Title: Part A: Cheesemaker Cf77 potato starch interactions with Faba Bean legumin and pea protein isolates

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Abstract: This literature review is on the topic of currently available papers and studies conducted on potato starch interactions with faba bean and pea protein legumins. Including a detailed summary of individual molecules, of the said interactions, complexes formed, and how can they be useful. The main focus is on how these complexes could then be utilized in the formation of vegan cheese, and how then the probable product would taste. As such this review will include detailed summary of gelatinization and retrogradation process as they are pivotal processes used in the gel formation. Lastly gaps in the current literature will be identified with the focus on how the starch- protein complexes could be further utilized, and then next steps will be given on how to proceed with the information found.

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Introduction

Recently there has been a noted increase in vegan food production due to the growing movement of producing and developing more ecologically friendly animal food produce alternatives. As dairy industry is one of the largest parts of the food sector there has been a constant effort put into developing new vegan alternatives for cheeses. Vegan cheese currently available on the market does a good job at mimicking the taste and feel of their dairy counterparts but there's still a large gap between them. As such new methods and recipes for vegan cheeses are being constantly tested and developed. In dairy cheese, the main ingredient used is milk and in milk, the milk protein casein is the main component used in the coagulation of the cheese (McGee, 2004). Altering the quality and amount of the protein will affect the texture, the feel and the taste of the cheese produced. Alternatively, in vegan cheeses starches and vegetable oils or proteins are used as main components in the cheese making process (Moreau, 2024). As such understanding the functional behaviour of the interactions between the plant proteins and the starches is crucial for the formulation of new vegan cheese alternatives.

As stated previously due to the growing demand for the vegan cheeses, this literature review will research and summaries the available information on the interactions between starches and legumin protein isolates used in vegan cheese production, with the main focus on potato starches and legumin proteins, including, faba bean and pea protein which will be discussed due to the availability and nutrient value (The Nutrition Source, 2019).

Additionally, questions like

Why is Cf77 Potato starch of interest?

What makes legumin proteins a valuable protein source?

and how do starch- protein interactions influence vegan cheese properties including texture, mouth feel, and taste?

Will be further explored and answered. To do so efficiently this review is organized into five sections starting with basic knowledge on starches and proteins of interest, then following into starch-protein interactions which will cover overall interactions, and more specific interaction based on the source of the protein involved, Variables affecting the said interactions, Gaps in Literature and implications for food product development, finishing in the conclusion.

1. Basic Knowledge on Starchers and proteins of interest.

1.1 Starch Basics

Starch is a polysaccharide consisting of a large quantity of glucose subunits jointed together by a glycosidic bond (Zhiguang et al., 2025), with its chemical formula being (C6H10O5)n. It is the most common carbohydrate present in the human diet, largely because it is produced by most green plants, although starch used in industry usually comes from rice, wheat, potatoes and maize in a form of white odourless powder. In nature starch is stored in a form of semi-crystalline granules. The size of the granules varies depending on the plant, from 2μm in rice up to 100μm in potatoes (Malin Sjöö and Lars Nilsson, Chimiste, 2018). Starch synthesis consists of four steps, them being granule initiation, coalescence of granules, phase transition and expansion (Bürgy et al., 2021), staring with singular glucose subunits, which bind via α-1,4-glycosidic bonds forming a large polymer called amylose, which then can be further modified using Starch branching enzymes introducing α-1,6-glycosidic bonds into the glucose chains, resulting the creation of amylopectin.

Amylose is a polysaccharide consisting of α-D-glucose units bonded together through alpha 1- 4 bond as stated above. It is a compact molecule with around 200 to 1000 alpha glucose units, which bond to form a helical structure (Malin Sjöö and Lars Nilsson, Chimiste, 2018). Amylose makes up approximately 20 – 30% of the starch granule. Due to the said structure, it has high resistance to digestion.

Amylopectin is a water insoluble highly branched polysaccharide consisting of up to 2000 to 200,000 alpha glucose units (Caballero, 2016). Due to the alpha 1 – 6 bonds between the glucose units branching occurs. As such structure of the molecule can be split further into two helical chains. Chain A being the unbranched chain with no 1-6 alpha bonds, while chain B is a chain where the bonds occur and is containing the branches which further extend the molecule (Dona et al., 2010). Due to the larger size of this molecule amylopectin's make up approximately 70 to 80% of the starch granules mass. As a highly branched polysaccharide, when dissolved amylopectin has low tendency of retrogradation (Ning et al., 2025).

1.2 Cf77 starch specific.

Potatoes are one of the most grown and consumed plants on the planet. This is due to many differing factors but mainly can be attributed to the adaptability of the plant which can grow in many different climates, while still be high yielding, and due to the vegetables produced having a high nutrient value, consisting of a high amount of carbohydrates. Due to the large quantities of potatoes being produced, potato starch is one of the cheapest starches available. Potato starches consist of large granules, which are usually 30μm to 100μm in size (Wang et al., 2016), with minimal amount of protein or fats present giving the starch a neutral taste with minimal foaming tendencies. The amylose to amylopectin ratio of the potato starch varies greatly depending on climate and species ranging from 11.4% to 30.55% of amylose and 63.4% to 99% of amylopectin in waxy potato species (Tong et al., 2023). Due to this potato starch is highly versatile and can be used in many different products, depending on the result wanted.

One such starch is the cheesemaker starch (Cf77) which is a modified Potato starch. It is a non-GMO product and is made up of Modified potato starch and starch sodium octenyl succinate. It was primary made for the purpose of replacing the Casein protein

in cheese production (Quadragroup.store, 2025), and as such is a good starch to use in cheese making.

1.3 Legumin Proteins

Legumin proteins are a family of insoluble hexametric conjugated globular proteins. As previously stated legumin protein consist of two trimers with the whole protein having a total of six subunits with each subunit being around 50 to 60kDa and consisting of two chains. Alpha hydrophilic chain and a beta hydrophobic chain which are bound using a disulfide bridge (Bailey and Boulter, 1970). Legumin proteins can be obtained from leguminous seeds including but not limited to lentils, chickpeas, peas and beans. Legumin primary function is the storage of angiosperms and gymnosperms in seeds (He et al., 2021), Due to its function it can be considered as an analogous to the mammalian milk protein Casein, with its unofficial name being "vegetable casein" (Rezaei, Maryam Sadat Safavi and Seyed Abbas Shojaosadati, 2019). As such legumin protein is very nutritious food supplement, containing high amounts of low-fat protein and essential amino acids with the specifics varying depending on the plant (Maphosa and Jideani, 2017).

Due to the presence of hydrophobic units, the legumin is insoluble in water but can be dissolved if the pH is slightly above or below the neutral point (Srivastava, 2002).

1.4 Faba Bean

Faba/fava bean is a plant belonging to pea and bean families and as such can be classified as a legume. It is cultivated as both a main crop and a cover crop and is used widely as a food source for both humans and animals across the world. Fava bean is a highly nutritious food source and is often used as a food supplement (protein supplement) in a powdered form containing 58% carbohydrates and 26% proteins (legumin and vicilin), with numerous nutrients like manganese, iron and vitamin B also being included (Sharan et al., 2020).

Vicilin protein is a trimeric legumin associated protein, with the primary function of protecting the seeds from fungi and other pathogens (San et al., 2024). It is an Allergan and as such faba bean is known to cause similar allergic reactions to peanut in some humans (Barre et al., 2008).

1.5 Pea protein

Pea protein is a protein supplement, made using green or yellow split peas (*Pisum sativum)* (Sandberg, 2011). Due to the large amounts of peas being grown it is widely available, and depending on the production method used in the production of the supplement pea protein supplement can either have high protein concentration if wet fractionation is used or low if dry fractionation is used.

Pea protein is highly nutritious, containing up to 30% proteins including up to 20% albumins and 80% globulins (Lam et al., 2016) including both legumin and vicilin proteins (Burger and Zhang, 2019). Carbohydrates consist of around 65% of the food supplement with majority of them being pea starches (Nadathur, Janitha P D Wanasundara and Scanlin, 2017). In addition, fibre, minerals vitamins and plant sugars are also present in smaller amounts. And as such pea "protein" is a very nutritious food supplement, and in comparison, with faba bean it is much less allergenic (Lam et al., 2016).

Due to the high nutritional value, and variety of production methods used in the pea protein production, pea protein is widely used in food industry as emulsifier, foaming agents and/or thickener (Sandberg, 2011).

