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Abstract

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A gradual shift towards a healthier and more sustainable diet with a higher quantity of plant-based products is suggested to be one of the most efficient ways to alleviate environmental pressure from the current food system. Pulses could play a crucial role in this shift due to the multi-faceted benefits they have on the environment and human health. This thesis aims to study the suitability of Swedish grown pulses as the raw material for new pulse-based foods and ingredients.

Flour from locally grown pulses (yellow peas, grey peas, faba beans, and white beans) prepared using different treatments (boiling, roasting, and germination) were analysed for their functional properties, nutrient content, and volatile compound composition. Protein isolates from locally grown pulses were prepared at a pilot scale using an alkaline extraction method, followed by isoelectric precipitation and were then analysed for proximate composition, thermal properties, and water holding capacity. The suitability of pulse flour in the development of cheese analogues and pulse protein isolates to produce high-moisture meat analogues (HMMA) was examined.

The results showed that the functional properties and nutrient content of flour from pulses were significantly affected by treatments prior to milling. Different treatments also affected the volatile compound profile of pulse flour to varying degrees. Plant-based cheese analogues with a firm and sliceable texture were successfully prepared using flour from boiled and roasted yellow peas and faba beans. The pulse-based cheese analogues could be categorised as functional foods owing to the high content of dietary fibre (7-8 g/100 g). Moreover, meat analogues can be produced using protein isolates from yellow peas and faba beans using high-moisture extrusion cooking. The target moisture content and extrusion temperature needed to be adjusted depending on the botanical origin of the protein isolate. Pulse-based cheese and meat analogues made from locally sourced materials could be utilised to increase the consumption of pulses in Sweden, which is currently very low.

Key words: legumes, pulse flour, functional properties, dietary fibre, folate, volatile compounds, cheese analogue, protein isolate, high-moisture extrusion, meat analogue.

Svensk sammanfattning

En gradvis övergång till en mer hälsosam och hållbar kost med en högre andel växtbaserade produkter föreslås vara ett av de mest effektiva sätten att minska klimatpåverkan från konsumtion av livsmedel. Baljväxter kan spela en viktig roll i detta skifte på grund av deras fördelar för både miljön och hälsan. Den här avhandlingen har målsättningen att studera lämpligheten hos baljväxter odlade i Sverige som råvara för nya växtbaserade livsmedel och ingredienser.

Mjöl från lokalt odlade baljväxter (gula ärtor, gråärtor, bondbönor, och vita bönor) behandlade med olika beredning metoder (kokning, rostning och groning) analyserades med avseende på funktionella egenskaper, näringsinnehåll och flyktiga ämnen. Proteinisolat från lokalt odlade baljväxter togs fram i pilotskala med en alkalisk extraktionsmetod följt av isoelektrisk utfällning och analyserades för sammansättning av makronutrienter, termiska egenskaper och vattenhållningskapacitet. Vidare undersöktes lämpligheten av baljväxtmjöl i utvecklingen av växtbaserade ostar och lämpligheten av proteinisolat från baljväxter för att producera köttsubstitut.

Resultaten visade att baljväxtmjölets funktionella egenskaper och näringsinnehåll påverkades avsevärt av kokning, rostning och groning. Dessa behandlingar påverkade också profilen av flyktiga ämnen. Växtbaserade ostanaloger med fast och skivbar konsistens framställdes med mjöl från kokade och rostade gula ärtor och bondbönor. De växtbaserade ostarna kan kategoriseras som funktionella livsmedel på grund av det höga innehållet av kostfibrer. Dessutom kunde köttsubstitut produceras med proteinisolat från gula ärtor och bondbönor genom våt-extrudering. Vattenhalten och extruderingstemperaturen behövde justeras beroende på råvaran i ingående proteinisolat. Växtbaserade ost- och köttsubstitut tillverkade av lokala råvaror skulle kunna användas för att öka baljväxterskonsumtionen i Sverige, som för närvarande är mycket låg.

NYCKELORD: Svenska baljväxter, mjöl, funktionella egenskaper, kostfiber, folat, flyktiga ämnen, växtbaserade ost, proteinisolat, våt-extrudering, köttsubstitut.

List of publications

Paper I – Flours from Swedish pulses: Effects of treatment on functional properties and nutrient content.

Ferawati Ferawati, Mohammed Hefni, and Cornelia Witthöft (2019),

Food Science and Nutrition, 7(12), pp. 4116-4126, https://doi.org/10.1002/fsn3.1280

Paper II – Characterization of volatile compounds in Swedish yellow and gray peas: Implications for new legume-based ingredients.

Ferawati Ferawati, Cornelia Witthöft, and Maria Bergström (2020),

Legume Science, 2(4), e55, https://doi.org/10.1002/leg3.55

Paper III – High moisture meat analogues produced from yellow pea and faba bean protein isolates/concentrate: Effect of raw material composition on texture properties.

Ferawati Ferawati, Izalin Zahari, Malin Barman, Mohammed Hefni, Cecilia Ahlström, Cornelia Witthöft, and Karolina Östbring (2021),

Foods, 10(4), 843, https://doi.org/10.3390/foods10040843

Paper IV – The application of pulse flour in the development of plant-based cheese analogues: proximate composition, colour, and texture properties.

Ferawati Ferawati, Mohammed Hefni, Karolina Östbring, and Cornelia Witthöft.

Manuscript

Author's contributions

Paper I – Ferawati, F was involved in the study design, performed the experiments and analyses, evaluated the results, and was responsible for writing the manuscript.

Paper II – Ferawati, F was involved in the study design, performed the experiments and analyses, evaluated the results, and was responsible for writing the manuscript.

Paper III – Ferawati, F designed the study, performed the extrusion experiments together with one of the co-authors (IZ), performed the analyses, evaluated the results, apart from the multivariate statistical analyses (MB), and was responsible for writing the manuscript.

Paper IV – Ferawati, F designed the study, performed the experiments and analyses, evaluated the results, and was responsible for writing the manuscript.

Other publications not included in the thesis

Röös, E., Carlsson, G., **Ferawati, F**., Hefni, M., Stephan, A., Tidåker, P., & Witthöft, C. (2018). Less meat, more legumes: Prospects and challenges in the transition toward sustainable diets in Sweden. *Renewable Agriculture and Food Systems*, (2010). https://doi.org/10.1017/S1742170518000443

Zahari, I., **Ferawati, F**., Helstad, A., Ahlström, C., Östbring, K., Rayner, M., & Purhagen, J. K. (2020). Development of high-moisture meat analogues with hemp and soy protein using extrusion cooking. *Foods*, *9*(6), 1–13. https://doi.org/10.3390/foods9060772

Contents

Introduction

The typical Western diet consists of a high proportion of animal-based products. The high consumption of resource-intensive foods, i.e., animal-based products, is putting a massive burden on both the food system and human health [1]. For example, the food system is responsible for 26% of global greenhouse-gas (GHG) emissions, with one-third of this coming from livestock and fish farms [2,3]. From a nutritional point of view, a diet with a high proportion of animal-based products is high in saturated fatty acid content but has a low content of dietary fibre, leading to an increased risk of coronary heart disease, diabetes, obesity, and some types of cancer [4]. Therefore, a transition towards a healthier and more sustainable diet containing more plant-based foods is suggested as one of the most efficient approaches for reducing the environmental pressure of the current food system [5,6]. Pulses are considered to be one of the most promising plant-based raw materials in this transition, owing to their nutrient content (high in protein, dietary fibre, vitamins and minerals) and positive impact on the environment (e.g., nitrogen fixation and low GHG emission).

The average daily meat and dairy consumption in Sweden is well above the global average [6]. In contrast, the average consumption of pulses is low in Sweden (12 g/person/day), although this differs between individuals [6,7]. Röös et al. [6] explored a transition scenario for Sweden in which half of the consumed red meat was replaced by cooked locally grown pulses. They found that the dietary changes created by incorporating more pulses and less meat into the diet would not negatively affect the nutrient intake on a population level [6]. However, there would be an improvement in the average total dietary fibre and folates intake after the proposed scenario [6]. The current average daily intake of total dietary fibre and folates in Sweden is below the recommended levels [8]. The environmental impact expressed as climate impact and land use linked to food consumption per capita would also be reduced by 20% [6].

Pulses are maturely harvested dried legume seeds (e.g., dry beans, dry peas, and lentils), excluding soybeans and green beans/peas [9]. The main pulse crops in

Sweden are faba beans and yellow peas. Though these crops are mainly used as animal feed [6], there is an increasing interest in using pulses as food. One limitation on increasing the use of pulses is the presence of the so-called anti-nutritional compounds which could decrease nutrient absorption. However, the adverse effects of anti-nutrients in pulses are currently being re-assessed [10]. Pre-treatments, such as boiling, are found to reduce the content of these compounds in pulses [11]. Additionally, pulses are reported to have good techno-functionalities that make them suitable for many food applications [12,13].

Another reason for the low consumption of pulses in Sweden might be due to the lack of attractive pulse-based products on the market. Also, only a few scientific studies have investigated the potential of ingredients derived from locally grown pulses in Sweden [14–16]. Therefore, this thesis attempts to gather scientific data to support the development of locally sourced, attractive, and versatile pulse-based ingredients and products of high nutritional value, which would be suitable for increasing the amount of pulses consumed in the diet and would promote sustainable farming and food production.

Aims

The purpose of this research was to study the suitability of locally cultivated pulses as the raw material for the development of new pulse-based foods and ingredients, which could potentially provide options for consumers in Sweden to increase their pulse consumption.

For this purpose, the aims of the research were:
To characterise flour from locally grown pulses prepared using different processing treatments in terms of functional properties (Paper I), nutrient content (Paper I), and volatile compound composition (Paper II).

To examine the suitability of ingredients prepared from locally grown pulses to produce high-moisture meat analogues (Paper III) and pulse-based cheese analogues (Paper IV).

Background

Pulses

Pulses are dry-harvested leguminous crops (*Leguminosae*) that include common beans, broad beans, peas, chickpeas, cowpeas, pigeon peas, lentils, and lupins [9]. Pulses do not include legumes containing a high fat content, such as soybeans and peanuts, or fresh beans and peas [9]. In recent years, pulses have been gaining global interest as an alternative source of plant protein due to their high protein content, low allergenicity, non-GMO cultivation practice [13], and their suitability for cultivation in a cold climate, such as in Europe.

The global production of pulses is still very low compared with cereals or soybeans (Figure 1). The world's pulse production reached 88 million tons in 2019 and was dominated by dried common beans, followed by chickpeas and dried peas [17]. Pulse production in Europe was around 9.5 million tons in 2019, requiring only 3% of the total available European cultivation area [17]. Dried peas contributed 55% of the total pulse production in Europe [17]. In Sweden, pulse production was as low as 0.13 million tons in 2019, also requiring 3% of the total available cultivation area, and was dominated by two crops, dried peas (46%) and dried faba beans (53%) [17]. Pulses are still an underutilized crop, thus, there is ample room to expand the cultivation, especially as in recent years there has been a high demand from consumers for sustainable and locally sourced food products.

Common beans (*Phaseolus vulgaris*), as the main produced pulse species, includes a wide range of varieties such as kidney beans, navy beans, pinto beans, and black beans. Myanmar, India, and Brazil are the top producers of dry common beans [17]. Dried peas (*Pisum sativum*) are the third most produced pulse crop globally. The primary producers of dried peas in 2019 were Canada, the Russian Federation, and China [17]. Dried faba beans (*Vicia faba*) are produced globally in much lower quantities than the common beans or dried peas. However, faba beans are gaining interest due to their high protein content, around 28% (db) [18]. Altogether, these pulses show potential as high-value plant-based ingredients.

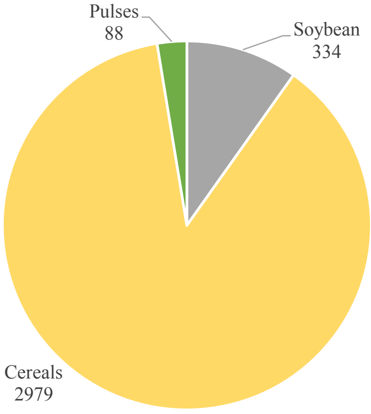

Figure 1. Global production of pulses, soybeans, and cereals in 2019 (million tons) [17].

Chemical composition

Pulses are good sources of protein, complex carbohydrates, vitamins, and minerals (Table 1). The protein content in pulses is twice as high than in whole grain cereals (barley, wheat, and oats) [9]. The amino acid profiles of pulses and cereals are complementary [19]. Pulses have a high lysine content but are low in methionine and cysteine (Table 2), which are the limiting amino acids. In contrast, cereals are high in methionine and cysteine but low in lysine [19]. Pulse proteins mainly consist of globulins (55-79%), followed by albumins (12-30%), prolamins, and glutelins (Table 3).

Table 1. The macro- and micronutrient composition of selected pulses (g/100 g edible portion on db)[1].

Nutrient	Peas	Faba beans	Common beans
Protein (g)	27	28	24
Fat (g)	3	2	2
Carbohydrate (g)	47	43	48
Total dietary fibre (g)	20	23	21
Iron (mg)	6	6	9
Folate (µg)	83	280	460

[1]Data was adapted from [18]

Table 2. The essential amino acid composition of selected pulses (g/100 g edible portion on db)[1].

