Technical Aspects of Plant-based Protein Coagulation for Cheese Analogues

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Tekniska Aspekten av Växtbaserade Protein Koagulering för Ost Analoger

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Sammanfattning

Den här rapporten visar på vikten av att utforska alternativa proteinkällor för att kunna producera hållbara och näringsrika ostanaloger. Att använda baljväxter som sojaböna och åkerböna kan vara ett viktigt steg mot att minska vårt beroende av animaliska produkter. Samtidigt är detta i linje med FN:s globala mål, utan att förflytta de planetära gränserna längre från de säkra gränsvärdena, utifrån dagens vetenskapliga underlag (Steffen et.al. 2015).

FN:s globala mål 12, *Hållbar Konsumtion och Produktion,* betonar vikten av att minska matsvinnet och produktionens avfall. Att komplettera traditionella produkter med alternativa, växtbaserade produkter kan vara en viktigt för att nå detta mål, och ostanaloger gjorda på baljväxt-protein kan bli en del av denna lösning.

Erhållna resultat visar att både sojabönor och åkerbönor innehåller proteiner som innehåller en komplett aminosyraprofil och en mängd olika bioaktiva föreningar som tros vara hälsofrämjande. Detta indikerar en stor potential för utveckling av nya proteinrika växtbaserade ostanaloger men det finns fortfarande mycket arbete att göra. Vidare forskning behövs för att förstå och övervinna de tekniska och nutritionella utmaningar som uppstår vid produktionen av baljväxtbaserade ostanaloger, som att uppnå de önskade smak- och textur profilerna. Emellertid visar denna rapport att det finns potential för baljväxtprotein som en viktig proteinkälla i framtiden som kan bidra till att uppnå FN:s globala mål för en hållbar framtid.

Abstract

This report highlights the importance of exploring alternative protein sources to produce sustainable and nutritious cheese analogues. Using legumes such as soybeans and fava beans can be a crucial step towards reducing our reliance on animal products. While also staying within the planetary boundaries, in line with the United Nations global goals.

UN global goal 12, *Responsible Consumption and Production*, stresses the importance of reducing food waste and production waste. Protein rich cheese analogues can be part of this solution by complementing traditional products with plant-based alternatives.

The results indicate that both soybeans and fava beans contain proteins that include a complete amino acid profile and a range of bioactive compounds that are believed to be health-promoting. This indicates a great potential for developing new protein-rich plant-based cheese analogues. However, there is still much work to be done. Further research is needed to understand and overcome the technical and nutritional challenges that arise in the production of legume-based cheese analogues, such as achieving desired taste and texture profiles. Nevertheless, this report shows that there is potential for legume protein to be an important protein source in the future and can contribute to achieving the UN's global goals for a sustainable future.

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Terminology

Anthropocentric	Considering human beings as the most significant entity of the universe.
Curd	A soft, white substance formed when “milk” coagulates, used as the basis for cheese.
Coagulum	A mass of coagulated matter.
Ecosystem services	Any positive benefit that wildlife or ecosystems provide to people.
The isoelectric point	Is the pH at which a particular molecule carries no net electrical charge.
Precursor	A substance from which another is formed, especially by the metabolic reaction.
Slurry	A semi-liquid mixture, typically of fine particles of manure suspended in water.
Tipping points	The point at which a series of small changes or incidents becomes significant enough to cause a larger, more important change.
Vegan	Contains no food or other products derived from animals.

Introduction

The plant-based food industry faces the challenge of responding to the increasing demand for innovative and nutritious plant-based products. Globally, dairy cheese is a widely consumed food and is produced in a variety of flavors, textures, and consumption varieties. Consequently, it's crucial for the non-dairy food industry to develop cheese analogues with a better nutritional profile that mimics the texture of dairy cheese. The saturated fat content of non-dairy soft cheese is usually high, 28% (Mefleh et.al. 2022). Therefore, producing alternative plant-based spreadable cheese products that can have the same fat content, but other preferable fat compositions, could give better nutritional values. Including lower content of saturated fat and higher amounts of unsaturated fat, such as monounsaturated and polyunsaturated would be advantageous (Mefleh et.al. 2022).