2. Variable affecting structure and Interactions of the molecules.

2.1 Factors affecting the structure of starch.

2.1.1. Gelatinization

Starch as a molecule is insoluble in water, but this does not mean it cannot interact with water. In the natural state, structure of starch as mentioned previously is composed of semi- crystalline granules, with different sizes and composition depending on the species and cultivation environment of the potato the starch was extracted from. In this form hydrogen bonding mainly occurs between the starch chains, and the starch granules are separate from each other. Due to the structural stability of the semi-crystalline structure of starch external variables are needed for the starch to start interacting with water. One such variable is heat If the temperature of the mixture the starch is in reaches high enough the tight helical structure of the granules will loosen and this will allow water to interact with the glucose molecules inside. This leads to the water being absorbed by the granules. This process causes the structure of the granule to become more random leading to the loss of the granule uniformity. As such the granules will start to swell. The swelling of the granules damages the structure leading to eventual leaching of amylose into the surroundings. The process ends with the granule disintegrating (Zobel, 1988). The temperature required to start the gelatinization process differs according to the starch which was used as shown in Figure 1. Figure 1 also show that the higher the amount of amylose there is in starch the higher the temperature required for reaction to start.

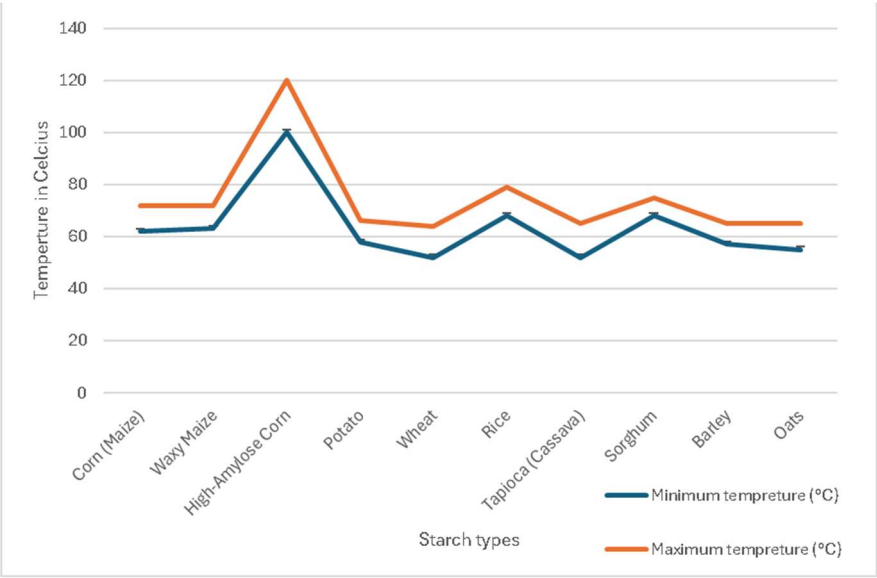

Figure 1: Temperature requirements for the process of Gelatinization to happen according to each starch type.

Temperature isn't the only factor affect this process. Amount of water is another factor. For the process to continue successfully, total volume of water needs to be equal to at least 30% of starches weight (Altay and Gunasekaran, 2006). The gelatinization may be partly successful if the volume of water is lower that the given number, if there is too little water dextrinization will take place.

Another factor affecting Gelatinization is pH. In acidic conditions hydrolysis will occur leading to the swelling of the granules to decrease, which results in thinner and weaker gel. In Alkaline conditions the temperature required for the gelatinization process to occur will increase but the starch may denature, resulting in a thinner or no gel (Slade

and Levine, 1987). The ideal pH for the gelatinization would vary depending on starch but it should be in the 6 to 7 pH range.

2.1.2 Retrogradation

Retrogradation is a process in which the now gelatinized starch will start to cool down. The process can take a long time depending on and the amount of starch used. During the said process the structure of the starch will slowly start to return to uniformity. Although the granules will usually not reform if the process was fully completed, a gel will form instead (Wang et al., 2015). During the process small amount of water will be expelled from the solution in the process called syneresis. Viscosity of the gel again depends on the starch. The higher the amylose percentage the stronger the gel is, and the more water will be expelled (Eliasson and Larsson, 1993).

2.2 Factors affecting the structure of legumin Proteins and their compatibility with potato starch.

2.2.1 Temperature

Temperature naturally causes proteins to denature, but if the process doesn't finish foam/gel can be formed from the said protein. This happens when the proteins begin to unfold and in a similar fashion to starches turn to gels or precipitate, this process Hower is irreversible if the temperature is too high. For pea proteins this temperature range spams from 60 °C to 85 °C, while for faba bean it ranges from 65 °C to 85 °C. Both protein supplements contain legumin proteins but the concentration of the legumin proteins is higher in faba beans as pea protein has more vicilin, as such faba bean protein is more stable in comparison to pea protein (O'Kane et al., 2005). Due to this to combine both the legumin proteins (either faba bean or pea protein) with potato starch a protein needs to be picked which will not denature before the process of gelatinization will finish, therefore the temperature needs to be lower than 64 Celsius (Liu, et al., 2020) as this is the maximum temperature specified for potato starch.

2.2.2 pH

Again, the pH can cause denaturation of the proteins while both type of legumin proteins function normally at around 6 to 8 pH. Both proteins start to aggregate at around 5 pH while if the pH exceeds 8, they tend to hydrolyse which would break down the gel (Schutyser et al., 2015). Therefore, the pH would need to stay above 6 so the proteins form gels and do not aggregate. In a similar fashion potato starch also is stable at 4 to 8 range (Evans and Haisman, 1982).

2.2.3 Salts

Salts like Naci, phosphates and divalent salts depending on their concentration tend to affect the structure and the proper protein function. At low concentrations both pea protein and fava bean protein are shown to be more stable, but if the ionic strength in the solution increases the solubility and emulsification capacity tends to increase also. If the ionic strength is too high the protein aggregates. Though divalent cations can promote gelation or aggregation depending on the temperature (Chambers, Carr and Lambert, 1990). While for potato starch the higher the salt concentration the more stable the starch is, which includes the divalent cations conversely, modified potato starches can have varied responses to salt levels. Though phosphates can

destabilise the starch gel and lead to increased syneresis (Evans and Haisman, 1982).

2.3 Buffers

If a study conducting an inve3stagtion into interactions between potato starches and pea proteins were to be made, distilled water would be a bad buffer for starches, legumin protein would be able to be dissolved in it but if the study were to focus on interactions between the two molecules the water would not be the optimal choice. Phosphate-Buffered Saline (PBS) buffer would be a better choice as it encourages gel formation in right conditions, as it contains salts which can both stabilize the molecules and help in gelatinization. DMSO would also be acceptable, but as it is toxic it is a secondary option, as PBS is usable, and safer to use.

3. Starch-Protein Interactions

3.1 General Mechanisms of interactions

There are five general interactions which can happen between a starch and a protein. First interaction type is hydrogen bonding. Due to the many polar groups found on the protein and because starch is made up of glucose subunits which all contain hydroxyl groups, hydrogen bonds/polar bonds are one weak but one of the most numerous bonds which can form between the two molecules. Due to the shear amount of the said bonds even if weak individually as the whole this type of interaction is very significant. Hydrogen bonds help in the stabilization of the complexes between the starches and the proteins and appear more commonly when the two molecules start the gelatinization process (Singh, Kaur and McCarthy, 2007).

Second type of interaction found can be the electrostatic interactions proteins usually contain charged regions due to differently charged amino acids residues found on their surface. These charged residues can interact with charged starch residues. Starch can be further modified to be more, or less charged, to facilitate stronger or weaker bonds. These bonds can reduce charges of the two molecules which can help and increase the rate of aggregation (Singh, Kaur and McCarthy, 2007).

Thirds type of interaction is a hydrophobic interaction. As starch is a hydrophilic when the glucose subunits are exposed to water, if protein contains hydrophobic amino acids residues this could facilitate a hydrophobic interaction between them. If so a complex between the two molecules can be made, though this type of interaction is the weakest one so far, and as such can be broken quite easily (Duodu and Emmambux, 2018).

Fourth interaction type is a physical interaction. In this case proteins which are much smaller can be trapped in the starch granules as they swell during the gelatinization. As such when trapped so other interactions may occur between the two molecules (Wu et al., 2025). All previously mentioned interactions are general interactions which could be found between any starch and most of protein molecules, that being said majority of them require the starch granules to lose their uniformity and be more open, therefore conditions need to be right for them to take place, like higher temperature, slightly altered pH and differing salt levels.