Amino acid	Peas	Faba beans	Common beans
Histidine	0.7	0.7	0.7
Isoleucine	1.1	1.1	0.9
Leucine	2.0	2.0	1.7
Lysine	1.9	1.8	1.5
Methionine	0.3	0.2	0.2
Phenylalanine	1.3	1.2	1.2
Threonine	1.0	1.0	1.0
Tryptophan	2.4	2.4	2.8
Valine	1.3	1.3	1.1

[1]Data was adapted from [18]

Table 3. Protein fractions of selected pulses[1].

Protein fraction (% of total protein)	Peas	Faba beans	Common beans
Albumins	18 – 25	20	12 – 30
Globulins	55 – 65	60	54 – 79
Prolamins	4 – 5	8	2 – 4
Glutelins	3 – 4	15	20 – 30

[1]Data was adapted from [20–22]

The fat content is relatively low in pulses (Table 1) and mainly consists of polyunsaturated fatty acids [18]. Pulses also have a high amount of dietary fibre, ranging between 20-23 g/100 g (Table 1). Aside from the macronutrients, pulses are rich in folates (83-460 μg/100 g) and other B-vitamins [23]. The predominant folate forms in pulses are 5-methyltetrahydrofolate (5-CH_3-H_4folate), formylfolic acid (10-HCO-PteGlu), and 5-formyltetrahydrofolate (5-HCO- H_4folate) [24,25].

Consumption and health effects

Due to the vital role of pulses in sustainable and health-promoting food systems, numerous countries, such as Bulgaria, Canada, Greece, India, Ireland, Spain, South Africa, the UK, and the USA, have included pulses in their dietary guidelines [26]. Bulgaria recommends the highest amount of cooked pulses in the diet, around 200-300 g/day, whereas other countries recommend 80-100 g cooked pulses/day [26]. Pulses are promoted in Swedish dietary advice, but no specific recommendation regarding the quantity is provided [8]. In Sweden, the actual average consumption of pulses is low, at around 12 g/day/person [8]. Increased consumption of pulses would improve the intake of dietary fibre and folates. These nutrients are currently consumed below the recommended level by the majority of the Swedish population [8].

There is evidence regarding the positive effects of pulse consumption on health, such as reduced blood cholesterol level, controlled blood sugar, prolonged satiety, reduced blood pressure, and thereby reducing the risk of coronary heart disease, obesity, and some types of cancer, this is attributed to a high content of resistant starch and dietary fibre [27,28]. Moreover, folates are essential for cell division, especially during pregnancy, to prevent neural tube defects [29], and folates are a critical vitamin of which the intake is low in Europe [30].

Klemcke et al. [31] suggested that a lack of knowledge of preparation methods and the health benefits of pulses is the barrier to increased pulse consumption amongst consumers in Europe. Also, the presence of anti-nutrients and a strong flavour limits the use of pulse-based ingredients in food products [11,32]. The presence of anti-nutritional compounds, such as phytates, trypsin inhibitors, lectins, tannins, and oligosaccharides, in pulses might interfere with nutrient absorption [11,33]. Phytates can strongly bind essential minerals such as iron, calcium, zinc and magnesium, and form a complex compound that cannot be absorbed [34]. Lectins may attach to intestinal cells and disrupt nutrient absorption; they may also cause the precipitation of red blood cells (hemagglutination) [34]. Protease inhibitors interfere with protein digestion, thus decreasing the availability of amino acids for absorption [34]. Oligosaccharides, which cannot be hydrolysed in the small intestine, are directly transported to the large intestine and fermented by the gut microbiota, leading to gas accumulation and digestive discomfort [34]. However, the adverse effects of these anti-nutrients are being re-assessed as several studies have indicated that some of these compounds might have positive effects on health [10].

Processing

Pulses have to be treated before consumption to reduce their anti-nutrient content, improve palatability, enhance the aroma, and enhance their nutritional value by increasing protein digestibility, amino acid and vitamin availability [11]. Traditional household methods, such as soaking, boiling, roasting, and germination, are commonly used to process pulses. Canning and extrusion can also be applied as industrial methods. Different treatments affect the content of nutrients and anti-nutrients in pulses to varying degrees, as summarized in Table 4. Soaking is a common preliminary step before further treatments, such as cooking or germination. Improved protein digestibility and an increased folate content in pulses have been reported after soaking. The increase in folate content might be due to *de novo* synthesis [35]. The anti-nutrient content decreases after soaking, due to the leaching of these compounds into the soaking water. Boiling of soaked pulse seeds enhances the nutrient content and continues antinutrient loss, possibly due to the leaching of the compound into the soaking and/or cooking media. Also, high temperatures during boiling leads to deactivation of the inhibitors [11]. Similarly, roasting and extrusion could improve protein digestibility and reduce the content of heat-labile anti-nutrients (i.e., amylase and trypsin inhibitors, and lectins). Germination has been a common practice for centuries to enhance the nutritional content in cereals, including folates [36,37]. Germination of pulses results in improved protein digestibility and folate content. Germination also reduces the anti-nutrients, such as trypsin inhibitors, phytic acid, and tannins, in pulses.

Table 4. The effects of processing on the content of nutrients and anti-nutrients in pulses.[1]

Treatment	Nutrients	Anti-nutrients
Soaking	↓2-6% protein ↑40-60% folate	↓4-20% trypsin inhibitor activity ↓4-27% α-amylase inhibitor activity ↓2–45% oligosaccharides ↓17-45% phytates ↓23-87% tannins
Boiling	↑16-27% total dietary fibre ↓24-32% folate	↓62-100% trypsin inhibitor activity ↓80-100% α-amylase inhibitor activity ↓100% hemagglutinin activity ↓15-62% oligosaccharides ↓5-58% phytates ↓24-99% tannins
Roasting		↓100% trypsin inhibitor activity ↓24-65% oligosaccharides ↓26-40% phytates ↓9-86% tannins
Germination	↑2-8% protein ↓38% total dietary fibre ↑40-240% folate	↓6-57% trypsin inhibitor activity ↓26-37% α-amylase inhibitor activity ↓5-77% hemagglutinin activity ↓83-100% oligosaccharides ↓23-76% phytates ↓18-76% tannins
Extrusion		↓69-100% trypsin inhibitor activity ↓100% α-amylase inhibitor activity ↓98-100% hemagglutinin activity ↓24% oligosaccharides ↓6-27% phytates ↓7-92% tannins

[1]Upward arrows indicate an increased content of compounds, whereas downward arrows indicate a decreased content of compounds after treatments. Data is summarized from the literature [6,38–47].

Pulse-based ingredients

Pulses are commonly consumed as whole cooked seeds in Europe and Sweden, but they can also be transformed into flour, which has more versatile food applications [48]. There is no commercial flour from locally grown pulses available in Sweden (Gunnerud U 2018, 7th June, oral communication). Pulse flour could be produced by milling pre-treated pulse seeds [49]. Pre-treatment, such as boiling, roasting, or germination, is applied before milling to reduce the content of anti-nutrients and the strong beany flavour, and modify the functional properties of pulse flour [49].

Due to the relatively high protein content (18-34 g protein/100 g), flour from raw pulses could be processed further by fractionation to extract and separate the protein [18,50]. The protein fraction is categorised as a concentrate (48-65% protein db) or isolate (≥ 75% protein db) [13]. The production of pulse protein concentrate/isolate is commonly performed in two ways, as depicted in Figure 2.

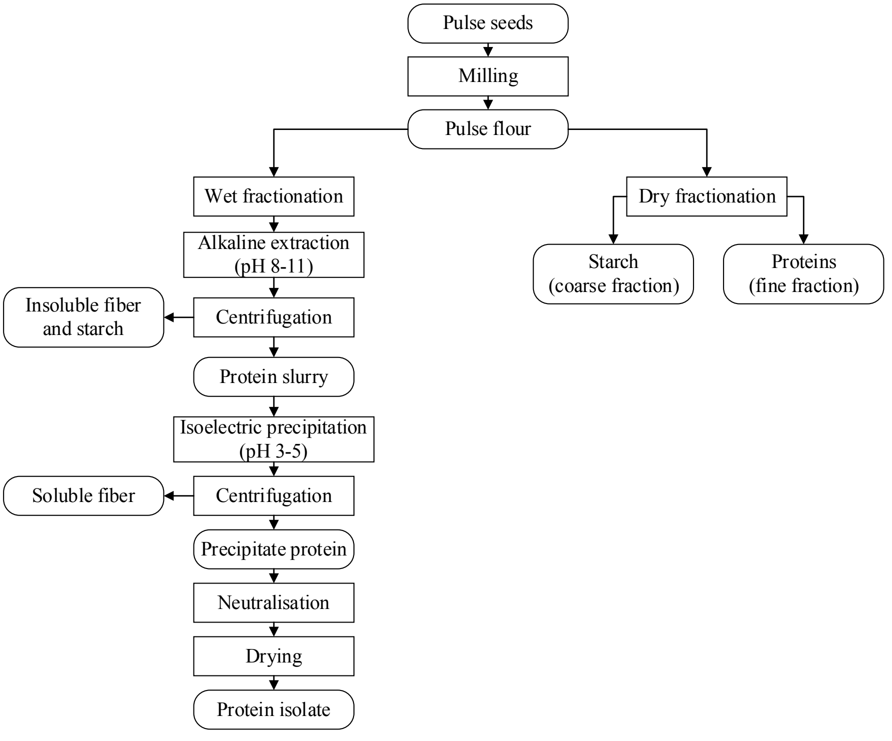

Figure 2. Processing pulse seeds into protein and starch fractions by wet fractionation and dry fractionation [51–53].

The dry fractionation uses a stream of air to separate the pulse flour by particle size and density [54]. Pulses are ground using a pin mill to obtain a fine grind of flour which is fed into a classifier with air circulation and separated according to their aerodynamic properties [55]. The fine fraction is the protein, whereas the coarse fraction is the starch [55]. The milling of the coarse fraction could be repeated to detach the remaining protein from the starch matrix [55]. The wet fractionation is performed using an alkaline solution to extract the protein, followed by iso-electric precipitation to concentrate the protein fraction [54]. The dry fractionation yields 49-70% protein content, while the wet fractionation yields 70-90% [50]. The advantage of dry fractionation is that it requires a low amount of energy and water. In contrast, the wet method generates higher protein purity, and water-soluble anti-nutrients are removed [50].

The starch fraction is the coarse fraction obtained after the milling and separation in the dry fractionation method (Figure 2). In the wet fractionation method, the starch fraction remains in the solid phase after alkaline extraction (Figure 2). The solid phase is then redissolved in water, and the pH is adjusted and filtered to obtain the starch fraction [52]. The fibre fraction could be obtained from the pulse seed coat (outer fibre) and the cotyledon (inner fibre) [53]. Outer fibre could be obtained from the dehulling of pulse seeds, whereas inner fibre is acquired through wet fractionation after the removal of protein and starch fractions [53].

Functional properties

Pulse flour and protein concentrate/isolates from peas, faba beans, and lupins have recently gained global interest as an alternative to soybean-based ingredients. Pulse-based ingredients could be used to produce pulse-based foods or as an added ingredient for increasing the nutritional value of a food product [56,57]. However, the use of pulse-based ingredients might affect the characteristics of the food. Hence it is necessary to understand the functional properties of pulse-based ingredients. These functional properties, including water-holding capacity, oil absorption capacity, emulsion activity and stability, foaming capacity and stability, and gel-forming properties, significantly influence the overall quality of the product [48]. These properties are essential in various food applications, such as meat products, snacks, bakery goods, dressings, desserts, and beverages [48,58].

Water absorption capacity (WAC) describes the ability to retain water in the food matrix, which plays an essential role in the moistness of the food, starch retrogradation, and staling during storage [59]. Pulse-based ingredients with a high WAC value are ideally utilized to produce custard, sausages, or dough because of their capacity to absorb water that contributes to the viscosity of the product [60]. Oil absorption capacity (OAC) reflects the ability to retain oil in the food matrix [61]. OAC affects the storage stability of the flour and the palatability of the product [61,62]. Pulse flour with a low OAC value are suitable for fried products to prevent excessive oil retention in the product [60]. Emulsion and foaming properties are related to the performance of the protein fraction in pulse-based ingredients [48]. Emulsion activity and stability reflect the ability of proteins to stabilize an emulsion and prevent destabilization mechanisms which cause phase separation [61]. Pulse-based ingredients with high emulsion activity and stability could be used as emulsifiers in colloidal food products (e.g. dressings, mayonnaise), bakery, and meat products [63]. Foaming capacity and stability show the ability of the protein to form a thin viscoelastic film that stabilizes air bubbles in suspensions and prevents breakdown [61]. Pulse ingredients with a high foaming capacity and stability values can be utilised to produce cakes, whipping cream, and mousse [51]. Least gelation concentration (LGC) shows the minimum concentration of pulse-based ingredients needed to form a gel [48]. The lower the LGC, the better the gelling properties [60]. Pulse-based ingredients with a low LGC could act as a thickening or gelling agent in puddings or sauce products [63].

Volatile compound composition

Another challenge for increasing the use of pulses as ingredients in food products is the presence of a strong beany flavour [64]. Characterisation of volatile compounds in pulses could provide essential information for food manufacturers in selecting the pulse-based ingredients and processing steps that decrease the beany flavour. Previous studies identified hexanal, 1-octen-3-ol, 2-pentylfuran, and 3,5-octadien-2-one as the markers of the beany flavour [65–69]. The aroma description of 1-octen-3-ol is earthy, green, oily, and fungal [70]. The aroma of 2-pentylfuran is described as green, earthy, and beany [70]. Another well-known odour, 3,5-octadien-2-one, is described as fatty, mushroom-like, and fruity [70]. Hexanal is also reported as a marker compound. However, it is described as lacking any beany characteristic as an individual compound but may cause a beany flavour in combination with other compounds [69–71]. The compounds mentioned above originate mainly from lipid degradation due to enzymatic and chemical reactions during post-harvest, e.g., storage and processing [72]. Thermal processing, in general, causes a reduced abundance of beany flavour compounds in pulses [65].

