt *et al.*, 2008). Both phytic acid and oxalic acid will affect the bioavailability of minerals and proteins negatively.

The effect of tempeh manufacturing on oat

The fermentation of oat with *R. oligosporus* leads to an increment of soluble nitrogen (Nowak, 1992). The increment is due to the proteolytic activity of *R. oligosporus* and the enzymes from the mould that take part in the fermentation.

There have been difficulties in production of an oat tempeh with a satisfactory texture (Berghofer *et al.*, 1998). Modifications of the manufacturing process does however seem to positively affect the texture and give the desired product. In a study by Eklund-Jonsson *et al.* (2006) it was reported that the mycelial growth showed good result and that the tempeh product was excellent.

Like in the case of lupins, germination have been applied to oat in an attempt to improve the nutritive content (Singh *et al.*, 2015). The concentration of soluble proteins increase when germinated and in a study by Tian *et al.* (2010) the protein concentration increased from 18,98 percent to 22,02 percent when being germinated.

Fermentation of oat with *R. oligosporus* decreased the levels of the phytic acid in a study by Eklund-Jonsson *et al.* (2006). The authors do however mean that the reduction is not enough, and that a further reduction would be important for improving the bioavailability of minerals in oats. A further reduction would improve the absorption of iron and zinc for the consumer. The treatments like dehulling, pearling and steaming can further reduce the levels of phytate (Eklund-Jonsson *et al.*, 2006).

4.2.3. Barley

Barley, *Hordeum vulgare,* is the second most produced crop in the Swedish farming system (Swedish Board of Agriculture, 2019). Just like wheat, barley is sowed both in autumn and in spring. That give two classifications; winter barley and spring barley. Spring barley is the dominating crop of the two and data from Swedish Board of Agriculture (2019) shows that in 2019 the distribution of land was 269 740 hectares for spring barley and 20 550 hectares for winter barley.

Most of the barley produced in Sweden is used as animal feed (Tidåker *et al.*, 2016). The barley used for human consumption is mainly used in the beer brewing industry and the direct human consumption is very limited. In Western countries' barley is commonly applicated as pearled, flaked, whole or milled barley, and used for making breakfast cereals, porridge, bakery flour blends, soups, stews and baby foods (Baik & Ullrich, 2008). The application of barley in food products is however not comparable to that of wheat, and few efforts have been made to develop food products containing barley. The use of barley for human consumption has decreased during the 19^{th} and 20^{th} century, and according to Newman and Newman (2006) this is due to an increased intake of wheat and rice.

Possibilities and drawbacks with barley

The nutritional content of barley differs depending on variety, but ranges between 49-66 percent starch, 9-22 percent crude protein and 14-28 percent dietary fibre (Oscarsson *et al.*, 1996).

The consumption of barley has a positive effect on human health through a low metabolic response, a high induced feeling of satiety compared to wheat, and the possibility to reduce the risk of cardiovascular diseases (Feng, 2006). The health effects come from the concentration of β-glucan, phytochemicals, and other health benefiting compounds.

Barley has a high concentration of phytate, limiting the human consumption (Eklund-Jonsson *et al.*, 2006).

The procedure for fermenting barley to tempeh is not identical to that of a traditional soybean tempeh-fermentation. Like the other cereals in this review, the concentration of starch is much higher in barley than it is in soybeans (Feng, 2006). Therefor the strain of mould, *R. oligosporus* commonly used, must be chosen carefully in order to get a satisfactory fermentation.

The effect of tempeh manufacturing on barley

The high phytate content in barley can be lowered through two processes: fermentation and germination (Feng, 2006). Malting will maintain the high content of β-glucan while reducing the levels of phytate with 50-80 percent. In a study by Eklund-Jonsson *et al.* (2006) it was shown that fermentation with the mould *R. oligosporus* could lower the concentration of phytate with 97 percent when fermenting whole grain barley.

Phytases can also be reduced in barley through fermentation induced by LAB in sourdough (De Angelis *et al.*, 2003). LAB induced fermentation can reduce the levels of multiple other antinutrients that are present in cereals (Leroy & De Vuyst, 2004), and the co-inoculation of LAB in barley tempeh-fermentation has positive effect on the phytate concentration (Feng *et al.*, 2007).

Tempeh manufacturing with barley as the raw material gives a firm cake with a desirable texture (Feng *et al.*, 2007).