3.2 Potato Starch and Pea Protein Interactions

Due to the ratios of legumin protein found in pea protein there are specific interactions which pea protein does with potato starches. As pea protein has a relatively high hydrophobicity it can form many hydrogen bonds with the starch granules. This in turn

improve the stability between the forming starch-pea protein complex, which can result in a formation of a firmer gel with tougher texture after gelatinization is finished, though process can be impacted by pH temperature and salts content present in the solution (Yang et al., 2024). Due to the formation of the complexes the resulting gel has a higher water retention capacity, with a decreasing syneresis of the gel.

3.3 Potato Starch and Faba Bean Protein Interactions

Fava bean proteins have slightly different ratio of legumin to pea proteins, due to the resulting differences, faba beans can form stronger protein to protein hydrogen bonds instead of protein to starch bonds resulting in firmer and more elastic gels in comparison to pea protein ones (Cao, Meinou Corstens and Schroën, 2024). Due to this change Fava bean protein requires more strenuous processing when combining with potato starch, requiring higher temperature, and due to the antinutrients present enzymatic treatments might be necessary, to remove the bad flavours (Arroqui et al., 2024).

4. Gaps in Literature

Even with the extensive studies in this topic due to the nature of it and it's necessity, there are still many gaps in the literature, Firstly There are not many studies found which focus on modified potato starches, nor are there many studies which focus exclusively on either legumin protein or vicilin protein, as majority of the studies focus on bulk proteins found in peas or in faba beans, and their compatriots. These studies if conducted could really help to understand how each of the said proteins closely interacts with starches and how the complexes are formed in detail. Secondly there are studies focused on physically characterising and the properties of the proteins, but there are not many studies found which include characterizing on the molecular scale the interactions between the protein and the starch which could again further the understanding of the complex formation. Thirdly there is a gap on understanding on the thermodynamic behaviour of the complexes formed between protein and the starch. Fourthly the role of legumins in other alternative foods creations like plant-based meat analogues is poorly understood due to the lacking number of studies on this topic especially so specifically on the high moisture extrusion process. This is important as starch-legumin complexes can not only be used in cheese formation but in other foods as well which should be research as there should always be alternative food sources available just in case. Another gap in the literature is on the usage of legumin -starch complexes in delivery systems and how would the process work, either in emulsion stabilization or encapsulation. Additionally, there is a lacking number of digestive studies on the legumin- potato starch complexes. And lastly there has been close to no studies on the Cf77 potato starch, neither it's structure nor how it behaves when interacting with legumin proteins.

5. Implications for Food product development

A) Nutrient	Faba Bean Protein	Pea Protein
Protein quality (PDCAAS)	~0.65–0.75	~0.75–0.82
Lysine	High	High
Methionine & Cysteine	Low (limiting amino acids)	Low
Anti-nutrients	Contains vicine/convicine (removed in isolates)	Phytates, saponins, lectins

B) Feature	Faba Bean	Pea
Flavour	More pronounced, slightly bitter/earthy	Milder, but still beany/earthy
Aftertaste	Can be metallic or grassy if untreated	Less intense than faba bean
Color	Slightly lighter	Light yellow to beige

Table 1: A) Showing the nutrient profile of the legumin proteins. B) Showing the sensory characteristics.

A) Nutrient	Potato Starch
Calories	~330–350 kcal per 100 g (mostly carbohydrates)
Protein	Very low (typically <0.5%)
Fat	Negligible (~0%)
Carbohydrates	~85–90%, primarily amylopectin and amylose
Fiber	None (not a source of dietary fiber)
Resistant Starch	Present in raw potato starch (~50–60%) — acts like dietary fiber (RS2)
Glycemic Index (GI)	High when cooked (gelatinized), Low in raw form due to resistant starch
Micronutrients	Trace amounts only; not a significant source

B) Feature	Potato Starch
Flavour	Very neutral — bland taste with almost no flavor
Odour	Odourless when pure; may have slight earthy note if not refined well
Color	Typically white to off-white; high-purity starch is bright white
Texture	Smooth, slippery, gives a glossy, clear gel when cooked
Aftertaste	None when purified; low-grade starch may have a faint earthy or potato aftertaste

Table 2: A) The nutrient profile of the potato starch. B) The sensory characteristics.

One of the aims of this literature review was to verify and identify how compatible and allegeable were the legumin proteins and potato starches for the creation of vegan cheese. As shown in Table 1 and Table 2, all ingredients researched are very nutritious, especially the pea and faba bean proteins, though pea protein has a slightly higher protein quality. While looking at the sensory characteristics Again pea protein has a cleaner pallet, making it easier to season. According to the previous information obtained Faba bean forms firmer and stronger gels due to the larger amount of legumin protein present while Pea protein forms softer gels. Therefore, from taste perspective Pea protein is better, but the cheese formed using it and the potato starch might be too soft while the faba bean protein would help with getting the cheese texture correct but might bring unpleasant flavour to the cheese. Bearing into account the fact that the Cf77 potato starch was specifically modified for cheese creation, more specifically a chewy cheese for pizzas This problem might be moot, and if the pea protein is added another healthier alternative to dairy cheese could be made.

6. Conclusion and Next steps

In conclusion formation of vegan “cheese” analogue is highly likely to be possible using the pea protein or fava bean protein and Cf77 modified potato starch. From the information gathered the pea protein might be a better choice as it is easier to process and has a better flavour. The legumin- potato starch complexes are known and understood but further research into how they behave would be recommended, along with how such complexes could be utilised further. In regard to next steps, a study on the interaction between the pea protein and the cf77 modified potato starch should be conducted, to check the validity of how likely a gel could be made using the to molecules and how such gel, and then the said gel behaviour.

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References/ Bibliography:

Altay, F. and Gunasekaran, S. (2006). Influence of Drying Temperature, Water Content, and Heating Rate on Gelatinization of Corn Starches. Journal of Agricultural and Food Chemistry, 54(12), pp.4235–4245. doi:https://doi.org/10.1021/jf0527089.

Arroqui, C., Noriega, M.J., Ibañez, F.C., Milagro, F.I., María José Beriain and Paloma Virseda (2024). Impact of a Dual Treatment on Resistant Starch Level and Techno-Functional Properties of Pea and Faba Bean Flours. *Starch - Stärke*, 77(2). doi:https://doi.org/10.1002/star.202400111.

Bailey, C.J. and Boulter, D. (1970). The Structure of Legumin, a Storage Protein of Broad Bean (*Vicia faba*) Seed. *European journal of biochemistry*, 17(3), pp.460–466. doi:https://doi.org/10.1111/j.1432-1033.1970.tb01187.x.

Barre, A., Sordet, C., Culerrier, R., Rancé, F., Didier, A. and Rougé, P. (2008). Vicilin allergens of peanut and tree nuts (walnut, hazelnut and cashew nut) share structurally related IgE-binding epitopes. *Molecular Immunology*, 45(5), pp.1231–1240. doi:https://doi.org/10.1016/j.molimm.2007.09.014.

Burger, T.G. and Zhang, Y. (2019). Recent progress in the utilization of pea protein as an emulsifier for food applications. *Trends in Food Science & Technology*, 86, pp.25–33. doi:https://doi.org/10.1016/j.tifs.2019.02.007.

Bürgy, L., Eicke, S., Kopp, C., Jenny, C., Lu, K.J., Escrig, S., Meibom, A. and Zeeman, S.C. (2021). Coalescence and directed anisotropic growth of starch granule initials in subdomains of Arabidopsis thaliana chloroplasts. Nature Communications, [online] 12(1), p.6944. doi:https://doi.org/10.1038/s41467-021-27151-5

Caballero, B. (2016). *Encyclopedia of food and health*. [online] Amsterdam: Academic Press. Available at: https://www.elsevier.com/books/encyclopedia-of-food-and-health/caballero/978-0-12-384947-2.

Cao, J., Meinou Corstens and Schroën, K. (2024). Interfacial and Bulk Properties of Potato and Faba Protein in Connection with Physical Emulsion Stability at Various pH Values and High Salt Concentrations. *Foods*, 13(23), pp.3795–3795. doi:https://doi.org/10.3390/foods13233795.

Chambers, S.J., Carr, H.J. and Lambert, N. (1990). An investigation of the dissociation and denaturation of legumin by salts using laser light scattering and circular dichroism spectroscopy. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1037(1), pp.66–72. doi:https://doi.org/10.1016/0167-4838(90)90102-l.