Development of pulse-based foods

A European survey in 2020 revealed that consumers still believe that there are not enough options for plant-based products available in retail stores [73]. Consumers request a greater variety of raw materials, flavours, textures, and product types [73]. There is still room for improvement in developing plant-based products. Locally cultivated pulses in Europe, such as peas and faba beans, could be explored further as a way to offer sustainable and diversified food products to consumers. Meat and dairy are the main contributors to protein supply in Europe and Sweden [17]. Therefore, plant-based alternatives for these food categories are worth exploring.

Plant-based meat analogues

Despite the high demand for plant-based meat analogues from consumers, optimization of texture properties remains a challenge. According to a survey, consumers demand plant-based meat alternatives to have a similar texture to meat and meat products, and to be made from non-GMO ingredients [74]. Several studies have reported that pulse protein isolates can be used as the raw material to produce meat analogues using extrusion technology [75–78]. Extrusion is a continuous thermomechanical process involving an extensive mixing of ingredients with water through the movement of screws in combination with high pressure, force, and temperature [79]. The melted mixture is then passed through a cooling die to shape the product [79]. The texture of the extrudate is dependent on several factors, such as the intrinsic properties of the raw materials, barrel temperature, ingredient feed rate, target moisture content, screw speed, screw configuration, and geometry and length of the cooling die [80].

An early type of meat analogue has been produced using the low-moisture extrusion technique since the 1960s. This type of meat analogue needs to be rehydrated before incorporation into food products and the texture resembles minced meat [81]. A relatively recent technology, high-moisture extrusion (HME), enables a product with a fibrous meat-like structure, known as a high-moisture meat analogue (HMMA) to be created from plant protein raw materials [81]. During the HME process, plant proteins are unfolded, aggregated, and realigned with heat, pressure, and force in the extruder barrel [81]. In the cooling die, as the temperature decreases, the protein molecules are cross-linked, leading to the formation of a fibrous meat-like structure [76,81]. The covalent disulphide bonds and, to a lesser extent, the non-covalent bonds between proteins are suggested to be essential in forming the fibrous structure of HMMAs [77].

Plant-based cheese analogues

According to a survey in Europe, consumers feel that plant-based cheeses cannot yet compete with their animal-based counterparts in terms of taste and texture [73]. Furthermore, most commercial plant-based cheese analogues are made from oil and starch, leading to concerns about their nutritional profile. Other nut-based products are seen as being too expensive [73]. Therefore, the use of pulses to produce plant-based cheese analogues seems promising due to their nutritional and economic value.

Plant proteins are substantially different from milk proteins in terms of structure and functional properties [82]. Plant proteins are larger and have a more complex quaternary structure than milk proteins [82]. Thus, plant proteins are unable to form a compact aggregate structure like the casein micelle structure in dairy-based cheese [82]. Therefore, it is very challenging to make plant-based cheeses with similar characteristics as traditional dairy cheese [83]. Stabilizers, such as carrageenan and xanthan gum, are commonly added in plant-based cheese analogue formulations to help to form the shape and improve the texture [84–86]. Carrageenan can be categorised into kappa, iota, and lambda carrageenan. Kappa carrageenan forms a strong and brittle gel in the presence of calcium salts, whereas iota carrageenan forms an elastic gel [87]. Lambda carrageenan does not form gel but can increase the viscosity of the mixture [87]. Xanthan gum is a thickening agent, and it is commonly combined with gelling agents to increase the elasticity of food gels [88]. The use of combined stabilizers may have a synergistic effect, improving the texture of plant-based cheese analogues [85].

Materials and methods

Materials

The pulse raw materials and reference products used in different studies are listed in Table 5. The pulse species were chosen due to their suitability for cultivation in Sweden and interest from local food industries.

Table 5. Pulse species and reference products used in the different studies.

Material	Paper
Yellow peas (*Pisum sativum* var. *clara*), harvested in 2016 and 2019	I, II, III, IV
Grey peas (*Pisum sativum*, unknown Latvian variety), harvested in 2016	I, II
White beans (*Phaseolus vulgaris* var. *T9905*), harvested in 2016	I, II
Faba beans (*Vicia faba* var. *alexia*), harvested in 2017	I, II
Faba beans (*Vicia faba* var. *gloria*), harvested in 2019	III, IV
Commercial yellow pea protein isolate	III
Commercial faba bean protein concentrate	III
Boiled chicken (reference)	III
Boiled beef (reference)	III
Commercial soybean HMMA (reference)	III
Gouda cheese (reference)	IV
Commercial starch and coconut-based vegan cheese analogue (reference)	IV

Preparation of pulse-based ingredients

Flour

Flour from yellow peas, grey peas, white beans, and faba beans were prepared using different treatments, as depicted in Figure 3. Flour from untreated (raw) pulses were also prepared by directly milling the seeds. These pulse flour were analysed (in duplicate from duplicate samples, $n = 4$) in terms of their functional properties: water absorption capacity (WAC), oil absorption capacity (OAC), emulsion activity and stability (EA and ES), foaming capacity and stability (FC and FS), and least gelation concentration (LGC) (Table 6). The total dietary fibre (TDF), resistant starch, total choline, and folate content were also examined (in duplicate from duplicate samples, n = 4) (Table 6). The results of these analyses are presented in Paper I. Moreover, the volatile compounds in different pulse flour were extracted using a headspace solid-phase microextraction technique and identified with gas chromatography-mass spectrophotometry (HS-SPME/GC-MS, in triplicate n = 3) (Table 6). The volatile compound composition of flour from untreated and boiled yellow and grey peas is presented in Paper II. Flour from boiled and roasted yellow peas and faba beans were used as the raw materials to develop cheese analogues (Paper IV).

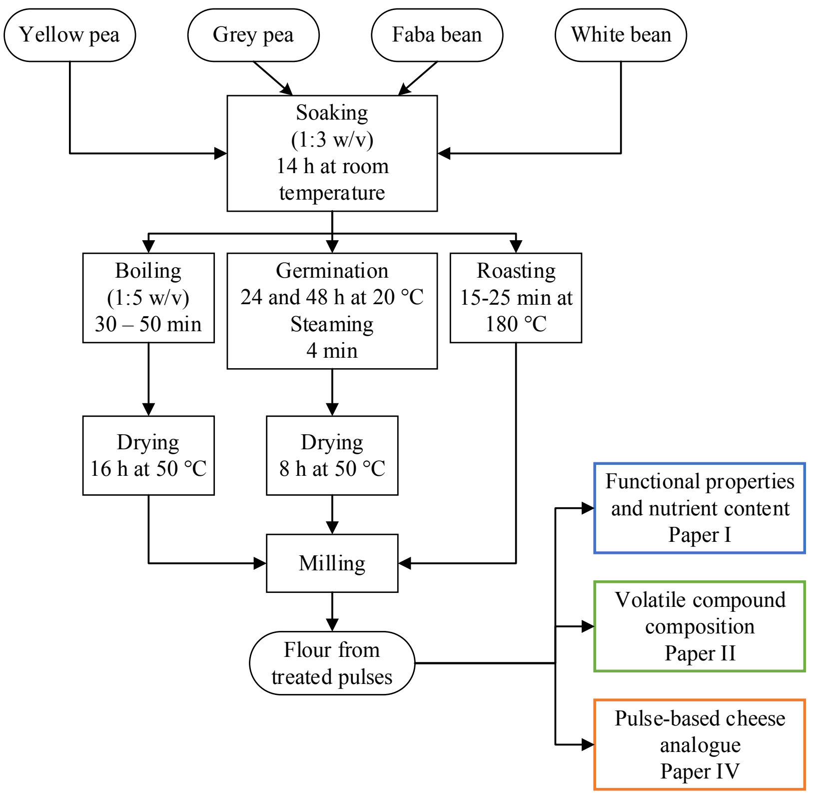

Figure 3. Preparation of flour from pulses using different treatments.

Protein isolates

The preparation of pulse protein isolates was performed as shown in Figure 4. The pulse protein isolates were analysed for proximate composition, water holding capacity, and thermal properties in triplicate (Table 6), and the results are presented in Paper III.

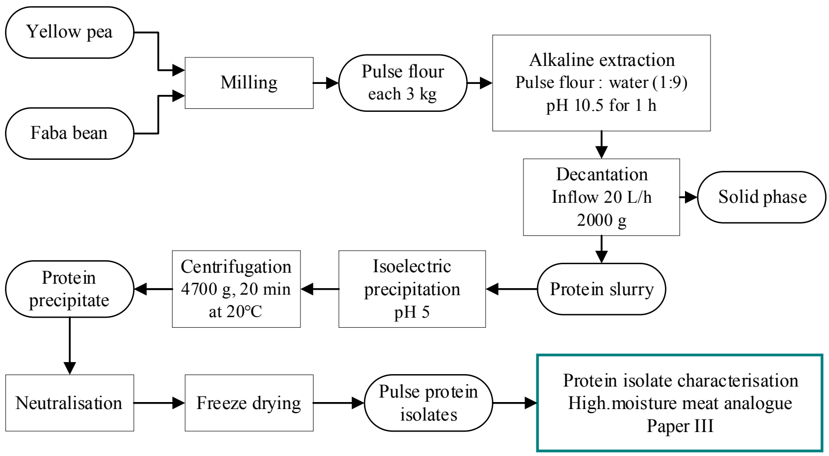

Figure 4. Preparation of protein isolates from different pulses using alkaline extraction followed by an isoelectric precipitation method.

Development of pulse-based foods

High-moisture meat analogues (HMMA)

The extrusion experiments were performed using a laboratory co-rotating twin-screw KETSE 20/40D extruder. The extruder barrel was segmented into four temperature zones, as depicted in Figure 5. A cooling die was attached to the end of the extruder to prevent expansion and facilitate fibrous texture formation. The aimed water content and ingredient feed rates were calculated according to the initial moisture content of each protein ingredient, the target moisture content for HMMA, and total mass flow.

Due to the limited amount of protein isolates prepared from local pulses, preliminary trials were conducted using commercial yellow pea protein isolate (YPI-com) to define the experimental settings (Figure 5a). Different temperatures above the denaturation temperature of the isolate were explored with a focus on heating zones 3 and 4 to determine the extrusion temperature range (Figure 5b). After that, different target moisture content in the final product and screw speed (Figure 5b) were adjusted until fibrous structures were formed in the extruded material exiting the cooling die. Selected extrusion parameters were also varied for the commercial faba bean protein concentrate (FBC-com) and protein isolates from local yellow peas (YPI-local) and faba beans (FBI-local). The detailed extrusion parameters are given in Appendix B,

Table A3 in Paper III. Boiled chicken breast, boiled beef, and commercial soybean HMMA were used as references. The references and HMMAs were analysed in terms of texture in triplicate (Table 6). The results are presented in Paper III.

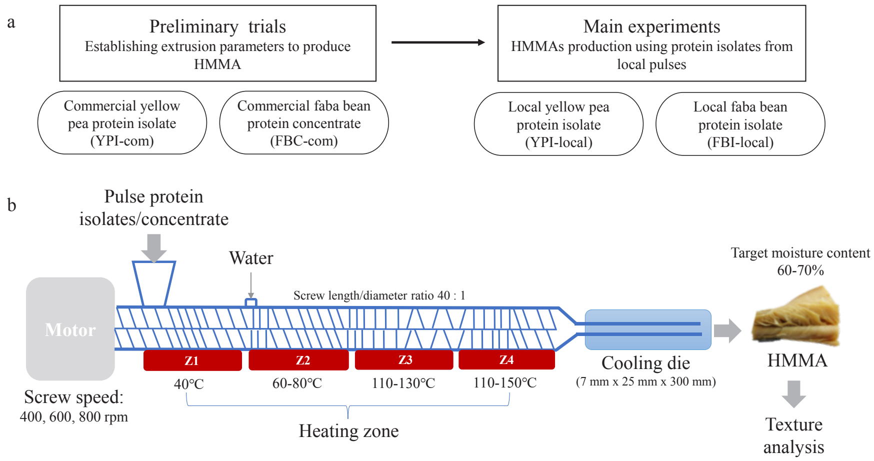

Figure 5. Study design of HMMA production (a) and schematic diagram of twin-screw extruder and parameters used in the study, Paper III (b).

Pulse-based cheese analogues

Pulse-based cheese analogues were prepared according to a patent by Thresher [89]. Preliminary assessments were conducted using flour from boiled yellow peas to determine the amount of water required (Figure 5). The formula with the chosen ratio of pulse flour to water was used as the basis for determining the concentration of the stabiliser. Kappa-carrageenan (KCG) was evaluated at four different concentrations (Figure 5). Additionally, combinations of kappa-carrageenan with iota-carrageenan (ICG) and kappa-carrageenan with xanthan gum (XG) were explored. The total concentration of the stabilisers was at 1% in the formula. All samples were subjected to texture analysis (Table 6).

Pulse-based cheese analogues were made using flour from boiled yellow peas (BYP), roasted yellow peas (RYP), boiled faba beans (BFB), and roasted faba beans (RFB) according to the formula derived from preliminary trials (Figure 5). Flour from boiled and roasted yellow peas and faba beans were chosen due to their superior gelling properties and flavours compared with flour from germinated pulses (Table 7). All formulation trials were carried out in duplicate. Two commercial samples, dairy cheese Gouda (AxFood Sverige AB, Sweden) and vegan cheese made from coconut oil and starch (Violife Foods, Greece), were used as references. The references and all samples were subjected to proximate, colour, and texture analysis in triplicate (Table 6). The results are presented in Paper IV.