In fact, the majority of plant-based cheese analogues on the market could be nutritionally better. For the reason that today's plant-based cheese has low protein content, usually less than 5% protein, while dairy-based cheese has over 5% protein, varying between cheese brands (Mefleh et.al. 2022). An improvement in protein content could be achieved by using legume proteins. However, there is a lack of knowledge about the ability of these proteins to mimic the technological properties of the casein matrix. Legume proteins do not form a coherent protein network. Therefore, the non-dairy industry usually uses non-protein ingredients, e.g. modified or non-modified starches, gums, and hydrocolloids, to meet the structural requirements and stabilize the fat with starch.

However, the knowledge of using legume proteins to mimic the technological properties of the casein matrix is something that should be pursued. Today dairy production faces similar environmental and animal welfare challenges as meat production (Världsnaturfonden, WWF 2022). Milk production in Sweden, including organic, is based on profit-making principles, and the environmental impact is discussed (Norlander 2012). At the same time, ruminants are able to convert feed with little or no value for people to food important in human diets. Dairy cows can also benefit biodiversity as they can graze regularly. However, it's important to investigate the possibility of producing cheese analogues, which could be used as a complement to the human diet.

The aim of this report is to investigate two different plant-based protein sources to determine whether these alternatives can lead to cheese analogue. It also aims to map the existing knowledge on the ability of legume proteins to mimic the coagulation- and technological properties of the casein matrix.

Material and Methods

The Primo database was used for the literature search. To some extent, Google Scholar was also used. Search terms used in various combinations included: analogues, casein, cheese,

coagulation, environment, fava bean, milk beverages, nutrition, plant-based, properties, protein, and soybean.
The main objective of this literature review is to provide an overview of the existing knowledge on plant-based coagulation methods for the production of cheese analogues. Since the knowledge on this subject is limited, the report also highlights the importance of obtaining this knowledge. In addition, the literature review was narrowed down to two bean alternatives, soy- and fava bean, for cheese analogue production, and compared with methods for casein coagulation.

The beans were chosen due to the fact that soy drink is similar to milk in terms of protein and nutrient composition. In addition, fava beans have the potential to be a Swedish alternative to soybean.

In order to compare the environmental impacts and the nutrient content of the different beans more concretely, delimitations were also made here. This delimitation was made with the help of the Global Goals from the UN, starting from one selected goal, number 12: *Responsible Consumption and Production*. Global Goal 12 is described later to illustrate their relevance for the production of cheese analogues.

Literature Overview

Non-dairy Cheese Analogues on the Market

Alternatives to dairy products are not exactly similar to dairy products, but rather something entirely different. In this case, technology and components are major limiting factors. The proteins found in milk include casein and fats. It is important to note that proteins have a special structure. Curds based on dairy milk are formed when bacteria or enzymes react with proteins during cheese production. On the market, there are cheese analogues made from plant-based substances such as coconut oil, starch, water, beta-carotene, and flavors. Generally speaking, plant-based cheeses manufactured from coconut oil and starch are nutritionally inferior to those made from dairy milk because coconut oil and starch does not contain protein. Additionally, dairy cheese contains calcium naturally, whereas plant-based cheeses do not. However it's an added ingredient in plant-based cheese analogues (McClements and Grossmann 2022).

In recent years, vegan cheese alternatives have grown rapidly and different styles such as mozzarella, cheddar, pepper jack, etc. have begun to appear. A non-dairy cheese alternative available is generally classified into two categories based on the processing methods used to produce them (Bachmann 2001). In order to produce a starch-based cheese product, the ingredients are simply mixed and it results in shredded or sliced cheese analogues (Goldstein and Reifen 2022).

Cheese products made from nuts are typically sold as spreads or blocks and are produced similarly to dairy cheese, which involves fermentation and aging (McClements and Grossmann

2022). These two varieties of cheese analogues cover a part of the market demand, but they lack certain qualities that the consumers are seeking. Since starch is used to mimic the melting and stretching properties of dairy cheese, starch-based cheese analogues usually contain very little protein (McClements and Grossmann 2022). In terms of nut-based cheese analogues, it contains a certain amount of protein and nutritional value, but it has a very different texture compared to dairy cheese and which makes it unsuitable for people with nut allergies (McClements and Grossmann 2022).