4.2.4. Rye

Rye, *Secale cereal*, is a tough crop that has the possibility to grow in cool temperate areas where it is difficult to cultivate other cereals (McKevith, 2004). It is a winter crop, meaning that it is sown in the autumn and harvested in the summer.

Rye is a major crop in the Scandinavian countries, Russia, Germany and Poland (McKevith, 2004). The harvest of rye was in 2019 229 800 tonnes in Sweden, which equals 3,75 percent of the total cereal harvest (Swedish Board of Agriculture, 2019).

When applicated in the food industry rye is used for bread production, in the production of crispbread and for fermenting alcohol (McKevith, 2004). Other application areas are animal feed.

Possibilities and drawbacks with rye

The dietary fibre content is higher in rye than wheat, and the nutrient content in rye has positive effects for human health (McKevith, 2004). This includes lowering the risks for diabetes, improving the gut health and decreasing the glycaemic response in consumers. Folate is a term used that include multiple forms of folic acid (Kariluoto *et al.*, 2006). Folates are co-factors in multiple enzymatic reactions and provides positive health benefits for the consumer. In Finland rye is the most important source of folate.

Rye contains the antinutrients trypsin inhibitors that can affect the digestibility of proteins negatively (McKevith, 2004). Trypsin inhibitors are however commonly deactivated through heat treatments (Bender, 2006), and in tempeh manufacturing boiling of the raw material is included (Nout & Kiers, 2005).

There are other antinutrients occurring in rye, but according to McKevith (2004) they are not of disturbance for human consumption since they are removed or destroyed during the processing of rye.

Rye contains the storage protein gluten, that causes allergic responses in humans with the disease's celiac disease and nonceliac gluten sensitivity (McKevith, 2004). This causes a limitation in the consumption, restricting the possible consumer of the product.

The effect of tempeh manufacturing on rye

When fermenting rye, the levels of folates, lignans, free phenolic acids, alkylresorcinols and total phenolic compounds increases (Katina *et al.*, 2007). Cereals are major sources of folate and the intake of folates are especially important for pregnant women, or women in child bearing age (Kariluoto *et al.*, 2006). Folates are co-factors in enzymatic reactions that take place in the human body, and the intake of folates lowers the risk of cardiovascular diseases (Kariluoto *et al.*, 2006).

The folate increase in rye during fermentation is mainly dependent to the growth of yeasts (Kariluoto *et al.*, 2004). It is therefore important that the starter culture for tempeh fermentation does not solely consists of isolated *R. oligosporus* but include multiple microorganisms. LAB has the possibility to synthesis folates, while at the same time compete with yeast for nutrients in the production of these (Kariluoto *et al.*, 2006). LAB also produces organic acids that inhibit the yeasts producing folate, making LAB both a positive and negative microorganism in terms of folate concentration. In a study by Kariluoto *et al.* (2006) four LAB strains commonly occurring in sourdough were investigated and it showed that all the strains depleted the levels of folate.

The fermentation of rye bran with LAB has proven to be an effective treatment that improves the bioavailability of minerals by degrading antinutrients present in rye (Katina *et al.*, 2007). The fermentation with LAB will further increase the antioxidative potential in rye (Liukkonen *et al.*, 2003).

4.2.5. General discussion regarding cereals as raw material for tempeh manufacturing

The consumption of cereals is not limited by the content of antinutrients to the same extent as legumes are. Factors that are limiting for cereals, and the effect of tempeh manufacturing on the limitations, are presented in table 9 below.

Table 9. An overview of limitations associated with cereals and the effect of the manufacturing steps