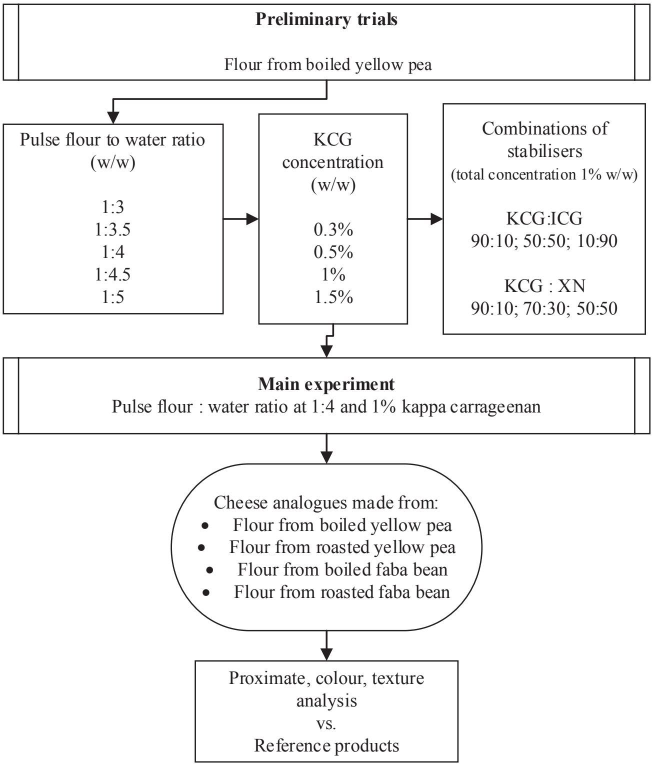

Figure 6. Study design of pulse-based cheese analogue development presented in Paper IV. KCG = kappa-carrageenan, ICG = iota-carrageenan, and XG = xanthan gum.

Analytical methods

Detailed analytical procedures in Table 6 are described or referred to in the Materials and Methods section of each paper.

Table 6. Methods of analysis used for the characterisation of pulse flour[1], pulse protein isolates, and pulse-based foods.

Analysis	Material	Description	Reference	Paper
Functional properties				
Emulsion activity (%)	Pulse flour	The sample was mixed with water. Oil was added, homogenised, and centrifuged. Emulsion activity = (vol. of emulsified layer / total vol. of emulsion) x 100	Kaur & Singh [90]	I
Emulsion stability (%)	Pulse flour	The sample was mixed with water. Oil was added homogenised, heated, and centrifuged. Emulsion stability = (vol. of emulsified layer / total vol. of emulsion) x 100	Kaur & Singh [90]	I
Foaming capacity (%)	Pulse flour	The sample was mixed with water and whipped. The foam volume was recorded. Foaming capacity = (vol. after whipping – vol. before) / (vol. before) x 100	Kaur & Singh [90]	I
Foaming stability (%)	Pulse flour	The reduction in foam volume was recorded over 2 hours. Foaming stability = (vol. foam at t (t = 20, 40, 60, 120 min) / initial foam vol.) x 100	Kaur & Singh [90]	I
Least gelation concentration (%)	Pulse flour	Suspensions of pulse flour at different concentrations (2-20%) were prepared in tubes, heated, and cooled. The gelation concentration was the lowest concentration at which the sample did not fall when the tube was inverted.	Sathe et al. [91]	I
Oil absorption capacity (g oil/g db)	Pulse flour	The sample was mixed with oil, stirred, and centrifuged. The weight of the sample before and after treatment was compared.	Kaur & Singh [90]	I

Table 6. continued.

Analysis	Material	Description	Reference	Paper
Water absorption capacity (g water/g db)	Pulse flour	The sample was mixed with water, stirred, and centrifuged. The weight of the sample before and after treatment was compared.	Kaur & Singh [90]	I
Water holding capacity (ml water/g db)	Pulse protein isolates	The sample was mixed with water, stirred, and centrifuged. The weight of the sample before and after treatment was compared.	AACC [92]	III
Proximate composition and other nutrients				
Ash (g/100 g db)	Pulse protein isolates, cheese analogues	The sample was incinerated in the muffle oven at 525 ºC for 16 hours.	AOAC [93]	III, IV
Carbohydrate (g/100 g db)	Pulse protein isolates, cheese analogues	The carbohydrate content was calculated by difference. Carbohydrate = 100 – moisture – ash – fat – protein.		III, IV
Fat (g/100 g db)	Pulse protein isolates, cheese analogues	The fat content in the sample was determined using a Soxhlet method.	AOAC [94]	III, IV
Folate (µg/100 g db)	Pulse flour	The sample was treated with α-amylase, protease, and rat serum (de)conjugase. Folate was quantified after purification using SAX using HPLC-UV/FLD as the sum of individual folate.	Hefni et al. [95]	I
Moisture content (g/100 g db)	Pulse flour, pulse protein isolates, cheese analogues	The sample was dried in the oven at 105 ºC for 16 hours.	AOAC [96]	I, III, IV
Protein (g/100 g db)	Pulse protein isolates, cheese analogues	The protein content in the sample was determined using a combustion method (N conversion factor 6.25).	AOAC [97]	III, IV
Resistant starch (g/100 g db)	Pulse flour	The sample was treated with α-amylase and amyloglucosidase. The resistant starch was solubilised with KOH and treated with amyloglucosidase. The resistant starch content was measured as glucose content using a spectrophotometer.	AOAC [98]	I

Table 6. continued.

Analysis	Material	Description	Reference	Paper
Total choline (mg/100 g db)	Pulse flour	The sample was treated with acid and 1-naphthyl isocyanate. The total choline was quantified using HPLC-FLD.	Hefni et al. [99]	I
Total dietary fibre (g/100 g db)	Pulse flour, pulse protein isolates, cheese analogues	The sample was treated with α-amylase, protease, and amyloglucosidase. The fibre residue was filtered, dried, weighed, and corrected for ash and protein content.	AOAC [100]	I, III, IV
Other analyses				
Colour properties	Cheese analogues, Gouda cheese, and commercial vegan cheese analogue	The surface colour of the sample was measured using a colorimeter.	Zahari et al. [101]	IV
Thermal properties	Pulse protein isolates	The sample was mixed with water and heated in a calorimeter instrument. The denaturation temperature (°C) and enthalpy were recorded (J/g).	Zahari et al. [101]	III
Texture properties (Force g)	HMMAs, cooked chicken and beef, commercial soybean HMMA, cheese analogues, Gouda cheese, and a commercial vegan cheese analogue	The sample was compressed using a cylinder probe and cut using a knife blade.	Zahari et al. [101]	III, IV
Volatile compound profile (total peak area)	Pulse flour	The sample in an Erlenmeyer flask was conditioned in a water bath to release the volatile compounds to the headspace. The volatile compounds were collected using DVB/CAR/PDMS SPME fiber and identified using GC-MS.	Oomah & Liang [67]	II

[1]Pulse species = yellow peas, grey peas, faba beans, and white beans. Type of treatment = boiling, roasting, and germination.

Statistical analysis

All data was expressed as mean ± standard deviation (SD), with duplicate measurements or more. One-way ANOVA and post-hoc Tukey's test were used to determine significant differences between the observed data in Paper I - IV. The level of significance was set to 0.05. ANOVA analyses were performed using R software version 3.4.1-2017 or Graphpad Prism software version 7.04-2017. The principal component analysis (PCA) was conducted using Graphpad Prism software version 9.1.2-2021 to examine similarities or groupings among tested pulse flour based on functional properties and nutrient content (Paper I). Multivariate analysis, principal component analysis (PCA), and partial least square analysis (PLS) were performed using Simca software version 16.0.1 to investigate the correlation between the raw material composition and extrusion parameters on the texture properties of HMMA (Paper III).

Results and Discussion

This thesis is based on four studies (Paper I – IV). Paper I and II focussed on the characterisation of flour produced from different pulse species and treatments in terms of functional properties (I), nutrient content (I), and volatile compound composition (II). The focus of Paper III and IV was to assess the suitability of pulse protein isolates in the development of meat analogues (III) and pulse flour in the development of cheese analogues (IV).

Characterisation of pulse ingredients

Flour

Functional properties and nutrient content

The data from functional properties and nutrient content analyses was subjected to principal component analysis (PCA), as shown in Figure 7. The first and second principal components (PCs) explained 37.9% and 25.3% of the variance, respectively. Therefore, the first two PCs represent 63.2% of the total variability. The loading plot of the two PCs provided information about correlations between measured functional properties and nutrient content. The properties that are close to each other on the plot are positively correlated while those which are in opposite directions are negatively correlated. Water absorption capacity (WAC) is negatively correlated with emulsion activity (EA) and foaming capacity (FC) (Figure 7). Furthermore, PCA showed a clear separation between flour from raw and treated (boiled, roasted, and germinated-steamed) pulses (Figure 7). Flour from raw pulses were located at the upper left quadrant of the score plot due to higher EA and FC. On the other hand, flour from treated pulses were mostly located at the right side of the score plot due to higher WAC, LGC (least gelation concentration), OAC (oil absorption capacity), TDF (total dietary fibre), total choline, and folate content. Flour from roasted and germinated-steamed white beans were separated from other treated pulse flour due to the high folate content.

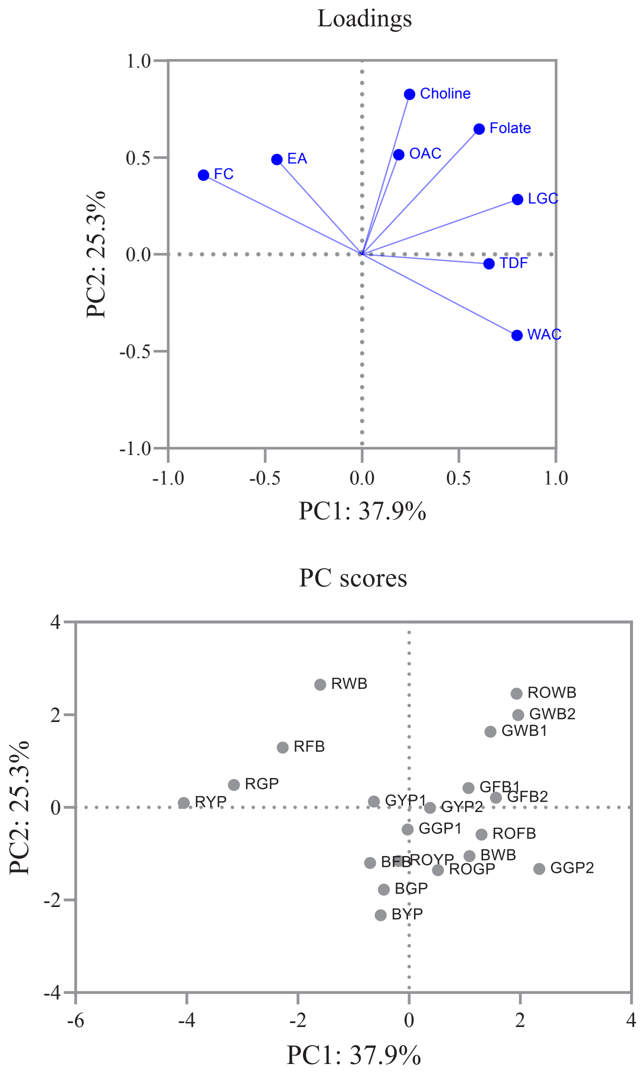

Figure 7. Principal component loadings (PC loadings) and principal component scores (PC scores) based on the functional properties and nutrient content in flour from raw and treated pulses. EA: emulsion activity, FC = foaming capacity, LGC = least gelation concentration, OAC = oil absorption capacity, TDF = total dietary fibre. Flour from raw yellow peas (RYP), raw grey peas (RGP), raw faba beans (RFB), raw white beans (RWB). Flour from boiled yellow peas (BYP), boiled grey peas (BGP), boiled faba beans (BFB), boiled white beans (BWB). Flour from roasted yellow peas (ROYP), roasted grey peas (ROGP), roasted faba beans (ROFB), roasted white beans (ROWB). Flour from germinated (24 h and 48 h)-steamed yellow peas (GYP1 and GYP2), germinated (24 h and 48 h)-steamed grey peas (GGP1 and GGP2), germinated (24 h and 48 h)-steamed faba beans (GFB1 and GFB2), germinated (24 h and 48 h)-steamed white beans (GWB1 and GWB 2).

The data (values) describing the functional properties of raw flour from locally grown pulses was within the ranges reported by others [61,102] for flour from raw beans and peas. All flour from raw pulses had a similar OAC (~1 g oil/g db), EA (~50%), and FC (~33%), but they differed in WAC and LGC (Figure 8). Among the raw flour tested, yellow pea flour had the lowest WAC (0.8 g water/g db) and LGC value (8 %) (Figure 7).

Independent of pulse species, all treatments (boiling, roasting, and germination) increased the WAC but impaired the EA, FC, and LGC of pulse flour compared with flour from raw pulses (Figure 8). The WAC of flour prepared from all treated pulses was higher by two to threefold (2-3 g water/g db) than in raw pulse flour (Figure 8), which is in line with previous findings for WAC in flour from boiled beans [103]. Flour from roasted faba beans had the highest WAC amongst samples.