Increasingly, consumers are seeking products that are high in protein, have dairy cheese-like textures, and are environmentally sustainable at the same time (Mefleh et.al. 2022).

Global Goal 12: Responsible Consumption and Production

Responsible consumption and production is one of the UN's global goals for 2030. Due to the finite nature of earth's resources, this is an essential goal. As humanity takes over the earth's natural resources and continues its development, the earth is becoming more and more anthropocentric. To ensure a secure future, Steffen and Rockström (2015) proposed a model based on nine planetary boundaries. Four of these limits have already been exceeded: climate change, loss of biodiversity, altered land use, and altered nitrogen and phosphorus biochemical flows (Steffen et.al. 2015).

The overexploitation of the earth's resources leads to a deterioration of the earth's resilience in the sense that the resources are exploited to such an extent that the resulting consequences occur and that the earth and nature do not have time to cope with them.This in turn leads to the condition of the earth being negatively affected and slowly but surely deteriorating. This is another thing illustrated in Rockström's model, Quadruple Squeeze, where the four "squeezes" all contribute to a deterioration of the earth's resilience (Rockström et.al. 2010). This means that it is increasingly difficult for the earth to recover from the consequences of man's continuous influence (Rockström et.al. 2010).

Loss of biodiversity is one of the four "squeezes" in the Quadruple squeeze model (Rockström et.al. 2010). This is due, among other things, to the fact that ecosystem services are necessary for human societies to develop. For example, it is about the production of ecosystem services such as food or wood that people receive from ecosystem, or about the support and regulation of ecosystem services such as photosynthesis and pollination, which are very important factors for the existence of human life (Rockström et.al. 2009).

Already 60 % of the most important ecosystem services are estimated to deteriorate, which will both affect the earth's resilience and which ecosystem services will be able to be used in the future (Rockström et.al. 2010). This shows the importance of preserving and restoring today's ecosystems and biodiversity and why this needs to be taken into account in food production and why alternatives in the human diet are needed.

To counteract this pressure, to which human anthropocentrism constantly contributes, a change needs to take place. This is in the form of a global change, among other things, in terms of people's production and consumption habits. Changes are required in order to not add additional pressure to the earth, changes that reduce carbon dioxide emissions, and an agricultural industry that better regulates the leakage of environmentally harmful substances.

More sustainable production and also consumption is therefore required. As humans are already consuming and producing in an unsustainable way, a continually growing population. The same production and consumption patterns would mean that the pressure on the earth is rapidly moving toward a completely unsustainable situation. Where tipping points replace each other and result in fatal consequences for life on earth with a domino effect (Steffen et.al. 2015).

The UN's global goal 12: *Responsible Consumption and Production* are about what is required to counteract these consequences, from the individual's role in the problem to what is required of global actors (Global Goal 12, 2015). In one aspect this can be accomplished by creating a cheese analogue that offers similar nutritional properties but emits less carbon dioxide than dairy cheese and can be a complement to the human diet.

Milk Traits and Technological Properties

Milk is a liquid product secreted by mammals where the milk in general has a pH between 6.6 to 6.7 (Walstra 2005). Its composition varies from species to species, but also between individuals. For human consumption bovine milk, buffalo milk, and milk from small ruminants are mainly used. Milk is an important raw material for various dairy foods worldwide and about 40% of Swedish cheese is made from Swedish milk (Från Sverige 2023).

The overall composition of bovine and small ruminant milk is similar in terms of energy, fat, saturated fat, carbohydrate, sugar, protein, and salt content. However, compared to buffalo milk, the overall composition and technological properties of bovine and small ruminant milk differ significantly. The reason is that buffalo milk fat and protein content is approximately 6 times respectively 1.5 times higher (Öja Gård 2023). Dairy products contribute significantly to daily calcium intake. A 100g serving of dairy cheese contains the entire daily calcium consumption, therefore two cheese slides (15g) provide around 15% (Arla Foods 2023).