Limitation	**Method**	**Course of action**	**Raw material**	**Effect**	**Reference**
Antinutrients	Soaking	Lower the concentration of antinutrients	Cereals	Soaking will decrease the levels of phytic acid in cereals	(Singh *et al.*, 2015)
	Boiling	Lower the levels of trypsin inhibitors	Rye	Trypsin inhibitors are deactivated by the heat treatment, which improves the bioavailability of protein in rye	(Bender, 2006)
	Fermentation with LAB	Reduce the levels of multiple antinutrients present in cereals	Cereals	Lowers the level of multiple antinutrients present in cereals	(Leroy & De Vuyst, 2004)
	Pre-treatments, including pearling, autoclaving and soaking with lactic acid combined with fermentation	Reduce the high levels of phytate present	Barley	Phytate concentration lowered by 97%	(Eklund-Jonsson *et al.*, 2006)
Minerals and antioxidative potential	Fermentation	Increase the content of vitamins and compounds with antioxidative potential	Rye	Fermentation with yeast present will increase the vitamins and compounds with antioxidative potential in rye	(Katina *et al.*, 2007)
	Fermentation	Increase the content of folates, and antioxidative potential in the raw material	Rye	Yeast and LAB present in the fermentation of rye increased the content of folates more than 100% and the antioxidative potential	(Liukkonen *et al.*, 2003)
Bioavailability and quality of protein and nitrogen	Fermentation	Increase the in vitro protein digestibility	Wheat	The in vitro protein digestibility was increased in wheat through fermentation of naturally occurring microorganisms	(Shekib, 1994)
	Fermentation	Increase the concentration of soluble nitrogen	Oat	The activity of *R. oligosporus* increased the concentration of soluble nitrogen in oat with over 165%	(Nowak, 1992)
	Fermentation and germination	Increase the concentration of soluble protein	Oat	Increment in the concentration of soluble protein when oat was germinated and fermented	(Tian *et al.*, 2010)

Like in the case of legumes, there are many benefits associated with the inoculation of mixed microorganisms in tempeh fermentation of cereals (Feng, 2006). With a mixed inoculation there is a possibility get more positive alterations in the fermentation. Since cereals are commonly utilized for bread production there are many studies made on the benefits of yeast and bacterial fermentation. One example is the fermentation of rye. When fermenting rye with yeast and LAB the mineral vitamin contents and antioxidative potential increases (Katina *et al.*, 2007; Liukkonen *et al.*, 2003).

No studies have been found where rye is fermented into tempeh. It is therefore difficult to conclude if rye is a suitable raw material for tempeh, and how this raw material will be affected in a tempeh manufacturing. The effects of using rye as the raw material when making tempeh should however be similar to other cereals in this study. It should foremost be similar to wheat and barley that have comparable nutritional composition. The manufacturing will most probably need to be altered to a traditional process. A modification of the manufacturing has been applied when making tempeh with barley (Feng, 2006), oat (Berghofer *et al.*, 1998), and when making wheat tempeh the texture has been a problem (Hachmeister & Fung, 1993).

Since wheat already is an established raw material and the most produced cereal in Sweden (Swedish Board of Agriculture, 2019) the application of wheat in tempeh could be appealing to consumers. The texture of wheat-tempeh was unacceptable according to Hachmeister and Fung (1993) and therefore a mixture of wheat with legumes could be a solution. It has been recommended to use a mixture of cereals and legumes since it gives a more appealing product (Lyimo, 2000; Rodríguez-Bürger *et al.*, 1998; Nowak, 1992).

This could be the case for all cereals. Combining cereals with legumes in tempeh would appeal to the consumer that is unfamiliar with legumes, while cereals are much more commonly occurring in the everyday life.

Barley is however not utilized for human consumption at a large extent (Feng, 2006). If the goal is to attract consumers who are unfamiliar with legumes, barley might not be the best alternative. There is however a possibility to increase the utilization of barley for human consumption by using barley as the raw material for tempeh, either solely or combined with other sources.

The germination of oat as a pre-treatment to tempeh manufacturing have been studied by Tian *et al.* (2010). The concentration of soluble protein increased by germination, as well as by fermentation. Germination could be an alternative for increasing the concentration of protein in all the studied cereals. There is however a concern raised regarding the effect of germination on the properties of the end product. This concern includes legumes that are the subject of germination as a pre-treatment as well, and further studies with the aim to investigate this concern are needed.

The intolerance and allergies connected to gluten is a limitation for all the studied cereals, with an exception for oat. If applying wheat, barley and rye in tempeh the possible consumers will be lowered. However, the limitation will not affect many consumers, as there is only around 1 percent of the population in the western countries that have celiac disease (Green *et al.*, 2015).

The protein concentration in the studied cereals is lower than in that of the studied legumes (Singh *et al.*, 2015; Sparvoli *et al.*, 2015). Since the purpose of tempeh in most cases is to work as an alternative protein source to meat, the obvious choice is to ferment legumes prior to cereals. There are however many factors speaking in favour of cereals as, the high yields, the already established production and fewer limitations caused by antinutrients compared to legumes . There are benefits and disadvantages with the raw materials from both classifications, and it is therefore not possible to point out one species solely being best possible raw material grown in Sweden for tempeh production.