A 14-34% reduction of EA in pea flour after all treatments was observed compared with raw pea flour (Figure 8). In contrast, the EA of flour from beans were not affected by any treatment, except for the flour from boiled white beans (Figure 8). The observed EA value of flour from all treated pulses was in accordance with the range (32-45%) reported by others [104–106]. All treatments also resulted in an 11-18% decrease in FC of flour from yellow peas, grey peas and faba beans compared to the raw flour (Figure 8). The FC value of flour from treated pulses was within the range reported in the literature for FC values of flour from boiled beans (30-36%) [103].

In general, flour from treated pulses had increased LGC up to twofold (10-16%) compared to the raw flour (Figure 8), confirming the findings of a previous study [103]. Among the tested flour, flour from germinated-steamed faba beans and white beans had the highest LGC. A higher LGC value corresponds to inferior gelling ability, meaning that a greater amount of flour is required to form a firm gel. For OAC, there was no difference observed in treated pulse flour (~1 g oil/g db) compared with raw flour, except for flour from boiled pulses, which had a 20% decrease in OAC (Figure 8).

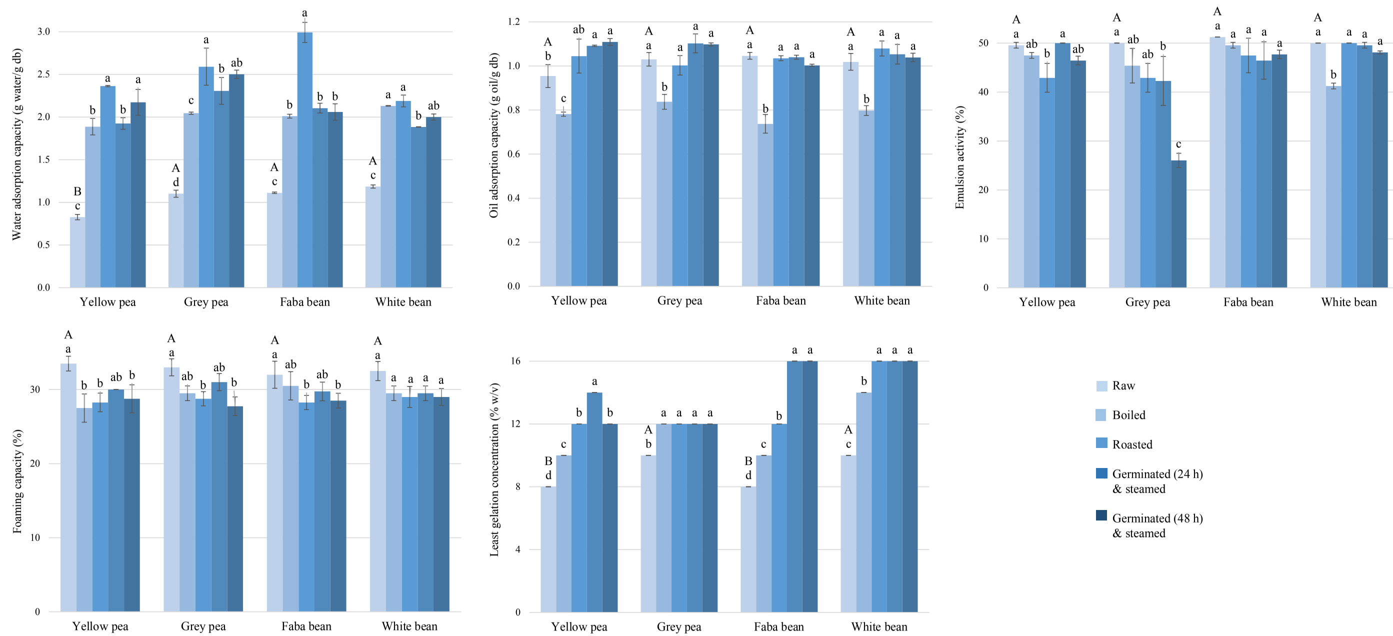

Figure 8. Water absorption capacity, oil absorption capacity, emulsion activity, foaming capacity, and least gelation concentration of pulse flour. Bars represent the mean ± SD (duplicate analyses from two independent trials, $n = 4$). Different lowercase letters within pulse species indicate a significant difference (Tukey's test, $p < 0.05$) between treatments. For raw flour, means with different uppercase letters indicate a significant difference (Tukey's test, $p < 0.05$) between pulse species.

All tested functional properties of pulse flour can be attributed mainly to the properties of protein [48]. It has been reported that the application of various treatments during flour preparation is expected to lead to changes in protein structure and functionality [60,106,107]. As a result, the functional properties were significantly affected [60,106,107]. It was suggested that protein denaturation leads to exposure of hydrophilic groups of the protein, thus increasing its ability to retain water [60,106]. With an increasing thermodynamic affinity of proteins for aqueous phase, the protein-protein interactions decrease, reducing the gelling ability [60]. Denatured proteins may become insoluble, making them unable to position themselves on the oil-water interface to form a film, thereby reducing both emulsion and foam formation and stability [48].

Data regarding the high contents of total dietary fibre (16-18 g/100 g db) and folates (73-245 μg/100 g db) observed in the raw pulse flour in the current investigation are well in line with data reported by others [25,108,109]. Generally, flour from raw beans had a higher nutrient content than flour from raw peas (Figure 9). Flour from raw beans had a 13% higher total dietary fibre (TDF) content than flour from raw peas (16 g/100 g db) (Figure 9). Also, the total folate content in flour from raw beans was two- to threefold higher than in flour from raw peas (Figure 9). In raw peas, the dominating folate vitamer was 10-formylformylic acid, in raw faba beans it was 5-methyltetrahydrofolate, and in raw white beans it was 5-formyltetrahydrofolate (Figure 9). The observed findings for folate content are within the range reported by Rychlik et al. [25] but lower than the value reported in the data base of the Swedish Food Agency (SFA). Folate data by Rychlik et al. [25] and from the study in Paper I was measured as the sum of individual vitamers (tetrahydrofolate, 5-methyltetrahydrofolate, 10-formylfolic acid, and 5-formyltetrahydrofolate) for which commercial standards were available. In contrast, for data from the SFA, a microbiological assay was used that measures total folates from all folate forms contained in the sample, which might explain the higher reported values [95,110]. The total choline content in flour from raw beans was 10-50% higher than in peas (136-141 mg/100 g db). The observed total choline content in flour from raw pulses was 20-40% higher than the sum of esterified phospholipids reported by Lewis et al. [111]. The lower value of the literature data [111] might be explained by the lack of individual choline standards when calculating the total choline [99].

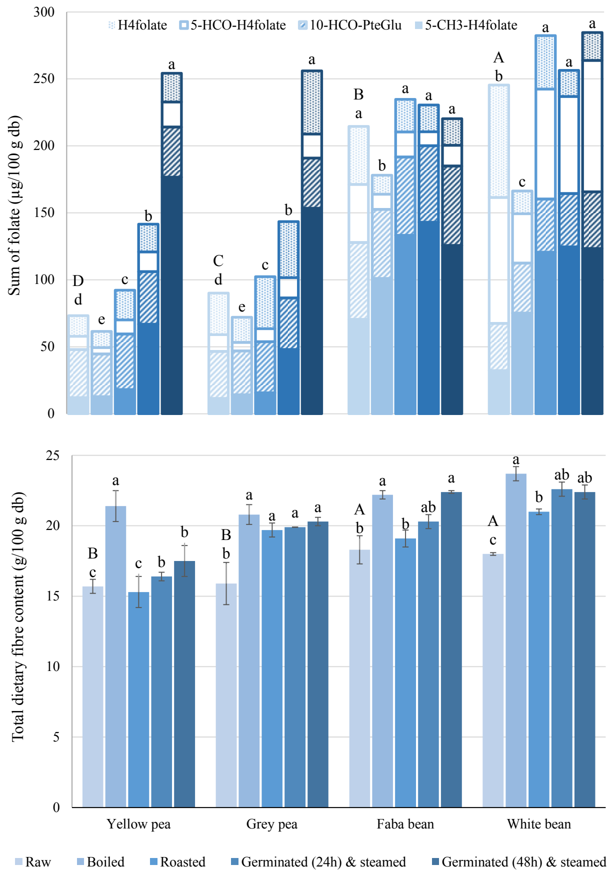

Figure 9. Total dietary fibre (g/100 g db) and folates (µg/100 g db) in flour from raw, boiled, roasted, and germinated pulses. Bars represent the mean ± SD (duplicate analyses from two independent trials, *n* = 4). Different lowercase letters within the same pulse species indicate a significant difference (Tukey's test, $p < 0.05$) between treatments. For raw flour, means with different uppercase letters indicate a significant difference (Tukey's test, $p < 0.05$) between pulse species. H_4folate = tetrahydrofolate, 5-HCO-H_4folate = 5-formyltetrahydrofolate, 10-HCO-PteGlu = 10-formylfolic acid, 5-CH_3-H_4folate = 5-methyltetrahydrofolate.

Moreover, the content of nutrients in pulse flour significantly depended on both the preparation method and pulse species, with flour from beans tending to have a higher nutrient content than flour from peas (Figure 9). This study showed that boiling resulted in a 20-33% increase in TDF content which was higher than after germination or roasting (Figure 9). A 15-30% rise of TDF content in pulses after boiling was also reported by Aguilera et al. [112], this might be attributed to the formation of retrograded resistant starch. Boiled pulses required a longer drying time (16 h), which might enhance the starch retrogradation, thus resulting in flour with higher TDF content than in those after other treatments. There was also a 17-27% reduction of total choline and a 15-32% reduction of folate content in pulse flour after boiling compared with raw pulse flour. These losses might be caused by leaching of choline and folate into the cooking water, as reported in previous studies [111,113,114]. The decrease of folate content was mainly due to a decrease of tetrahydrofolate and 5-formyltetrahydrofolate (Figure 9).

The increase of folate content after germination was mainly due to an increase of 5-methyltetrahydrofolate (Figure 9), which is in accordance with previous findings [41,115]. Germination notably enhanced the folate content by up to twofold in peas but only by 2-14% in beans. This increase is probably caused by *de nov*o synthesis of folates during germination as reported by others [35]. In several studies, an up to threefold increase in total folate content was observed after the germination of pulses [41,115,116]. Roasting increased the TDF content in all pulse species but at a lower magnitude than boiling (Figure 9). Folate content was also higher in flour from roasted pulses compared to the raw pulses (Figure 9). The net increase of folate was because roasting did not lead to leaching of folate from the pulse seeds in the way boiling did.

Volatile compound composition

Information on the volatile compound profile of pulses can provide the basis for selecting suitable treatments to process pulses prior to milling when developing food products with pulse ingredients with a subtle beany flavour. In the study, HS-SPME/GC-MS analysis was used to identify as many volatile compounds as possible in pulse flour prepared using different treatments.

In flour from raw pulses, hexanal, nonanal, and 1-hexanol were the dominating volatile compounds (Figure 10a), confirming findings by others [66,117]. The volatile composition in raw faba beans differed from that of other species due to the absence of hexanal. The compounds 1-octen-3-ol and 2-pentylfuran were found in a high abundance in flour from all raw pulses (Figure 11a), whereas 3,5-octadien-3-one was found in high abundance only in yellow pea and white bean flour (Figure 11a). The compounds hexanal, 1-octen-3-ol, 2-pentylfuran, and 3,5-octadien-3-one are known markers for a strong beany flavour [65–68].

Flour made from boiled peas and white beans had a one- to twofold increased total peak area for hexanal and nonanal, while 1-hexanol was not detected compared with flour from raw pulses (Figure 10b), confirming the findings by others [32]. Flour from boiled faba beans had a high abundance of hexanal, which had not been detected before in raw flour (Figure 10a and 10b). A high peak area of hexanal in flour from boiled pulses might not necessarily indicate a stronger beany flavour because hexanal lacks a strong beany character by itself, but it is an indicator of heat treatment [69–71]. Furthermore, there was a decrease in the abundance of 1-octen-3-ol, 2-pentylfuran, and 3,5-octadien-2-one after boiling (Figure11b), which agrees with previous findings by Mishra et al. [65]. The decrease of these compounds might suggest a diminishing beany flavour in flour from boiled pulses compared with flour from raw pulses (Paper II) [118].

The composition of volatile compounds in flour prepared from germinated pulses differed between pulse species (Figure 10c). There was a decrease in the total peak area of hexanal and nonanal and an increase in 1-hexanol after germination, compared with raw pulse flour (Figure 10c). Also, there was a notable abundance of esters in flour from germinated yellow peas (33% of total peak area) and faba beans (52% of total peak area), but only a small abundance of esters found in grey peas and white beans (Figure 10c). The most prominent ester identified was butanoic acid-3-methyl-ethylester, which is described as strong fruit-like, earthy, and green [70]. Also, 3,5-octadien-2-one was higher after germination in yellow peas and white beans than in the raw flour (Figure 11c). Hexanal, 1-octen-3-ol, and 2-pentylfuran were also present in a high abundance in all pulses (Figure 11c). It might indicate that flour from germinated pulses had a strong beany flavour, which agrees with findings from a preliminary internal taste evaluation where flour prepared from germinated pulses were described as having a strong beany flavour and green, bitter taste (data not shown). Similar findings of unpleasant flavours, described as grassy, green, and off-odour, in germinated lentils have been reported by Troszyńska et al. [119].