Milk fat, lactose, and milk proteins are the most valuable compounds in milk. These fractions are important for cheese manufacturing. When making cheese the process includes removing the water part from the milk and concentrating the fat and protein. One of the important technological properties of milk is its ability to be coagulated.

Milk contains two main types of protein which are casein and whey. Casein proteins are the ones of most interest in the cheese manufacturing process (Fellow 2017). Caseins are organized in structures called micelles. There are four types of casein, alpha s-1, alpha s-2, beta, and kappa, that make up the micelles (Walstra 2005). Kappa casein is the most important during the cheese-making process. Kappa casein forms a chain surface around the casein micelle.

These parts of kappa casein are negatively charged meaning that the micelles are repelling each other.

In order to make cheese, the negatively charged part of the kappa casein has to be affected in order to form curd after the aggregation of micelles. Milk coagulation can occur in three different ways: enzyme coagulation, acid coagulation, and acid and heat coagulation. The last one mentioned is used for fermented products such as yogurt.

During enzymatic coagulation, casein micelles are modified by rennet hydrolyzed casein to a limited extent, then aggregated by calcium. In traditional methods, rennet is extracted from the calf abomasum and consists of two proteases chymosin and pepsin that make up the gastric tract. Additionally, there is microbial and vegetable rennet available, including *Rhizomucor miehei* are able to produce proteolytic enzymes, Galium species, dried caper leaves, nettles, thistles, and mallow, among others (Mefleh et.al. 2022).

Rennet enzymes hydrolyze the kappa casein on the micelle surface and remove the negatively charged part of the protein, which happens when chymosin cleaves the Phe_{105}- Met_{106} bond of kappa casein (Walstra 2005). There are three phases to the chymosin-induced coagulation of milk. During the primary phase, the enzymatic hydrolysis of kappa casein into para kappa casein and casein macropeptide occurs, with the hydrophilic casein macropeptide part being released into the whey. It results in a negatively charged group loss and decreased steric stability.

Walstra et al. (2005) demonstrate that reducing the colloidal stability of the micelles by 70 percent leads to the onset of spontaneous, secondary aggregation. Through hydrophobic bonds, molecular chains form a three-dimensional network, which further solidifies through calcium cross-linking. The third phase involves syneresis which indicates more contraction through cross-links to expel whey from the casein network. In order to enhance coagulation, pH must be reduced, calcium concentration must be increased, and temperature must be increased, aggregation cannot occur below 20 ˚C. However, the efficiency of syneresis increases with increasing temperature and pressure, for example when stirring.

Acid curd, acid-fixed, lactic curd, and lactic acid fixation are all terms that refer to the use of acid to curdle milk. By lowering the pH of milk, casein micelles' properties are altered during acid coagulation. Casein micelles aggregate as they approach the isoelectric point of pH 4.6 (Walstra 2005). The acid dissolves the calcium bonds from the casein micelles, by changing the surrounding charge. In this case, the goal is to neutralize the negative charge that surrounds the casein micelles. In milk, all these negatively charged micelles repel from each other because they all have a negative charge. Because of this charge, caseins are often called "polar." The addition of acid neutralizes the surface of the micelles, causing them to interact with each other and aggregate. Aggregation is loosely connected through a porous network (Walstra 2005).

Generally, fermented milk products are manufactured using milk that has undergone a severe heat treatment of 90˚C for 5 to 10 minutes. Which improves the properties of the milk as a substrate for the bacterial culture and ensures that the coagulum of the final end product will be firm (Walstra 2005). As whey denatures at a temperature in excess of 60˚C, its disulphide bonds either associate with the casein micelles or form soluble aggregates (Walstra 2005). As a consequence of the interaction between denatured whey protein associated with casein micelles and each other, the curd becomes viscose (Walstra 2005). This is because there are more and stronger bonds in the acid gel (Walstra 2005). The active involvement of denatured whey protein in structure formation will also result in a rise in the concentration of proteins in the gel network (Walstra 2005).