Dona, A.C., Pages, G., Gilbert, R.G. and Kuchel, P.W. (2010). Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers*, 80(3), pp.599–617. doi:https://doi.org/10.1016/j.carbpol.2010.01.002.

Duodu, K.G. and Emmambux, M.N. (2018). CHAPTER 11. Starch–Protein and Starch–Lipid Interactions and Their Effects on the Digestibility of Starch. *Food chemistry, function and analysis*, [online] pp.218–234. doi:https://doi.org/10.1039/9781788012799-00218.

Eliasson, A.-C. and LarssonK. (1993). *Cereals in breadmaking : a molecular colloidal approach*. New York: M. Dekker.

Evans, I.D. and Haisman, D.R. (1982). The Effect of Solutes on the Gelatinization Temperature Range of Potato Starch. Starch - *Stärke*, 34(7), pp.224–231. doi:https://doi.org/10.1002/star.19820340704.

He, Z., Mattison, C.P., Zhang, D. and Grimm, C.C. (2021). Vicilin and legumin storage proteins are abundant in water and alkali soluble protein fractions of glandless cottonseed. *Scientific Reports*, 11(1). doi:https://doi.org/10.1038/s41598-021-88527-7.

Lam, A.C.Y., Can Karaca, A., Tyler, R.T. and Nickerson, M.T. (2016). Pea protein isolates: Structure, extraction, and functionality. Food Reviews International, 34(2), pp.126–147. doi:https://doi.org/10.1080/87559129.2016.1242135.

Liu, H., Wang, S., Wang, J. et al. (2020). An insight into the ultrastructural and physiochemical characterization of potato starch: a review. American Journal of Potato Research, 97(2), pp. 131–147.

McGee H (2004) [1984]. "Milk and Dairy Products". On Food and Cooking: *The Science and Lore of the Kitchen (2nd ed.)*. New York: Scribner. pp. 7–67.

Malin Sjöö and Lars Nilsson, Chimiste (2018). *Starch in food : structure, function and applications*. Duxford: Woodhead Publ

Maphosa, Y. and Jideani, V.A. (2017). The Role of Legumes in Human Nutrition. Functional Food-Improve Health through Adequate Food. [online] doi:https://doi.org/10.5772/intechopen.69127.

Moreau, E. (2024). *What in the World is Vegan Cheese, Anyway? Can it Actually Replace 'Real' Cheese?* [online] Organic Authority. Available at: https://www.organicauthority.com/recipes/what-is-vegan-cheese-made-of.

Nadathur, S.R., Janitha P D Wanasundara and Scanlin, L. (2017). *Sustainable protein sources*. London Academic Press.

Ning, J., Fu, B., Tang, X., Hao, Y., Zhang, Y. and Wang, X. (2025). Starch retrogradation in starch-based foods: Mechanisms, influencing factors, and mitigation strategies. *International Journal of Biological Macromolecules*, [online] 300, p.140354. doi:https://doi.org/10.1016/j.ijbiomac.2025.140354.

O'Kane, F.E., Vereijken, J.M., Gruppen, H. & van Boekel, M.A.J.S., 2005. Gelation behavior of protein isolates extracted from 5 cultivars of *Pisum sativum* L. *Journal of Food Science*, 70(2), pp. C132–C137. doi:10.1111/j.1365-2621.2005.tb07073.x

Quadragroup.store. (2025). *Quadra*. [online] Available at: https://www.quadragroup.store/products/cheesemaker-cf77.

Rezaei, L., Maryam Sadat Safavi and Seyed Abbas Shojaosadati (2019). Protein Nanocarriers for Targeted Drug Delivery. *Elsevier eBooks*, pp.199–218. doi:https://doi.org/10.1016/b978-0-12-814031-4.00008-8.

The Nutrition Source. (2019). *Legumes and Pulses*. [online] Available at: https://nutritionsource.hsph.harvard.edu/legumes-pulses/.

Sandberg, A.-S. (2011). Developing functional ingredients: a case study of pea protein. Functional Foods, pp.358–382. doi: ://doi.org/10.1533/9780857092557.3.358.

San, S., Cherian, R. M., Rabil, A., Souparnika Pamban, Nair, B. G., Pai, J. G., & Nair, S. S. (2024). Vicilin, a chitin binding protein from Sesbania grandiflora: Structural insights and functional potential as an antifungal agent. *International Journal of Biological Macromolecules*, 290, 138850–138850. https://doi.org/10.1016/j.ijbiomac.2024.138850

Schutyser, M.A.I., Pelgrom, P.J.M., van der Goot, A.J. and Boom, R.M. (2015). Dry fractionation for sustainable production of functional legume protein concentrates. Trends in Food Science & Technology, 45(2), pp.327–335. doi:https://doi.org/10.1016/j.tifs.2015.04.013.

Sharan, S., Zanghelini, G., Zotzel, J., Bonerz, D., Aschoff, J., Saint-Eve, A. and Maillard, M. (2020). Fava bean (Vicia faba L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), pp.401–428. doi:https://doi.org/10.1111/1541-4337.12687.

Singh, J., Kaur, L. and McCarthy, O.J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review. *Food Hydrocolloids*, 21(1), pp.1–22. doi:https://doi.org/10.1016/j.foodhyd.2006.02.006.

Slade, L. and Levine, H., 1987. Recent advances in starch retrogradation. In: D. French, ed., Industrial Polysaccharides: *Genetic Engineering, Structure/Property Relations and Applications*. New York: Elsevier, pp.387–430.

Srivastava, L.M. (2002). *Plant Growth and Development*. Elsevier.

Tong, C., Ma, Z., Chen, H. and Gao, H. (2023). Toward an understanding of potato starch structure, function, biosynthesis, and applications. *Food frontiers*, 4(3). doi:https://doi.org/10.1002/fft2.223.

Wang, C., Tang, C.-H., Fu, X., Huang, Q. and Zhang, B. (2016). Granular size of potato starch affects structural properties, octenylsuccinic anhydride modification and flowability. *Food Chemistry*, 212, pp.453–459. doi:https://doi.org/10.1016/j.foodchem.2016.06.006.

Wang, S., Li, C., Copeland, L., Niu, Q. and Wang, S. (2015). Starch Retrogradation: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, 14(5), pp.568–585. doi:https://doi.org/10.1111/1541-4337.12143.

Wu, Z., Zhang, X., Ma, M., Sui, Z. and Corke, H. (2025). The distribution of starch granule-associated proteins and their effects on starch properties: A review. *Food Hydrocolloids*, pp.111755–111755. doi:https://doi.org/10.1016/j.foodhyd.2025.111755.

Yang, L., Guo, X., Qin, Y., Ji, N., Dai, L. and Sun, Q. (2024). Different effects of pea protein on the properties and structures of starch gel at low and high solid concentrations. *International Journal of Biological Macromolecules*, 269, pp.132060–132060. doi:https://doi.org/10.1016/j.ijbiomac.2024.132060.

Zhiguang, C., Haixia, Z., Min, C., Fayong, G., & Jing, L. (2025). The fine structure of starch: a review. *Npj Science of Food*, *9*(1). https://doi.org/10.1038/s41538-025-00414-x

Zobel, H.F. (1988). Starch Crystal Transformations and Their Industrial Importance. *Starch - Stärke*, 40(1), pp.1–7. doi:https://doi.org/10.1002/star.19880400102.

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Part B: Characterisation of CF77 potato starch and pea protein and an analysis of how interactions between the two molecules could be affected by heat

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Keywords: Cf77 Starch, Pea Protein, Characterization, Dynamic light scattering, Analytical Ultracentrifugation, Heat interactions, Pea protein buffer suspension.

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Abstract: Animal based food products are the biggest contributors to the emission of greenhouse gases in the food sector. Looking into healthier alternatives which do not contribute to the global climate crisis is very important. One such product which is recently on a rise is vegan cheese and as such this study will focus on analysing the characteristics of cheesemaker Cf77 potato starch and pea protein for their viability and usage in vegan cheesemaking, and the effect excessive heat could have on the interactions between them. To do so Analytical ultracentrifugation and dynamic light scattering was used, to look at the polydispersity of the said molecules, and how the interactions between them will differ under different ratio of each component.