A short steaming (4 mins) was performed after germination to decrease the off flavour. It was observed that esters and alcohols were reduced by 57-76% after steaming in all pulses, but there was an increase in hexanal and nonanal in faba beans (Figure 10d). Volatile composition in all germinated pulses after steaming was similar, although some esters remained in yellow peas (Figure 10d). A longer steaming time would probably have decreased the esters in yellow peas. The abundance of the marker compounds 1-octen-3-ol, 3,5-octadien-2-one, and butanoic acid, 3-methyl, -ethyl ester decreased after steaming while 2-pentyfuran was about the same (Figure 11d). Therefore, germination combined with steaming may produce pulse flour with an enhanced nutrient content (Paper I) and reduce the beany flavour.

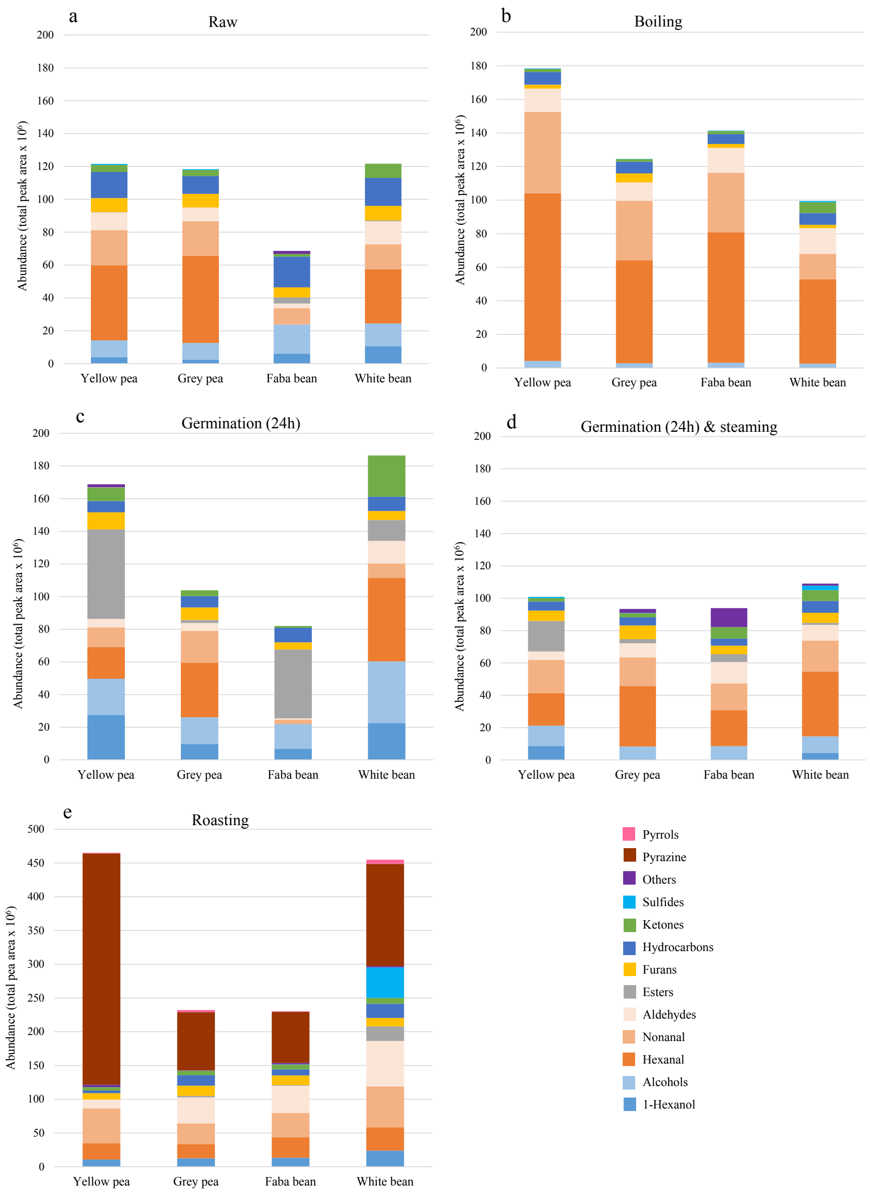

Figure 10. Volatile compound composition in flour from raw (a), boiled (b), germinated (c), germinated-steamed (d), and roasted (e) pulses. Bars represent the means of total area counts from triplicate analyses. The graph for the treatment roasting has a different y-axis scale due to a much higher total peak area identified (> 450 x 10^6).

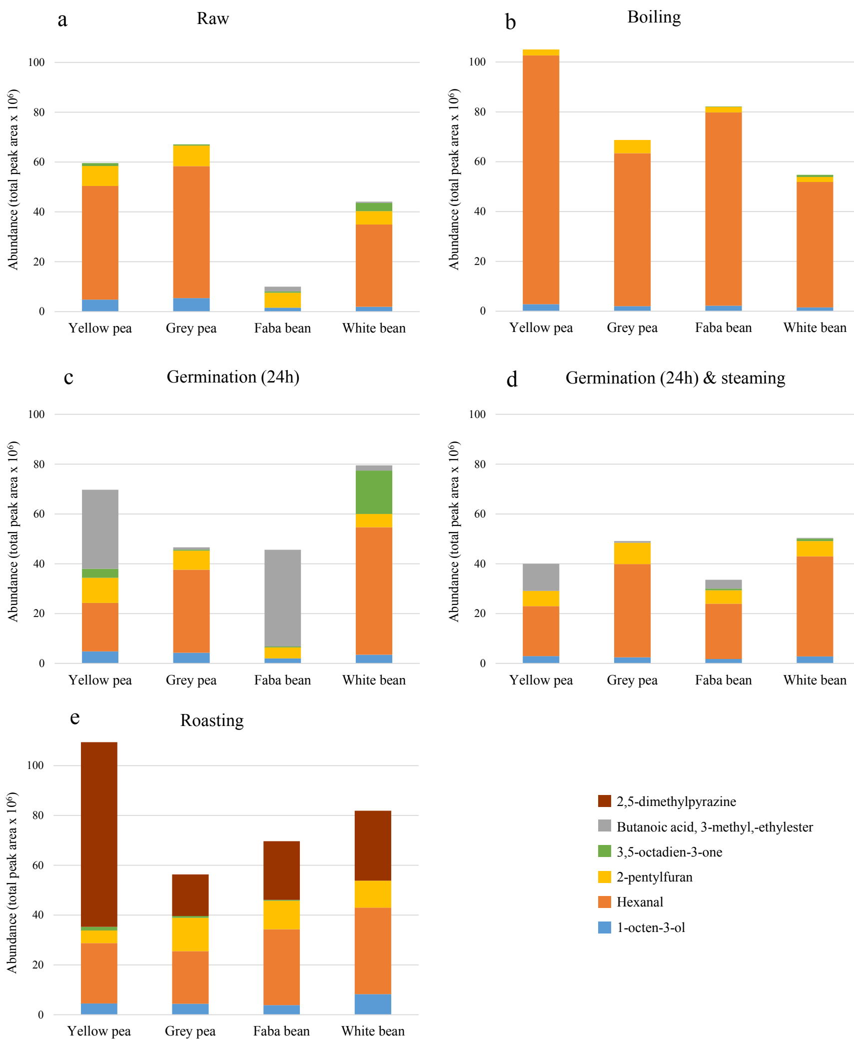

Figure 11. Markers for a strong beany flavour in flour from raw (a), boiled (b), germinated (c), germinated-steamed (d), and roasted (e) pulses. Bars represent the mean of total area counts from triplicate analyses.

Flour made from roasted pulses had a notable abundance of pyrazines (Figure 10e), which is in line with the findings in previous studies [72,120]. The volatile compound 2,5-dimethylpyrazine was found in a high abundance in all pulses (Figure 11e). Pyrazines have been reported to be the typical volatile compounds formed during the heating of food due to the Maillard reaction [121]. In general, the odour characteristics of pyrazines are described as cocoa, roasted, and nutty [70]. Pyrazines are considered as desirable food flavours that could potentially mask the beany flavour of pulses [72]. Flour from all roasted pulses had an increased abundance of 2-pentylfuran compared with the raw pulse flour and flour from boiled or germinated pulses (Figure 11e), and the compound dimethyl-disulfide was also identified in particularly high abundance in white beans after roasting. The abundance of 2-pentylfuran and dimethyl-disulfide was far below the total peak area of pyrazines; hence the unpleasant off flavour from these compounds might be masked.

Application of pulse flour in foods

In summary, the results presented in Paper I and II imply that pulse flour with particular functional properties, enhanced nutrient content, and low beany flavour can be obtained by utilizing the appropriate treatment technique before milling (Table 7). The results from this research indicated that flour from raw pulses are not suitable to be used in food products due to the high abundance of strong beany flavour compounds (Table 7). Furthermore, based on data by others, various treatments prior to milling (i.e., soaking, boiling, roasting and germination) are required to significantly reduce the anti-nutrient content in the pulses [43–45]. Hence, it is recommended using flour from treated pulses to ensure safety and diminish the undesirable beany flavour in pulse-based food products [122,123]. Nonetheless, flour from raw pulses might be used in limited food products, for example, pasta or extruded snacks [122,124].

Due to the high TDF content, flour from boiled pulses could potentially be used in the development of foods with a low glycaemic index and with a subtle beany flavour (Table 7). Anderson et al. [125] reported that a fixed-sized pizza meal made with flour from cooked pulses could give lower postprandial glycaemic response in healthy young men compared with the control meal made of whole-wheat. Flour from roasted pulses could be used in foods in which a nutty aroma is desirable (Table 7). For example, several studies have reported that incorporation of flour from roasted pulses (15-30%) in the formulation of various types of wheat bread improved the flavour and sensory acceptance of the product while having a minimal effect on the end product quality (volume and crumb structure) [126,127]. In addition, flour from roasted pulses could also fortify the total dietary fibre and folate content in the product.

The use of flour from germinated-steamed pulses as a single additive for gelation might be limited due to their poor gelling ability (Table 7). However, flour from germinated pulses can still be used as a fortification ingredient. For instance, in theory, incorporating flour from germinated-steamed peas by 5% in a plant-based spread formulation (initial TDF content 0.9 g/100 g and folate content 10.7 µg/100 g [128]) would increase the TDF content by 86–107% and the folate content by 62-115%.

Table 7. Effect of treatments on functional properties, nutrient content, and the volatile profile of pulses.

Treatment	Functional properties[1]	Nutrient content[2]	Volatile compound profile[3]	Food application[4]
None (Raw pulses)	Lowest WAC Highest EA, FC Lowest LGC	TDF 16-18 g/100 g db folate 73-245 µg/100 g db	High abundance of beany flavour compounds[4]	Limited use in food
Boiling	Lowest OAC ↑ WAC ↓ EA ↓ FC ↑ LGC	Highest TDF ↓ folate	↓ beany flavour compounds[4]	Bakery, dairy alternatives, snacks, soups, batters
Roasting	Highest WAC ↓ EA ↓ FC ↑ LGC	↑ TDF ↑ folate	↓ beany flavour compounds[4] ↑ pyrazines (desirable aroma)	Bakery, dairy alternatives, snacks, soups, custards
Germination (24h) & steaming	Highest LGC ↑ WAC ↓ EA ↓ FC	↑ TDF Highest folate	↓ beany flavour compounds[4]	Bakery, snacks Unsuitable for gel-based products

[1]For data for each parameter, refer to Figure 7. An upward arrow (↑) indicates an increased value or abundance compared with flour from raw pulses, whereas a downward arrow (↓) indicates a decrease value. Highest/lowest = highest/lowest value of all flour. WAC = water absorption capacity, OAC = oil absorption capacity, EA = emulsion activity, FC = foaming capacity, LGC = least gelation concentration, TDF = total dietary fibre.[2]The value for each nutrient refers to Figure 8. db = dry basis. [3]Beany flavour compounds = hexanal, 1-octen-3-ol, 2-pentylfuran, and 3,5-octadien-3-one. [4]Summarized from [106,123,126,127,129–132].

With respect to limitations, the antinutrient content and the bioavailability of nutrients in pulse flour were not investigated in the present study. For example, the phytate content in pulse flour could be of interest due to phytates ability to reduce the bioavailability of some essential minerals. Also, several studies have reported that phytates are less susceptible to heat treatment than other antinutrient compounds but are significantly reduced by bioprocessing [42] (Table 4). Therefore, further studies on the topics mentioned above would be of interest to provide further scientific data to motivate the use of pulse flour in food applications.

Protein isolates

Protein concentrates/isolates from soybeans are the main raw material of the vast majority of meat analogues in the market [13]. However, there has been an increased interest in using protein isolates from pulses to substitute soybean protein. Pulses are of interest due to their high protein content, low risk of allergenicity and non-GMO cultivation practice [13,101]. In the present study (Paper III), pilot scale-produced and commercial protein concentrate/isolates from pulses were analysed for proximate composition, water holding capacity, and thermal properties [133].

The protein content differed significantly between commercial and local faba bean concentrate/isolate (Paper III, Table 1). Protein isolate from local faba beans (FBI-local) was found to have the highest protein content (88% wb) of all samples. Commercial faba bean concentrates (FBC-com) had the lowest protein content (56% wb). This difference might be explained by the different fractionation techniques used. Protein isolates from the local pulses and commercial yellow pea protein isolate (YPI-com) were produced using a wet fractionation technique that yielded higher protein purity than the dry fractionation used for FBC-com (Figure 2), as also observed by previous studies [134,135]. The dry fractionation also resulted in a high carbohydrate (15% wb) and fibre content (10% wb) in FBC-com.