Plant-based Cheese Analogues

Cheese analogues can also be prepared entirely using plant-based ingredients. In order to produce cheese analogues, electrostatic repulsion between proteins must be overcome to induce gelation and curd formation. A thermal or isoelectric precipitation process is usually used to facilitate aggregation (McClements and Grossmann 2022). It is still necessary to conduct further research to find the best method and to increase the yield of cheese analogues products, even if the sensory characteristics of the cheese analogues are favorable (Mefleh et.al. 2022).

Soy and Coagulation of Soy Proteins

Soybean (*Glycine max)* is one of the most cultivated crops for the production of vegetable protein and oil. The majority of soy is used for animal feed in the production of meat, milk, eggs, and farmed fish. The soybean is energy-rich in dried form and contains about 40% protein, 35% carbohydrates, and 20% fat (Goldstein and Reifen 2022). This means that the soybean is well suited both as human as well as animal food and might be used as a base for cheese analogues that are more nutrient richer than the vegan cheese available on the market (McClements and Grossmann 2022).

Soybeans are primarily grown in tropical countries but also in the USA, Canada, and Ukraine to name a few non-tropical zones. However, soybeans are transported long distances before they reach the Swedish market. The emissions for one kilogram of dried soy transported by boat are approximately 0.6 kilograms of carbon dioxide equivalents (Goldstein and Reifen 2022). In order to reduce the environmental impact of soybeans, cultivation of soybeans would have to take place more locally, for example by Swedish farmers. However, it would entail modified varieties that might be difficult and inefficient production because the Swedish climate is not optimal for those origin crops (Goldstein and Reifen 2022).

In order for soybean protein to be used to make cheese analogues, it must be processed before they form a curd. The dried soybeans must first be soaked in water, then milled and cooked. The slurry is separated into a solid paste called okara and soy drink. Then salt, coagulants such as calcium and magnesium chloride, and sulphate are added to the soy drink to separate the curds from the water phase. In some cases, acid coagulants such as citric acid or glucono-δ-lactone (GDL) are used. The soy drink is poured into molds to allow the carbohydrate-containing water phase to drain (McClements and Grossmann 2022).

Traditionally, soybean curd is known as tofu. The industrial production of tofu uses GDL as a coagulant. Kohyama et al. (1995) reported that GDL is an acid precursor capable of cleaving gluconic acid in solution and then dissociating gluconic acid molecules to produce protons, which in turn lowers the pH of soy drink from a pH 7 to a pH between 4 and 5. When the pH approaches the isoelectric point (pH between 4 and 5) of the soy proteins, the random aggregation of the neutralized proteins leads to the formation of a stable gel through hydrophobic interactions and the formation of hydrogen bonds (Kohyama et al. 1995).

Fava Bean and Coagulation of Fava Proteins

The fava bean (*Vicia faba L.*) is an annual dicotyledonous legume that has been cultivated for millennia in Asia, Africa, the Mediterranean, and the Scandinavian region, but little is known about it and therefore it is hardly used in Western countries diets (Martineau-Côté et al. 2022). It is well adapted to different climates, including boreal types, and can therefore grow without problems in colder regions. Fava bean is energy-rich in dried form and contains about 25-35% protein, 40% carbohydrates which is mainly starch, and 1% fat (Goldstein and Reifen 2022). In addition to its high protein content and balanced amino acid profile, the fava bean contains bioactive components with health-promoting properties, including bioactive peptides and phenolic compounds (Martineau-Côté et al. 2022).

The emissions for one kilogram of dried fava bean cultivated in Sweden is approximately 0.16 kilograms of carbon dioxide equivalents (Goldstein and Reifen 2022). Therefore, it's emerging as a sustainable, high-quality plant protein source with the potential to meet the growing global demand for more nutritious and healthier foods.

For fava bean protein coagulation to occur the pH needs to be lowered to the isoelectric point, which is a pH between 5.0 to 5.5 (McClements and Grossmann 2022). This can be achieved by adding citric acid or glucono-δ-lactone. It can also be achieved by adding enzymes such as vegetable rennet or transglutaminase to the slurry to achieve a curd (Goldstein and Reifen 2022). However, there are technical challenges to the development of fava bean protein to form emulsion gel foods (McClements and Grossmann 2022).