5. Conclusion

The aim of the project was to review available literature on raw materials grown in Sweden to evaluate their suitability as raw material for tempeh manufacturing. The selection of raw materials was limited to legumes and cereals.

The result showed that legumes had a high content of antinutrients, a small production in Sweden and in many cases low yields, while cereals have a lower protein content and most of the cereals are already established in food products. Through tempeh manufacturing the content of some antinutrients would decrease, while other would be eliminated. Additionally, the content of available protein increases though fermentation, both in legumes and cereals.

From the literature survey the conclusion has been made that a mixture of microorganisms, including mould, LAB and yeast, provides many beneficial properties, compared to a tempeh manufactured with an isolated strain of *R. oligosporus*. The mixture of raw material for tempeh manufacturine, e.g. both cereals and legumes, provides high protein content, reduced production cost and appeals to consumers unfamiliar with legumes.

Hopefully the review will provide relevant information to the food industry regarding possible applications for Swedish cultured raw materials, and their potential to be utilized in tempeh manufacture by increasing the bioavailability of nutrients and decreasing limiting antinutrients.

5.1. Recommendations for future research

Further research within the subject could be focused on alternative raw materials that are excluded from this review, like oilseeds, that could be manufactured into tempeh. The climate impact of using Swedish crops, and what the possibilities are for producing tempeh in Sweden are also important aspects for future research that were excluded in the study.

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Acknowledgements

I want to give a special thank my supervisor Daniel Johansson for all the great ideas and for helping me to form my master's thesis. Also, my co-supervisor Su-Lin Hedén for helping me with starting this project and finding alternative ways when problems occurred.

I want to thank Lotta and Ellie for our breakfasts and for the company during the writing process.

Finally, I want to thank my family and friends supporting me along the way.

Appendix 1

Popular scientific summary

The world population is growing, leading to higher demands on global food production. There is a need to produce more food using less resources. Consumers around the world are demanding more animal protein, a source that includes meats, dairy products and eggs. The production of animal proteins has large resource uses and emission impact, leading to an acceleration of climate changes.

By replacing parts of our intake of animal proteins with alternative plant protein sources, like legumes and cereals, the negative effects from global food production can be lowered while the population would be provided with protein-rich food products. When replacing the intake of animal proteins, innovative ideas and products containing plant protein can help aid the cause, one example being tempeh.

Tempeh is a traditional Indonesian food product, where soybeans are fermented with mould. The growth of the mould will result in a cake of soybeans and mould mycelium that can be sliced, cooked and consumed as an alternative to meats. Soybeans is however not the sole alternative for tempeh manufacturing, other legumes, cereals, oilseeds etc. can be used and many of them are grown in Sweden.

In this master's project alternatives to soybeans will be presented for manufacture of tempeh. All the alternatives are grown in Sweden today, however in varying quantities. The alternatives that are included in the project are either legumes or cereals, other sources are excluded due to the delimitations of the project. The aim is to present what the possibilities and drawbacks with these raw materials are, and what effect tempeh manufacturing will have on the raw material, focusing on the nutritional content.

The legumes included in the project are common beans, fava beans, lupins and peas. Legumes have in common that the protein content is high, while the content of antinutrients limits for the human consumption.

Antinutrients are compounds that causes limitations to the raw material where they are found, including low digestibility of nutrients, bad taste, or even harmful effects to the consumer.

The tempeh manufacturing steps lower the levels of antinutrients and can alter the content of nutrients. The alteration of nutrients can change the protein and vitamin content to become more beneficial for human consumption. The human consumption of legumes is limited in Sweden, and it can be difficult to appeal to Swedish consumers with a legumes-based tempeh.

The cereals included in the project are wheat, oat, barley and rye. All the cereals have an established production in Sweden, and the amount produced is much higher than that of legumes. If using cereals for tempeh manufacturing the material costs could be decreased, a positive trait for the producer. Cereals do however have a low protein content, and in tempeh they will not provide the same desirable protein content as legumes do. The amount of consumed cereals is high in Sweden and consumers are familiar with most of the varieties, which could make cereal tempeh more appealing than legume tempeh.

An alternative is combining cereals and legumes in tempeh, an alternative that provides both a lower production cost compared to legume tempeh and a high protein content compared to cereal tempeh.

The goal with this review is to provide the food industry with relevant information that could help to increase the intake of plant protein in the human diet and thereby lower the climate impact of the food industry.