YPI-com and FBI-local had a negligible fat content, but YPI-local and FBC-com had a fat content of 3% wb (Paper III, Table 1). YPI-local and FBI-local were prepared using the same method, but there was a significant difference in the fat content, which might be due to a different fat location in the seed matrix [136] affecting fat retention during the extraction. The YPI-com, YPI-local, and FBI-local had around three-fold higher water holding capacity (3 mL/g wb) than FBC-com (1 mL/g wb). Similar findings have been reported by De Angelis et al. [135] for water holding capacity in dry- and wet-fractionated pea proteins. Proteins are more denatured during the wet isolation process than the dry fractionation, leading to changes in protein structure that might increase the exposure of hydrophilic groups, increasing the ability to hold water [54].

There were different numbers of endothermic peaks detected for yellow pea and faba bean proteins. YPI-local had three peaks (72 ºC, 89 ºC, 116 ºC) and only one peak was identified in FBI-local at 97 ºC. YPI-com had four peaks (67 ºC, 88 ºC, 102 ºC, 132 ºC), whereas FBC-com had three peaks (77 ºC, 95 ºC, 128 ºC) (Paper III, Appendix B, Figure A2). A different number of peaks obtained might be due to the different compositions of protein and non-protein components in each tested sample [137,138]. The endothermic peak at 88–89 ºC (yellow peas) and 95–97 ºC (faba beans) might correspond to the denaturation temperature of globulins [55,139]. The highest melting temperature for yellow pea protein was 132 ºC and for faba bean protein it was 128 ºC. So, to achieve a complete melt of the food mixture, which is critical in the production of meat analogues, the cooking temperature at the hottest zone in the extruder should be set above 132 ºC [76].

Development of pulse-based foods

Pulse-based cheese analogues

The preliminary trials using flour from boiled yellow peas were performed to determine the suitable amount of flour, water, and stabiliser in the formula. The preliminary trials showed that a firm and sliceable cheese analogue was successfully produced using a pulse flour to water ratio of 1:4 (w/w) and 1% (w/w) kappa-carrageenan (KCG) in the formula. This flour to water ratio and KCG concentration were used to prepare the final pulse-based cheese analogues from flour from boiled yellow peas (BYP), roasted yellow peas (RYP), boiled faba beans (BFB), and roasted faba beans (RFB).

The developed pulse-based cheese analogues had distinct differences in colour (Figure 11). The BYP cheese analogue, with the highest L*, a*, and b* value amongst the samples, was the most similar to Gouda cheese compared with other samples. Noticeable dark spots, caused by seed husks, could be observed in the BFB and RFB cheese analogues (Figure 12). Overall, cheese analogues made of flour from boiled pulses (BYP and BFB) were less red (lower a* value) but lighter and more yellow (higher L* and b* value) than the cheese analogues made from roasted pulse flour (RYP and RFB). This might be due to Maillard reactions during the roasting of pulses, leading consequently to a brownish coloration [140] of the cheese analogues.

In general, the chewiness and hardness of the cheese analogues were affected by both the pulse species (yellow peas or faba beans) and the type of preparation of the pulse flour (boiled or roasted) (Figure 13). The cheese analogues made from faba bean flour (BFB and RFB) had a higher hardness and chewiness than the samples made from yellow pea flour (BYP and RYP) (Figure 13). This might be due to the higher protein content in faba beans (26 g/100 g db) than in yellow peas (19 g/100 g db). The protein-protein interactions increase at a higher protein concentration, leading to the formation of a firmer gel [141].

Moreover, the cheese analogues made from flour from boiled pulses (BYP and BFB) had a lower value of hardness and chewiness than cheese analogues made from flour from roasted pulses (RYP and RFB) (Figure 13). This might be due to a different degree of starch gelatinization and protein denaturation caused by different treatments used to process the pulses before milling. Roasting uses hot air circulation and was done in a shorter time, possibly resulting in lower gelatinization of starch and denaturation of protein than hydrothermal treatment (such as boiling) [131,142]. Flour made from roasted pulses might have less gelatinized starch and denatured protein [131] than flour made from boiled pulses. Therefore, during the preparation of pulse-based cheese, the ungelatinized starch and undenatured protein fraction in roasted pulse flour started to gelatinize, denature and build a gel network upon cooling. Thus, it resulted in a harder gel texture than the samples made from flour from boiled pulses. No literature data on the texture characterisation of cheese analogues made from pulse flour is available.

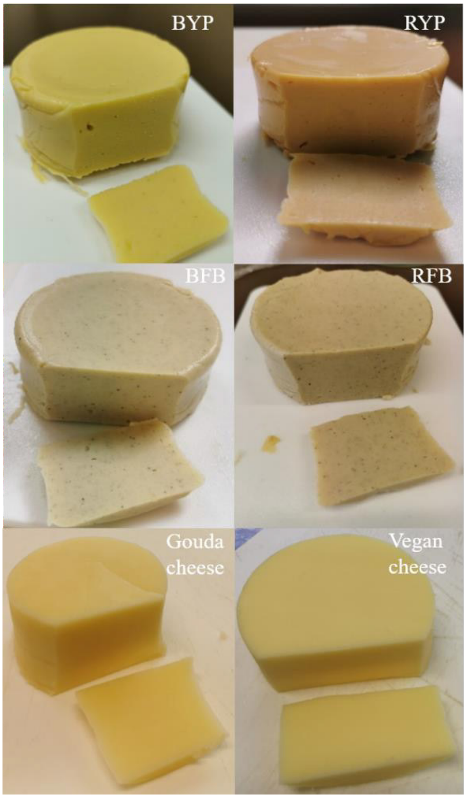

Figure 12. Pulse-based cheese analogues from boiled yellow pea flour (BYP), roasted yellow pea flour (RYP), boiled faba bean flour (BFB), and roasted faba bean flour (RFB), reference samples Gouda cheese and a commercial vegan cheese analogue.

All developed pulse-based cheese analogues had a greater hardness than (dairy) Gouda cheese. All pulse-based cheese analogues, and the referenced Gouda, were softer and less chewy than the commercial vegan cheese analogue (Figure 13). The harder texture of the pulse-based cheese analogues compared with the Gouda cheese might be due to a higher content of carbohydrate (starch) [143] and the use of kappa-carrageenan in the pulse-based cheese analogues [84]. The very high hardness and chewiness in the commercial vegan cheese analogue compared with other samples might be due to the high proportion of starch in the composition which immobilises the water in the matrix, forming a tough texture in the cheese analogue, as observed by Noronha et al. [143].

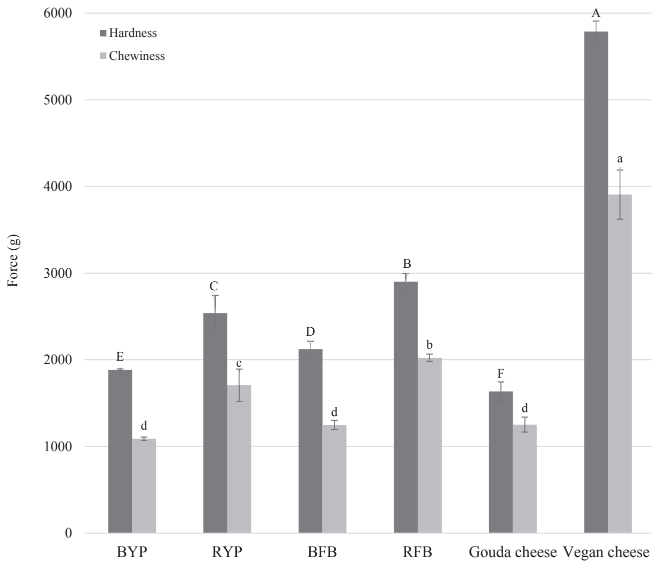

Figure 13. Hardness and chewiness of pulse-based cheese analogues (flour:water ratio 1:4 and 1% kappa-carrageenan) and references. Bars represent mean ± sd (triplicate analyses from two independent trials, $n = 6$). Different uppercase letters between samples indicate a significant difference (Tukey's test, $p < 0.05$) in hardness. Different lowercase letters between samples indicate a significant difference (Tukey's test, $p < 0.05$) in chewiness. Cheese analogues from boiled yellow pea flour (BYP), roasted yellow pea flour (RYP), boiled faba bean flour (BFB), and roasted faba bean flour (RFB).

Pulse-based cheese analogues had a 70-80% lower protein, a 60-64% lower fat, and had a three to four-fold higher total dietary fibre content (TDF) content than the Gouda cheese (Figure 14). The casein and fat in milk are concentrated during dairy cheese production, resulting in a high protein and fat content [144]. The TDF content in the developed cheese analogues was derived mainly from the pulse seed husks. The commercial vegan cheese analogue was made from coconut oil and starch. Therefore, it mainly consisted of carbohydrate and fat, without any protein, and an insignificant amount of TDF (Figure 14). The pulse-based cheese analogues could be labelled as high-fibre products according to European regulation (at least 6 g fibre/100 g product) [145]. Increased intake of TDF has been associated with risk reduction of cardiovascular disease, improved weight management, and enhanced growth of beneficial gut microbiota [33].

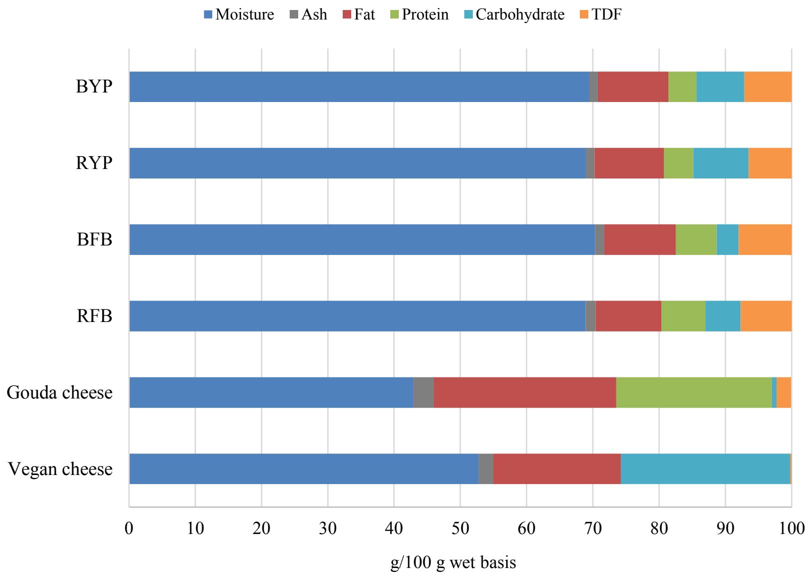

Figure 14. Proximate composition of pulse-based cheese analogues and references. The data for each nutrient is presented as mean (g/100 g wb, triplicate analyses from two independent trials, $n = 6$). Cheese analogues from boiled yellow pea flour (BYP), roasted yellow pea flour (RYP), boiled faba bean flour (BFB), and roasted faba bean flour (RFB).

High-moisture meat analogues (HMMA)

HMMAs with a fibrous layered structure from YPI-com were successfully produced using the target moisture content of around 67%, an extrusion temperature at 40-80-130-150 ºC (Z1-Z2-Z3-Z4), and a screw speed of 400, 600, and 800 rpm (Paper III) [133]. Some adjustments were made to enable the production of HMMAs from FBC-com using a target moisture content of 62%, an extrusion temperature at 40-60-130-150 ºC (Z1-Z2-Z3-Z4), and a screw speed of 400, 600, 800 rpm. These parameters were used to produce HMMAs from local pulse protein isolates. The fibrous structure of HMMA was judged using visual observation upon tearing the samples.

The production of HMMAs from YPI-local (81% protein wb) followed the extrusion parameters for YPC-com due to a similar protein content (79% wb). HMMA samples with prominent layered fibrous structures were successfully produced from YPI-local and FBI-local using the extrusion parameters summarized in Table 8. For FBI-local (denaturation temperature 97 ºC), the extrusion parameters used for YPI-com and FBC-com were initially applied, but there was no output flow and the cooling die was jammed. Therefore, the temperature in Z3 and Z4 was lowered in combination with a high screw speed (800 rpm) to allow a consistent flow. Lin et al. [146] summarized that proteins with a low denaturation temperature could easily be denatured and texturized by pressure and force in the extruder at a lower extrusion temperature. A similar mechanism might occur in the study (Paper III), possibly explaining why a fibrous structure formed even though the extrusion temperature was lower for FBI-local.

The commercial soybean HMMA had notably lower hardness and chewiness than any HMMA produced, as well as other references (chicken and beef) (Table 8). The HMMA made from yellow pea proteins had a similar hardness and chewiness as boiled chicken and beef. In contrast, all HMMA samples produced from faba bean proteins were harder and chewier than the references (Table 8). The crosswise cutting strength of HMMA made from YPI-com, YPI-local, and FBC-com (1427-2237 g) was generally higher than the lengthwise cut (1260-1813 g) (Table 8), meaning that the fibers were more textured in a longitudinal direction. On the other hand, the lengthwise cutting strength (3397 g) was higher than the crosswise strength (2423 g) in HMMA made from FBI-local (Table 8). Hence, the fibers were more aligned in a crosswise direction (parabolic pattern). This fiber pattern of HMMAs made from FBI-local was similar to the reference samples with a parabolic pattern (Table 8). HMMA made from FBI-local was extruded at 130 ºC (at the hottest region, Z4), whereas all other samples were extruded at 150 ºC and hence had fibers in a lengthwise direction. These results confirmed the findings of Osen et al. [76], who studied the extrusion of HMMA made from pea protein. Osen et al. argued that the melt viscosity decreases as the extrusion temperature increases, leading to a higher flow velocity at the core of the flow profile, resulting in more lengthwise-oriented fibers during solidification [76].