The first challenge is the fact that fava beans contain about 40% starch, which can gelatinize during the protein denaturation phase of emulsion gel production (Jiang et al. 2020). During starch gelatinization, starch, and water are exposed to heat, which causes the starch granules to swell. As a result, the water is gradually and irreversibly adsorbed. This results in a viscous and transparent texture. Starch-based gels are thermoreversible, meaning that they do not melt when heated. However, heating can cause evaporation of the water and shrinkage of the gel. In addition, gelatinized starch forms gels that are sticky, pasty, or too stiff and differ from the texture of tofu or yogurt (McClements and Grossmann 2022). Another way to avoid this is to break down the starch enzymatically before using the slurry to produce cheese analogues (Jiang et al. 2020).

The second challenge is that dry fava bean flour contains less than 2% lipids, and aqueous processing lowers this concentration even further. For this reason, the standard amount of lipids for cheese analogues production is 3%, which is an obstacle to production (Jiang et al. 2020). However, this could be achieved by emulsion gels in which the acids are associated with the formation and structure of their components. For example, oil droplets can serve as fillers, and increasing the oil content increases the strength of the emulsion gels (Jiang et al. 2020).

Nutrient Density to Climate Index

In addition to the fact that these products contain different nutrients and are manufactured differently. Consequently, a calculation of the protein nutrient density for each calorie consumed was carried out (Appendix). Figure 1 illustrates the difference in protein content between these cheese analogues and dairy cheese in relation to the climate Index (CarbonCloud 2022). Since soybean is transported from Brazil in this particle example, the carbon dioxide equivalent is higher than for fava beans (CarbonCloud 2022). The carbon dioxide equivalent in Figure 1 also includes the processing of isolating the legume protein, and therefore, the value is higher than previously mentioned (CarbonCloud 2022). It is ideal to produce a cheese analogue with a high nutritional density and a low carbon dioxide equivalent. It should be near the x-axis and as distant as possible from the origin. Presently, no dairy cheese or cheese analogues are available in the danger zone due to low nutritional density and high carbon dioxide equivalent (McClements and Grossmann 2022).