1. Introduction

Due to ever increasing climate crisis, the awareness of it has become much larger to the general population. In the food sector the animal-based products are the largest contributors towards the greenhouse gas emissions (Ritchie and Roser, 2023). Therefore, due to the increasing awareness of the said problem, rising percentage of population in developed countries start to identify as vegans/vegetarians. As such there has been a major demand increase for vegan cheese (www.futuremarketinsights.com, n.d.) as an alternative to the animal-based products which dominate the current market. Ingredients used to make vegan cheese are derived from vegetables, and mainly consist of protein, plant milks and fats, but could also include seeds, nuts, beans, starches and yeast (Moreau, 2024). Due to the lack on animal proteins and compounds found in the vegan cheese the taste, mouth feel, and nutrition composition can differ greatly in comparison to the vegan alternatives, which is a major problem, as most of the vegan cheese available does not contain much nutritional value, nor does it taste or feel similar (Craig, Mangels and Brothers, 2022) which can discourage people from purchasing them.

To combat the said problem, there has been two methodologies created in creation of new vegan cheeses. Combining/modifying existing known products mentioned above to create a vegan cheese with much closer resemblance in taste, behaviour and nutrition composition to animal-based products, this primary consists of mixing starches and plant-based proteins available (Dobson and Marangoni, 2023). Conversely, the other method relies on using cloning and recombination of genes into pet vectors in yeast cells. Following this method artificially made animal-based cheese with no negative connotation caused by greenhouse gasses emitted during its production could be made (Real Vegan Cheese, n.d.). To contribute to the research done based on the first methodology mentioned, characteristics of two viable molecules (CF77 cheese-making potato starch, and Pea protein) will be studied.

Cf77 cheese making potato starch is a modified potato starch produced by KMC, specially designed to replace casein and the role it plays in a typical cheese. Resulting cheese produced containing the CF77 is then mainly used in vegan cheeses made as pizza toppings or as normal everyday cheese slices. The Cf77 is made up of two components a modified Potato starch and Starch sodium Octenyl Succinate (Quadragroup.store, 2025). Starch is a polysaccharide composed from amylose and amylopectin which in turn are made up of glucose monomers which bind in an alpha 1,4 linkages (Melissa Petruzzello, 2019). Potato starches are usually larger than other plant-based starches having granules which can reach 150 μL (Xu et al., 2021) as such on average molecular weight of potato starch is around 10^8g/mol, while other starches are closer to 10^7g/mol. Due to its size potato starches gelatinase easier and have higher water binding capacity (Dupuis and Liu, 2019). Therefore, potato starches can be modified more extensively. The second component of the Cf77 is Starch sodium Octenyl succinate, this is an additive added to a starch which can act as emulsifier, binder, thickener and stabilizer (NguyenStarch, 2023). Though it was shown to have low toxicity, it cannot be absorbed by the gut but in turn gets hydrolysed and fermented inside the intestines by the microbes found there (Mortensen et al., 2017).

Pea protein is a compound of molecules consisting of around 25% proteins including albumins, globulin, prolamin and glutelin (Lam et al., 2016), and fat, vitamins, phytic acids, polyphenols, minerals and oxalates (Lu et al., 2019). 60% of the pea protein consists of carbohydrates and it is high in dietary fibre (Nadathur, Janitha P D Wanasundara and Scanlin, 2017). As such it is very rich in nutrients and can be eaten directly as a dietary

supplement when in powder form. Depending on extraction method the % of protein found in the powder can be modified. Due to these factors, a cheese made with pea protein would have similar nutrition content to animal-based cheese, excluding calcium. The pea protein has high denaturation point, it being between 79-87°C (ChemicalBook, 2025). Due to the pea proteins compound diversity, a cheese made with it, was found to be able to have similar physical properties of animal-based cheeses (Science.ku.dk, 2025).

To further analyse characteristics of these two molecules golden standard analytical ultracentrifugation (AUC) technique was used. The technique allowed for further look into the behaviour of both polysaccharides and proteins in their native hydrated state. Dynamic light scattering was used as it doesn't alter the sample and was used to monitor how the sizes of the molecules chang, and their corresponding aggregation behaviour. Viscometer was used to determine the viscosity of the molecules. Once the characteristics of individual molecules were analysed Dynamic light scattering was used again to observe if substances containing different ratios of the molecules had any noticeable differences to the interactions between them. Once the interesting ratios were narrowed down, Auc again was used to observe these interactions in more detail. This was repeated do see if heat treatment of the substance prior to the experiments had any effect on the observed results.

2. Materials & Methods

2.1 Characterization of Cf77 and pea protein

2.1.1 Buffer preparation

Two PBS buffer solutions were prepared and used during the experiments, first 0.1M PBS buffer with a pH of 7 was made using 4.595g of Sodium Phosphate Dibasic Dodecahydrate, 1.561g of Monopotassium phosphate, and 2.923 of sodium chloride, which were dissolved one at a time in 1 litre of distilled water. The second buffer used was a 0.1M PBS buffer with a pH of 7.8 and was made using; 5.79g of Sodium Phosphate Dibasic Dodecahydrate, 0.197g of Monopotassium phosphate, and 2.923 of sodium chloride, which were dissolved one at a time in 1 litre of distilled water. Both buffers were then stored in a fridge.

2.1.2 Dialysis of Starch

CF77 cheesemaker potato starch was dissolved in 0.1 M, pH7 PBS buffer, to obtain 5mg/ml solution. The solution was then dialysed in the same buffer using BioDesign™ Cellulose Dialysis tubing strips with 14,000 Da molecular weight cut off for a duration of 24 hours. The sample was then stored in the fridge.

2.1.3 Suspension of pea protein in PBS buffer

The pea protein used was dissolved in different buffers including, 0.1 M, Ph7.8 PBS buffer, pH 7 PBS buffer, and distilled water to obtain 5.0mg/ml solutions. One test solution was then split into two containers; one being left for 24 hours in fridge while rest were then left in an incubator for 24 hours at temperatures ranging from 35.0 to 49.5°C

2.1.4 Checking sample concentration.

ATAGO DD7 Digital differential Bench-top Refractometer was used to check if the samples made, reached required concentrations. The concentration of the dialysed starch sample was compared against the dialysate. While the concentration of the pea protein samples were compared against buffers the pea protein was dissolved in. Refractometer was calibrated using the respective buffer for each new sample, the measurements were taken at 25°C, after the samples were filtered using Sartorius Minisart 5 µL filter.

2.1.5 Sedimentation Velocity

Beckman XL-I Analytical ultracentrifuge was used to obtain sedimentation velocity (SV) (Schuck, 2003) for each molecule. Seven, 500µL samples of Cf77 starch were dissolved in 0.1 M PBS at concentrations of (0.5, 1, 1.5, 2, 2.5, 3 and 3.5 mg/ml) and then were used to determine SV of cf77 starch. Seven, 500µL samples of pea protein were used to determine the SV of the pea protein. Three of the samples contained pea protein dissolved in distilled water at concentrations of (0.3, 0.6, 0.9mg/ml). Four of the samples contained the pea protein dissolved in 0.1 M, Ph7.8 PBS buffer at concentrations of (0.3, 0.45, 0.6, 0.9 mg/ml). All samples were ultracentrifuged at 30,000 RPM, temperature 20°C. The data gathered was processed using SEDFIT v15.01b (Schuck, 2000).

2.1.6 Dynamic light scattering (DLS)

DLS was performed on 6 samples of CF77 starch diluted with the 0.1 M pH 7 PBS buffer at concentrations of (6.36, 3.18, 1.59, 0.79, 0.39, 0.19 mg/ml). DLS was performed on 12 samples of Pea protein, 6 of them being dissolved in 0.1M, pH7.8 PBS buffer at concentrations of (0.96, 0.76, 0.61, 0.49, 0.39, 0.19 mg/ml), and the other 6 being dissolved in distilled water at concentrations of (0.96, 0.77, 0.61, 0.49, 0.39, 0.20 mg/ml) The samples were filtered using 5 µL filter to remove unwanted contaminants including dust. The instrument was at 20°c while taking measurements. All cuvettes used were disposable plastic cuvettes.

2.1.7 Relative Viscosity

Using narrow capillary Ubbelohde viscometer the relative viscosity of both molecules was measured. Cf77 starch sample was diluted in the 0.1 M, pH 7 PBS buffer to obtain the samples with concentrations of (0.2, 0.4, 0.6, 0.8, 1, 2 mg/ml) which were then used in the experiment. Pea protein sample was diluted with the 0.1 M, pH 7.8 PBS buffer to obtain concentrations (0.2, 0.4, 0.6, 0.8, 0.96 mg/ml). The relevant viscosity of both buffers was also measured. All measurements were taken once the temperature of the sample lowered to 20°C.