Table 8. Extrusion parameters, texture profile, and appearance of HMMAs produced from pulse protein concentrate/isolates[1].

Raw material	Extrusion parameters	Screw Speed (rpm)	Texture profile				Appearance
			Hardness (g)	Chewiness (g)	Cut cross (g)	Cut length (g)	
YPI-com	Target moisture content: 67%	400	2021 ± 164	2389 ± 123	2237 ± 47	1749 ± 55	
		600	1570 ± 94	1824 ± 147	1427 ± 70	1466 ± 163	
	Temperature: 40-80-130-150 ºC (Z1-Z2-Z3-Z4)	800	1818 ± 103	2131 ± 139	1623 ± 59	1260 ± 71	
YPI-local	Target moisture content: 67%	400	1805 ± 176	2177 ± 175	1846 ± 140	1451 ± 220	
		600	1796 ± 148	2252 ± 210	1828 ± 275	1780 ± 32	
	Temperature: 40-80-130-150 ºC (Z1-Z2-Z3-Z4)						
FBC-com	Target moisture content: 62%	400	3002 ± 222	3780 ± 160	1520 ± 51	1644 ± 61	
		600	3631 ± 21	4522 ± 74	1697 ± 27	1813 ± 61	
	Temperature: 40-60-130-150 ºC (Z1-Z2-Z3-Z4)	800	3339 ± 90	4009 ± 93	1788 ± 44	1547 ± 14	

[1]All values shown are mean ± SD (triplicate analysis, $n = 3$). YPI = yellow pea isolate, FBC = faba bean concentrate, FBI = faba bean isolate.

Table 8. continued.

Raw material	Extrusion parameters	Screw speed (rpm)	Texture profile				Appearance
			Hardness (g)	Chewiness (g)	Cut cross (g)	Cut length (g)	
FBI-local	Target moisture content: 62% Temperature: 40-60-110-130 ºC (Z1-Z2-Z3-Z4)	800	2309 ± 152	2648 ± 188	2423 ± 109	3397 ± 180	
Commercial soybean HMMA			408 ± 39	500 ± 45	1204 ± 119	1553 ± 52	
Boiled chicken			1679 ± 86	2332 ± 148	2102 ± 131	3207 ± 227	
Boiled beef			1087 ± 113	1645 ± 238	1821 ± 383	3743 ± 123	

With respect to raw material composition, the ash, fibre, fat and protein content, and water holding capacity of the protein concentrate/isolates were suggested to be one of the most important factors affecting the texture of HMMAs, according to partial least square analysis (Figure 15). HMMAs made from FBC-com had the highest hardness of all samples investigated (Table 8). FBC-com had the highest content of ash (mineral), total dietary fibre, and carbohydrate but had the lowest protein content compared with the other raw materials (Paper III, Table 1).

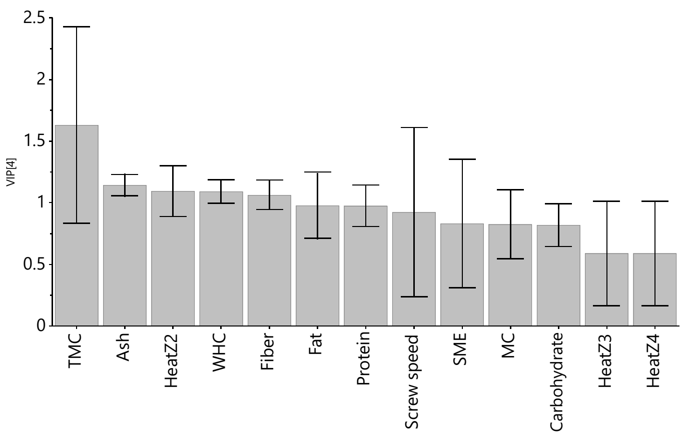

Figure 15. Variable of importance plot (VIP ≥ 1) showing the main variables affecting the texture of HMMA. TMC = target moisture content of HMMA, WHC = water holding capacity, MC = moisture content of protein concentrate/isolates. For complete multivariate analysis results, refer to Figure 4 in Paper III.

The disulphide bonds between proteins are suggested to play a main role in forming the texture of the meat analogue produced from soybean protein [147]. However, an essential role of minerals (divalent calcium ions) in the texture formation of casein-based meat analogue has also been reported [148]. This suggests that disulphide bonds might not be the only important factor in the texture formation of meat analogues and that there may be a contribution from ionic bonds. HMMAs made from protein concentrate/isolate from faba beans had a greater hardness than the samples made from yellow peas (Table 8). This difference might be explained by the higher mineral content in faba bean protein concentrate/isolate which contributes to more ionic bonds, hence increasing the hardness of HMMAs. Moreover, Pietsch et al. [149] explained that when the raw material has a high proportion of non-protein components (polysaccharide, ~30% db), the texturization of the meat analogue is more influenced by protein-polysaccharide interaction during extrusion. The high extrusion temperature might enhance the compatibility of the interaction between the protein and polysaccharides, resulting in a product with a firmer texture [150,151]; This is in line with the observed findings.

Furthermore, the target moisture content, extrusion temperature, and screw speed also influence the texture properties of HMMA, according to the multivariate analysis (Figure 15). There have been reports of similar findings [152,153] in which the hardness, chewiness, and cutting strength of HMMAs made from lupin and soy protein decreases with increased moisture content. The viscosity and internal temperature of food mix decreases as the moisture content increases, leading to less protein cross-linking and, therefore, a softer product [152,153]. The effect of different screw speeds on the texture of HMMAs was found to be inconsistent (Table 8 and Table A4, Appendix C, Paper III). At higher target moisture contents (69-70%), increased screw speed led to an increase in the hardness and cutting strength of HMMA, but at lower moisture contents (≤68%), there was an indistinct relationship between the screw speed and the texture of HMMA. On the other hand, Palanisamy et al. [153] reported that a higher screw speed (400-1600 rpm) increased cross-linking and polymerisation and thus increased the cutting strength of HMMA from lupin protein. These different observations might be due to differences in the raw materials and extrusion parameters used.

Application of pulse ingredients in cheese and meat analogues

Improved public awareness of the benefits of incorporating more plant-based foods in the diet has driven the growth of this food sector throughout Europe [73]. Plant-based meat and dairy alternatives are dominating the plant-based food sector [73]. Although there are several commercial soybean-based meat and cheese analogues on the market, there is consumer demand to see a greater selection in the store [73]. Thus, there is still ample room for product development in these food categories.

There is no reference available on the use of pulse flour to produce cheese analogues, other than a patent by Thresher [89]. Most published studies have focused on partial replacement of dairy ingredients with plant ingredients or the development of soft-cheeses and spread based on soybeans [86,154–157].

The results presented in Paper IV show that firm and sliceable cheese analogues could be produced using a mixture of pulse flour and water with a 1% addition of kappa-carrageenan to the formula. This result was in line with the findings of Hanáková et al. [156], who reported an improved (greater) hardness in the caseinate-based cheese analogue when 1% kappa-carrageenan was incorporated (Table 9). Furthermore, in Paper IV, flour from treated whole pulse seeds were utilized as the raw materials, in contrast to Oyeyinka et al. [157], who used filtered soy milk and discarded the fiber fraction. Thus, pulse-based cheese analogues could be categorised as foods with a high fiber content. To our knowledge, this is the first study on the development of pulse-based cheese analogues which has investigated both the textural and nutritional profiles of cheese analogues.

Table 9. Main findings of studies on cheese analogue production using plant ingredients.

Study	Ingredients and preparation method	Main findings
Oyeyinka et al. [157]	Water, soybeans, coagulant Coagulation of soymilk, draining	• Product: soy-based soft cheese • Nutrient profile: 20% wb protein, 7% wb fat, 0.3% wb dietary fibre • Sensory trial: acceptance score 7 out of 9

Hanáková et al. [156]	Water, casein, butter/vegetable fat, 1% stabilizer (kappa-carrageenan, iota-carrageenan, locust bean gum, arabic gum)
	
	Mixing of the ingredients, cooking, cooling
		• Product: casein-based cheese analogue
		• No nutrient profile
		• Texture properties: firm and sliceable
		  • Kappa-carrageenan was the preferred stabilizer
		  • Addition of 1% (w/w) kappa-carrageenan led to increased hardness in all samples compared with the control

Paper IV	Water, pulse flour, vegetable fat, 1% stabilizer (kappa-carrageenan) Mixing of ingredients, cooking, cooling	• Product: pulse-based cheese analogues • Nutrient profile: 4-6% wb protein, 10% wb fat, 7-8% wb dietary fibre • Texture properties: firm and sliceable • 1% (w/w) kappa-carrageenan was the optimum level to produce firm and sliceable cheese analogues from different pulse flour

However, the study in Paper IV has some limitations. For instance, the flavour aspect of pulse-based cheese analogues is not optimized yet. Flavour is an important sensory property and, together with texture and appearance, affects the quality of the cheese [158]. In dairy cheese, the flavour is formed due to the proteolytic activity of the starter culture during the ripening stage [159]. A future study could be considered to explore the possibility of incorporating starter cultures to enhance the flavour of pulse-based cheese analogues. This will complement the findings in Paper IV and hence providing a complete overview of the development of pulse-based cheese analogues. In addition, a consumer study, as performed in previous research [157] could be done to evaluate the sensory profile and product acceptance of the developed pulse-based cheese analogues.

Table 10. The main findings of studies of HMMA production using different pulse proteins.

Study	Materials	Main findings
Osen et al. [76]	Three commercial YPI (83-87% protein db)	• Target moisture content significantly affected HMMA texture • Lengthwise fiber formation increased with temperature at the last extruder zone
do Carmo et al. [160]	Commercial FBC (64% protein db)	• Target moisture content significantly affected HMMA texture • Lengthwise fiber formation increased with temperature at the last extruder zone • Higher extrusion temperature improved water/oil binding capacity and cooking yield of HMMA. • HMMA extruded at 130-140°C with ~54% target moisture content had better sensory profiles than other samples
Paper III	Commercial YPI (84% protein db) Commercial FBC (62% protein db) Local YPI (83% protein db) Local FBI (90% protein db)	• Ash, fiber, protein content, water holding capacity of protein material affected HMMA texture • Target moisture content, screw speed, and extrusion temperature affected HMMA texture • Lengthwise fiber formation increased with temperature at the last extruder zone • FBI (alkaline extraction) can also be used for HMMA production

YPI = yellow pea isolate, FBC = faba bean concentrate, FBI = faba bean isolate, HMMA = high-moisture meat analogue.

Results in Paper III were in line with the findings of Osen et al. [76] and do Carmo et al. [133] (Table 10), regardless of the different raw materials and extrusion parameters used. All three studies found that the target moisture content had a significant effect on the texture of HMMA, with a higher target moisture content resulting in a softer product. Moreover, HMMA samples with a predominant lengthwise fibrous structure were obtained when the temperature at the last zone of the extruder was increased to 140-160 ºC.

Contrary to other studies that used protein raw material from one pulse species, the present study investigated the feasibility of two protein raw materials (yellow peas and faba beans) in the production of HMMA, thus providing more complete data. There is no commercial protein isolate (>75% protein) from faba beans available in the market and, to our knowledge, this is the first study that has investigated the production of HMMA from faba bean isolate obtained through the alkaline extraction method.

Nonetheless, the present study (Paper III) has not investigated the effect of extrusion on the sensory properties of HMMAs, as demonstrated by do Carmo et al. [160] (Table 10). HMMAs obtained after extrusion require further formulation to produce a final product, such as plant-based chunks or shredded meat. Therefore, a future study on the sensory characterisation of HMMAs would be needed to determine the desired attributes for the final product. A descriptive sensory analysis, such as quantitative descriptive analysis (QDA), could be performed to obtain detailed information on product characteristics (e.g., texture, flavour, and appearance) [161].

Conclusions and further studies

The results of this research imply that it is possible to apply different methods (boiling, roasting, and germination-steaming) to process pulses before milling to obtain flour with specific functional properties, high nutrient content and less beany flavour. Flour from boiled and roasted yellow peas and faba beans are suitable for use in the production of cheese analogues with a firm and sliceable texture. The pulse-based cheese analogues can be categorised as functional foods due to a high dietary fibre content. Moreover, protein isolates from Swedish yellow peas and faba beans are suitable raw materials to produce meat analogues (HMMAs) with a fibrous layered structure. As shown in the results, the texture properties of HMMAs were significantly affected by the intrinsic properties of the protein concentrate/isolate as well as extrusion parameters.

This thesis has shown that ingredients produced from locally cultivated pulses in Sweden, i.e., flour and protein isolates, have a promising potential to be used as raw materials in developing plant-based foods such as meat and cheese analogues. In addition, these products could serve as a vehicle to raise the pulses consumption in Sweden, which is currently very low.

This research has some limitations and there are some aspects to consider that could be of interest in the future development of pulse-based ingredients or foods as stated below:

- The content of antinutrients, such as phytates, is of interest due to the heat resistant nature of this compound and its effect on the nutritional quality of pulse flour.
- Pulse flour and protein isolates have been shown to have a high nutrient content. Further investigation regarding the bioavailability of these nutrients is needed to provide complete scientific data to increase the use of pulse ingredients in foods.
- Flavour attributes are an important aspect determining the acceptance of a new product by consumers. Thus, flavour development and sensory studies on pulse-based cheese and meat analogues should be considered.

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