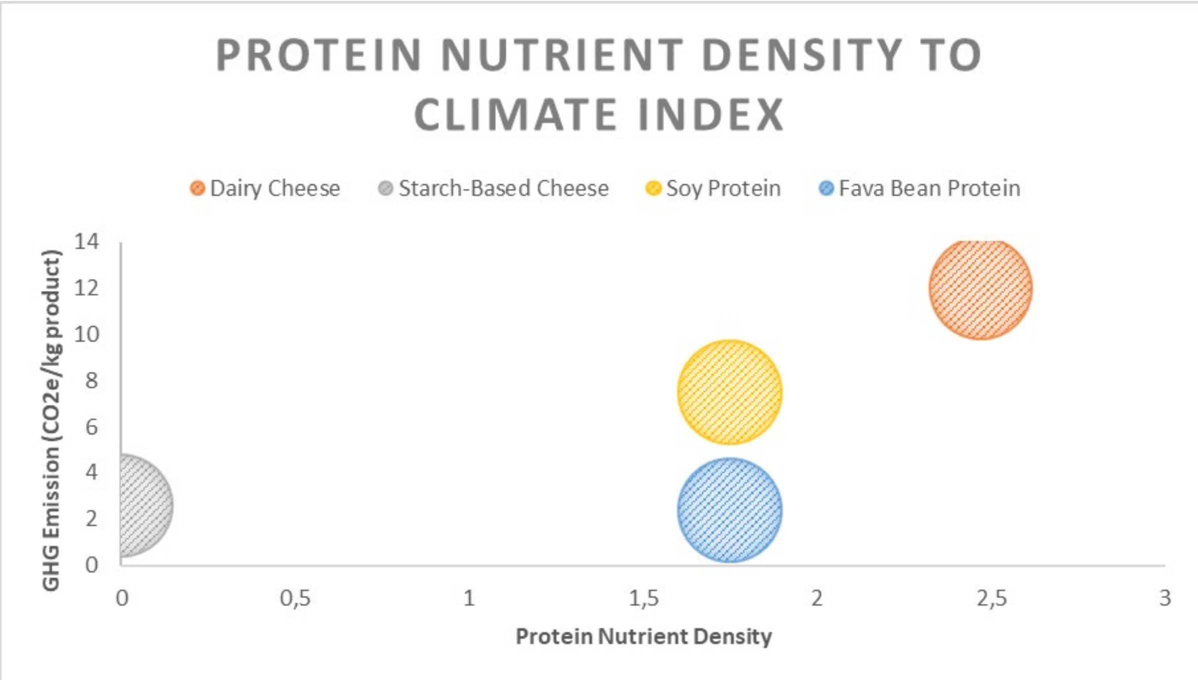

Figure 1: Protein Nutrient Density to Climate Index by Dana El Soudi

Discussion

Soy- and fava beans have proteins with a complete amino acid profile, are low in fat, and are free from saturated fat and cholesterol (Goldstein and Reifen 2022). They are an ideal source of protein to increase the nutrient density of foods. This makes them perfect candidates for the production of cheese analogues with similar protein content as dairy cheese (McClements and Grossmann 2022). However, the challenges lie in the technological limitations for the reason that dairy cheese methods do not apply to plant-based cheese analogues. Instead cheese analogues production on the market today are produced by completely different methods and ingredients than dairy products. Therefore, they are not comparable to dairy cheese (McClements and Grossmann 2022).

This report mentions that *Responsible Consumption and Production* is one of the seventeen global goals. The development of dairy-based alternatives is one of the parts of this process (Globala målen 2015). While alternatives to milk, cream, and yogurt are already available, cheese analogues are at a different stage of development (McClements and Grossmann 2022). The few products that exist do not yet have the wished properties, in the form of texture and meltability. In this case, it depends on the production method which does not use the casein protein from animal milk, and the ingredients do not contain casein either (McClements and Grossmann 2022). In contrast to dairy cheese, cheese analogs on the market are thickened and emulsified during the manufacturing process to achieve the same properties (Goldstein and Reifen 2022).

The main difference is that dairy cheese is composed of matrices between caseins and fat. Here the calcium bonds interact with caseins forming a cheese mass commonly called gel or coagulum (Walstra 2005). When using bean protein, a number of different factors must be considered to achieve the same properties as dairy cheese (Goldstein and Reifen 2022). Temperature, pH, processing conditions such as high pressure, and enzymes such as transglutaminase are some of the factors that make cheese analogues solidify, melt, and stretch (McClements and Grossmann 2022). The transglutaminase can be used to form covalent cross-links between legume proteins to increase the mechanical strength of the production, making it more similar to the texture of dairy cheese (Goldstein and Reifen 2022).

The pH of the slurry affects many of its physicochemical, biochemical, and sensory properties, including susceptibility to microbial growth, rate of chemical reactions, the solubility of ingredients, interaction with ingredients, texture, taste, and appearance (McClements and Grossmann 2022). For plant-based foods, such as cheese, it may be necessary to increase gel strength by adding cross-linking agents (Goldstein and Reifen 2022).

During the study, it has become very clear that casein is the component that gives dairy cheese its unique properties, and that's the hurdle for plant-based cheese analogues. Casein is found in mammals, but not in plant-based products. Taking advantage of microbial fermentation methods, the non-dairy company may be able to produce proteins with the characteristic of casein in a cost-effective plant-based dairy production method to meet the increasing demand for non-dairy cheese alternatives (Fellows 2017). Another group of scientists at Malmö University in Sweden is currently working on a way to mimic casein abilities with plant-based protein (Malmö University 2021).

Laboratory Scale Versus Industrial Scale

The most important functional properties the proteins need to have for plant-based cheese analogues are emulsification, gelation, and water holding (McClements and Grossmann 2022). However, proteins may also play an important role in the quality of the end product. Consequently, manufacturers should select the most appropriate legume protein, or combination of different legume proteins, to obtain the physicochemical, functional, and sensory attributes required (Goldstein and Reifen 2022). This often relies on having a good understanding of the protein solubility under different pH, ionic strength conditions, interfacial activity, and stabilization behavior, their thermal denaturation temperatures, their gelation properties, their capabilities to retain liquids, and their accessibilities to enzymatic modifications such as proteolysis and crosslinking (McClements and Grossmann 2022).