2.2 Interactions study

2.2.1 Sample preparation

The cf77 starch and pea protein were mixed and dissolved in 0.1 M, Ph 7.8 PBS buffer to make samples with different ratios of each molecule. These included ratios of 1:1, 1:2, 1:3. The samples were split two and half were left in the fridge while the rest were incubated overnight at 37.5°C.

2.2.2 Checking sample concentration

ATAGO DD7 Digital differential Bench-top Refractometer was used to check if the samples made, reached required concentrations. The refractometer was calibrated using the pH 7.8 PBS buffer each time the sample was swapped. All measurements were taken at 25°C, after the filtration of each sample using Sartorius Minisart 5 µL filter.

2.2.3 Dynamic Light Scattering

DLS was performed on each sample. To obtain a concentration gradient for each sample, Half of each sample was discarded after the DLS finished then was replaced with same amount of the buffer used in the said sample creation, this was repeated 5 times, so each sample had 6 measurements at different concentrations.

2.3 Cooked vs uncooked interaction study

2.3.1 Sample preparation

Three different types of samples were prepared. These being a pure 5mg/ml pea protein, pure 5mg/ml CF77 starch and a sample with a ratio of 1:1 of cf77 and pea protein. All samples were dissolved in 0.1 M, pH 7.8 PBS buffer. 1ml of 2 separate 1:1 ratio samples was put into separate Eppendorf tubes, while rest of the samples were boiled at gradually increasing temperature until the said temperature reached 82.0°C. This took 3 minutes, in a water bath.

2.3.2 Checking sample concentration

An ATAGO DD7 Digital differential Bench-top Refractometer was used to check if the samples made, reached required concentrations. The refractometer was calibrated using the pH 7.8 PBS buffer each time the sample was swapped. All measurements were taken at 25.0°C, after the filtration of each sample using Sartorius Minisart 5 µL filter.

2.3.3 Dynamic Light scattering

DLS was performed on all samples to obtain a concentration gradient the same procedure was done as explained in 2.2.2.

2.3.4 Dynamic light scattering Temperature trend

To obtain results on how different temperatures affect the DLS results of the sample with the ratio of cf77 to pea protein being 1:1, A temperature trend SOP was used. The temperature would start at 20.0°C and with each DLS measurement gradually increase to 82.0°C. This DLS was performed on Three samples One was pre boiled, and two were not boiled as explained in 2.3.1. reusable glass cuvette was used during this experiment.

2.3.5 Sedimentation velocity

A Beckman XL-I Analytical ultracentrifuge was used to obtain SV (Schuck, 2003) for two samples. Three of the sub samples contained pre-boiled pea protein mixed with cf77 in a ratio of 1:1 dissolved in 0.1 M, Ph7.8 PBS buffer at concentrations of (0.45, 0.9, 1.8mg/ml). Four of the sub samples contained un-boiled pea protein mixed with cf77 in a ratio of 1:1 dissolved in 0.1 M, Ph7.8 PBS buffer at concentrations of (0.5, 0.8, 1.2, 1.8 mg/ml). All samples were ultracentrifuged at 40,000 RPM, temperature 20°C. The data gathered was processed using SEDFIT v15.01b (Schuck, 2000).

3. Results

3.1 Characterization of the Molecules

3.1.1 Samples used

The Cf77 sample dissolved fully, resulting in the samples final concentration being 6.36mg/ml.
The Pea protein was more difficult to dissolve. As shown in Table 1 pea protein did not dissolve in pH7 PBS at room temperature nor after incubation. It did dissolve with similar success in distilled water, and in PBS buffer with a higher pH. Pea protein usually denatures at much higher temperatures and due to this the incubation temperature used was 49.5°C to make sure some of the protein dissolved. PBS buffer was used due to its stabilizing and isotonic properties.

Buffer used	Buffer used (ml)	Pea protein (mg)	Incubated	Incubated temp (°C)	Final Concentration (mg/ml)
PBS pH7	20	100	no	-	Too low
water	20	100	yes	49.5	0.96
PBS pH7	20	100	yes	49.5	Too low
PBS Ph7.8	20	100	yes	49.5	0.96

Table 1: The composition and techniques used during the creation of the pea protein samples used in future experiments.

3.1.2 Dynamic light scattering

Based on data presented in Figure 1 singular peak could be observed for the CF77 sample, this indicates the molecule is monodispersed. Both graphs show similar sized peaks, with the volume graph having the peak at 584.9 nm^2/s and the intensity graph having it at 646.8 nm^2/s. with no other peak recorded for all concentrations. Due to the unchanging size of the peak present even at different concentrations. CF77 could exhibit week aggregation.

Pea protein is largely polydisperse as shown in Figure 1. The polydispersity can be seen in both distilled water and PBS samples, though the PBS sample is less so. According to the Figure 2 there is a larger peak around 10 nm^2/s - 100 nm^2/s and another one closer to 1000 nm^2/s. When looking at the volume vs size graph the peaks hight is not influenced by concentration and as such pea protein could have weak aggregation.

Figure 1: The volume vs size on the left and intensity vs size on the right. A) two graphs representing DLS of cf77 in PBS (0.1M pH7). B) two graphs representing the DLS results of Pea protein dissolved in distilled water. C) two graphs representing the DLS results of Pea protein dissolved in PBS (0.1M pH7.8). All measurements were done at 20.0°C.

3.1.3 Sedimentation velocity

Sedimentation velocity experiment determined two species within cf77 which can be confirmed according to the literature (Quadragroup.store, 2025). based on peak sizes, them being ~50%, 3.6S and ~50% 2.2S.
Pea protein can be seen to behave in similar fashion in both buffers. There is a singular prominent peak at around 1.6S though that is
slightly higher in PBS buffer. The pea protein in PBS has an addition small peak around 3.4S though it is only exhibited by one concentration
The Sedimenation coefficient of Pea protein in distilled water was no extrapolated back to infinite dilutions due to the small number of data points.

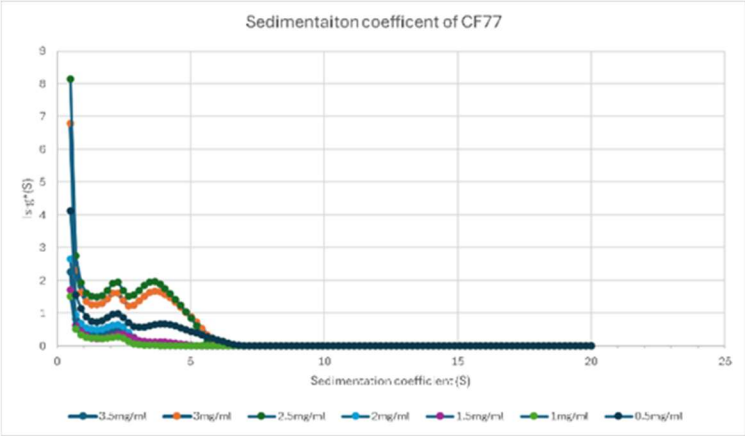


Figure 2a: Sedimentation velocity of Cf77 potato starch, based on concentration dependant sedimentation coefficient distribution.

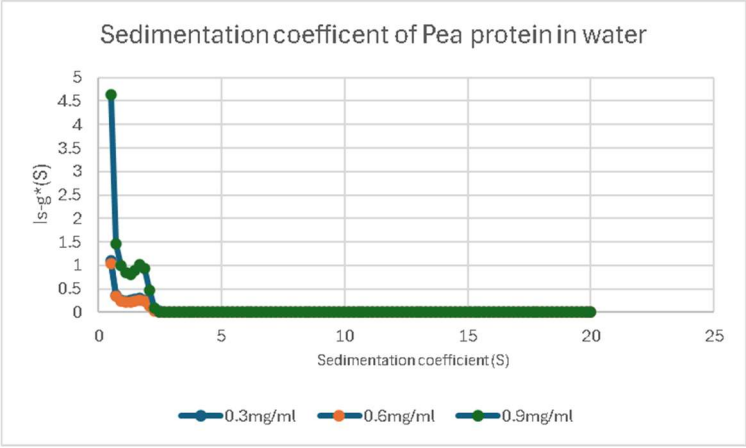


Figure 3a: Sedimentation velocity of Pea protein in distilled water, based on concentration dependant sedimentation coefficient distribution.

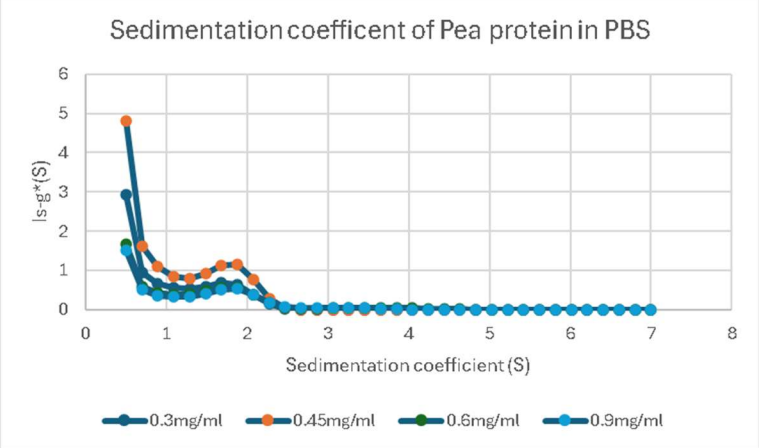


Figure 4a: Sedimentation velocity of Pea protein in pH7.8 PBS buffer, based on concentration dependant sedimentation coefficient distribution.

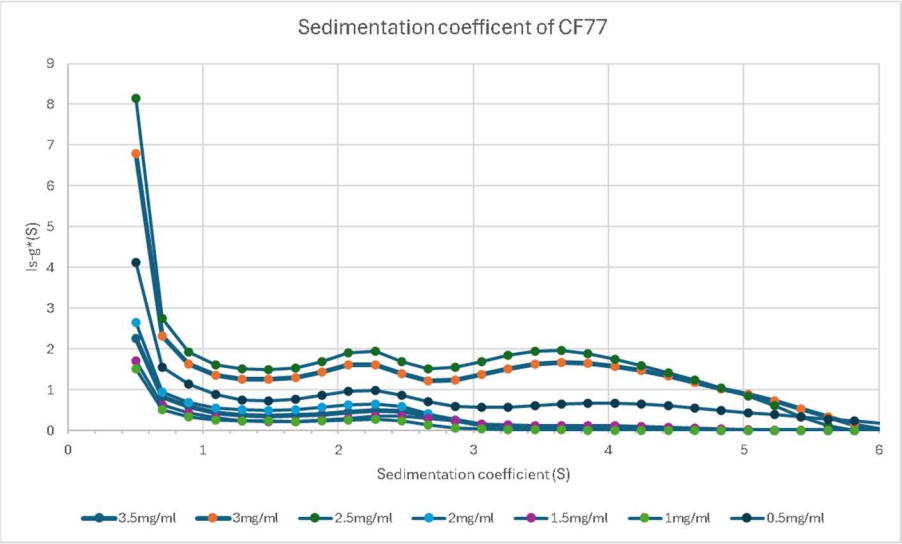


Figure 2b: Sedimentation velocity of Cf77 potato starch, based on concentration dependant sedimentation coefficient distribution with no residues.

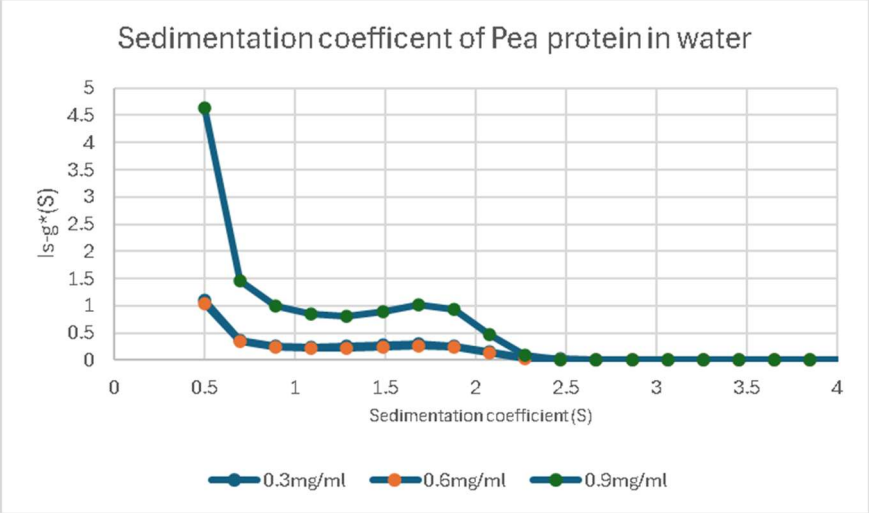


Figure 3b: Sedimentation velocity of Pea protein in distilled water, based on concentration dependant sedimentation coefficient distribution with no residues.

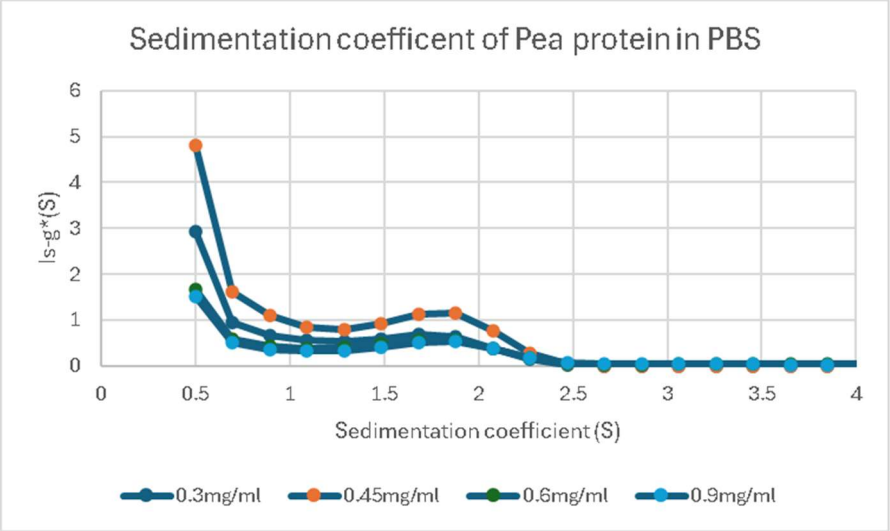


Figure 4b: Sedimentation velocity of Pea protein in pH7.8 PBS buffer, based on concentration dependant sedimentation coefficient distribution with no residues.

Figure 5: showing sedimentation coefficient of CF77, in accordance with standard solvent conditions, with concentration extrapolated back to zero

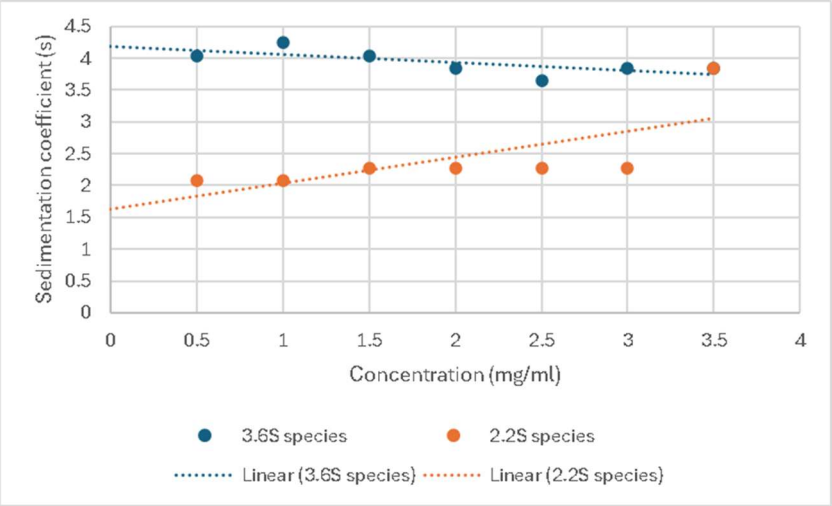
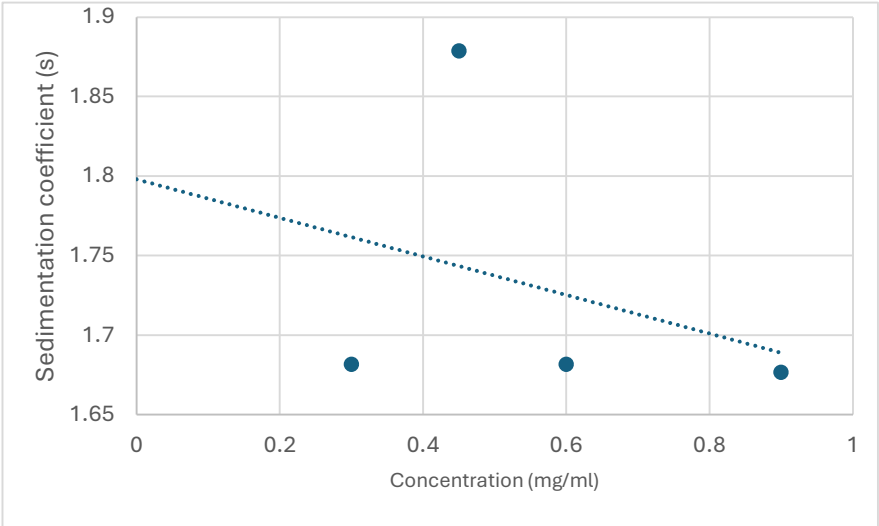


Figure 6: showing sedimentation coefficient of Pea protein in PBS, in accordance with standard solvent conditions, with concentration extrapolated back to zero



3.1.4 Relative viscosity

Unfortunately, it was not possible to obtain reliable values with conventional Ostwald capillary viscometers due to the low concentrations available and the heterogeneous nature of the proteins.