Fats and oils also play an important role in determining the physicochemical, functional, sensorial, and nutritional attributes of plant-based cheeses. In addition, they can act as solvents for nonpolar flavor molecules, which is important for designing the overall flavor profile of plant-based cheeses (McClements and Grossmann 2022). Ideally, the fats in plant-based cheese products should provide similar textural attributes as in dairy cheeses. It is therefore important to understand and control the solid fat content of plant-based cheese formulations to obtain the desired characteristics (Jiang et al. 2020).

At the moment the coagulation of plant protein for the production of soft cheese analogues seems to be able to occur but due to the limitation of understanding and knowledge in this area, the coagulation occurs randomly. For this reason, legume proteins from different legumes have different characteristics and work differently with additives. However, with the right amount of protein, carbohydrates, lipids, and other additives such as salt, pH controllers, crosslinking agents, and preservatives coagulation will occur (McClements and Grossmann 2022).

It is at the moment not possible to produce hard plant-based cheese analogues, as these analogues would be similar to tofu in texture and taste (McClements and Grossmann 2022). To be able to produce hard cheese analogues, a better understanding and methods to be able to control the coagulation process, maturation, and sensory properties of the plant-based protein are needed (Goldstein and Reifen 2022).

Depending on what kind of cheese analogues the consumer wants, there are different methods to form plant-based cheese curds (Walstra 2005). Therefore combined methods can be used to create plant-based gels with different textural and sensorial characteristics, which has important consequences for designing plant-based cheese analogues that range from soft to hard. It is important to investigate the mechanisms that are involved in plant protein aggregation to mimic the properties of casein gels, and thus be able to make cheese analogues.

Conclusion

The cheese analogues products are not made in the same way as dairy cheese. Instead, a different combination of starch and fat is used to try to imitate cheese. However, starch does not react to temperature changes in the same way as casein. This literature study focused on the possibilities of producing cheese analogues from legume proteins. It is clear from many different scientific papers that knowledge in this area is still limited. However, due to unsustainable production and consumption, it is important to find plant-based alternatives, especially if the world population wants to achieve global goals 12: *Responsible Consumption and Production,* in time.

Ongoing research has shown that it is possible to aggregate and coagulate legume proteins. We do, however, not fully understand exactly how aggregation occurs. Further research is thus necessary.

However, the knowledge e.g., where exactly a protease cleaves the amino acids in plant-based proteins aimed for the cheese analogues, might change the whole cheese analogues production. The conclusion is that it is possible to produce cheese analogues, but there is still a long way to go to produce high-quality end products.

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Electronic Source

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Appendix

Calculation for Protein Nutrient Density

According to the Swedish food agency, Livsmedelsverket the daily recommended protein is between 50-70 grams. (I choose to use 60 g in my calculation for the reason it is the middle value).

<u>Formula</u>:
Step 1: Divide the Nutrient (protein) amount by the RDA (Livsmedelsverket).
Step 2: Divide the calories amount of the product per 100 g by the total daily calories (2000 kcal).
Step 3: Divide the number from step 1 by the number from step 2.

Dairy Cheese:
25 g protein/ 60 g daily recommended protein = 0.4166
337 kcal/ 2000 kcal (total for a day)= 0.1685
0.4166/0.1685= **2.47**

Bean Cheese:
13 g protein/ 60 g daily recommended protein = 0.2166
248 kcal/ 2000 kcal (total for a day)= 0.124
0.2166/0.124= **1.75**

Starch-based Cheese:
0 g protein/ 60 g daily recommended protein = 0
270 kcal/ 2000 kcal (total for a day)= 0.135
0/0.135= **0**

Carbon dioxide equivalent

Values received from CarbonCloud.
Dairy cheese: 12 kg CO_2e//kg (Sweden)
Soy Protein: 7.5 kg CO_2e//kg (Brazile)
Fava Bean Protein: 2.4 kg CO_2e//kg (Sweden)
Starch-based Cheese: 2.6 kg CO_2e//kg (Sweden)