Future work would require higher concentrations and also coupling a viscometer to a separation medium, such as a chromatographic column (e.g. in a so-called SEC-MALS-visco-star type of viscometer)

3.2 Interaction studies

3.2.1 Samples used

The buffer used was the 0.1M pH7.8 PBS buffer. This was because CF77 was shown to easily dissolve in PBS as shown in 3.1.1, which coincided well with Pea protein solubility as shown in Table 1. Samples made were primary in 3 different ratio groups 1:1, 1:2, and 1:3. Incubation was done on half of the samples to see if it was required and as Table 2 shows, the incubation increased the concentration of dissolved molecules in the sample by nearly half. Therefore, all required samples were then incubated. The ratios were decided by the powder weight instead of molecular weight, as such the ratios might not be accurate.

Buffer used	Buffer used (ml)	Cf77 starch (mg)	Pea protein (r	Incubated	Incubated temp (°C)	ratio Cf77 vs Pea protein	Final Concentration (mg/ml)
PBS pH7.8	20	100	100	No		1 to 1	1.674
PBS pH7.8	20	67	134	NO		1 to 2	1.462
PBS pH7.8	20	134	67	NO		2 to 1	1.708
PBS pH7.8	20	100	100	yes	37.8	1 to 1	2.956
PBS pH7.8	20	67	134	yes	37.8	1 to 2	3.848
PBS pH7.8	20	134	67	yes	37.8	2 to 1	2.785
PBS pH7.8	20	150	50	yes	37.8	1 to 3	2.962
PBS pH7.8	20	50	150	yes	37.8	3 to 1	2.04

Table 2: The composition, techniques used, and final concentration of the samples used.

3.2.2 Dynamic light scattering

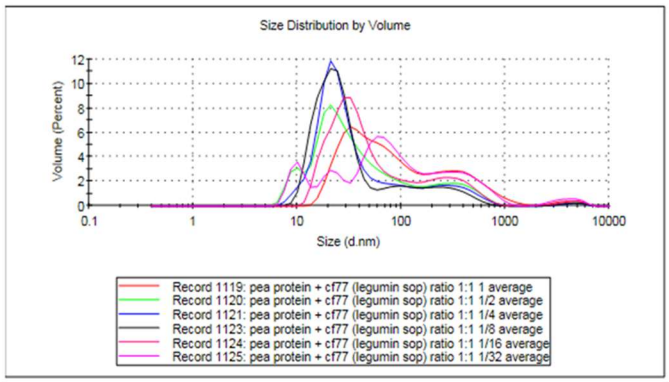
As shown in Figure 8 dynamic light scattering was used to take measurement of substances which contained different ratios of both cf77 and pea protein. Sample A was shown to have only one peak on the intensity graph, that peak ranged from 50 nm^2/s to 1000 nm^2/s, and due to the mono-dispersity there is a high probability that the two molecules formed a compound. Sample B was highly polydisperse and with multiple peaks present. Sample C was similar in results to sample A with a singular peak ranging from 40 nm^2/s to 1000 nm^2/s, but an even more uniform appearance on the volume graph. Sample D had similar results to sample C with one peak present in the intensity graph ranging from 30 nm^2/s to 1000 nm^2/s but had more varied peaks on the volume graph. Sample E had similar results to sample B with many different peaks on the intensity graph, though on the volume graph there were two strong peaks on ranging from 30 nm^2/s to 90 nm^2/s and another from 500 nm^2/s to 1000 nm^2/s. Sample F had two strong peaks one on both graphs one ranging from 50 nm^2/s to 110 nm^2/s and the other from 500 nm^2/s to 1200 nm^2/s. Sample G had two peaks on the intensity graph, the largest one ranging from 50 nm^2/s to 1000 nm^2/s with a small other peak in 8000 nm^2/s while the volume graph had three peaks 20 nm^2/s to 100 nm^2/s 100 nm^2/s to 1000 nm^2/s and the 8000 nm^2/s peak, showing strongest poly-dispersity so far. Sample H had similar peaks to sample G on the intensity graph with additional peak ranging from 10 nm^2/s to 100 nm^2/s, but the volume graph was different with the strongest peak ranging from 5 nm^2/s to 50 nm^2/s and a weaker peak at 500 nm^2/s to 1000 nm^2/s.

A

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 146.0	Peak 1:	60.18	68.6	38.06
PdI: 0.356	Peak 2:	415.6	29.3	255.9
Intercept: 0.916	Peak 3:	4072	2.1	1099

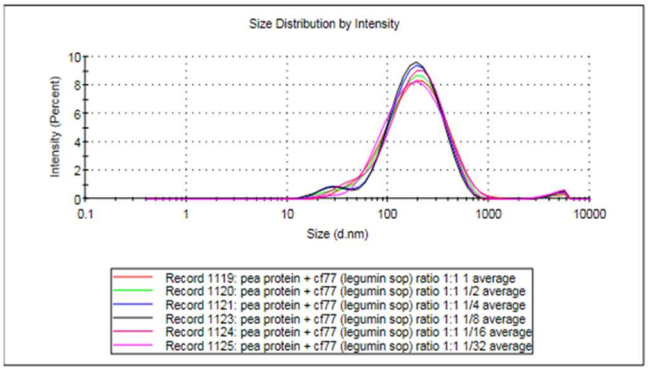
Result quality Good



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 146.0	Peak 1:	226.8	98.8	158.5
PdI: 0.356	Peak 2:	4406	1.2	932.6
Intercept: 0.916	Peak 3:	0.000	0.0	0.000

Result quality Good

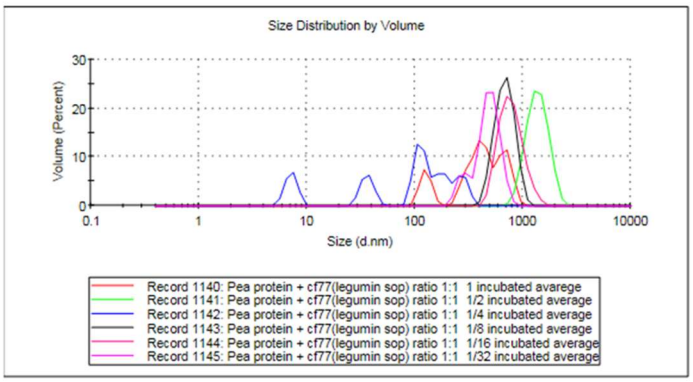


B

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 2942	Peak 1:	127.4	15.5	15.51
PdI: 0.918	Peak 2:	394.4	50.8	83.97
Intercept: 0.679	Peak 3:	669.7	33.8	107.3

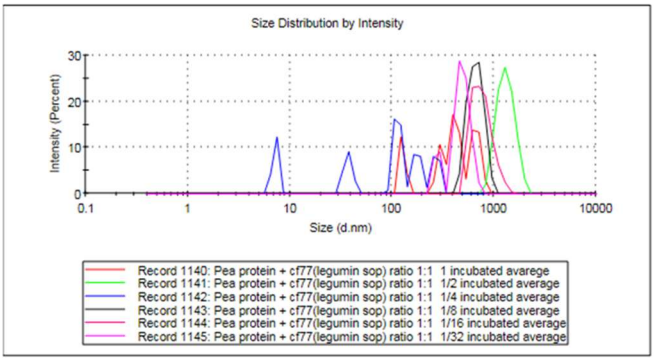
Result quality Refer to quality report



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 2942	Peak 1:	419.1	36.5	52.02
PdI: 0.918	Peak 2:	664.6	30.4	77.05
Intercept: 0.679	Peak 3:	305.2	17.9	28.68

Result quality Refer to quality report

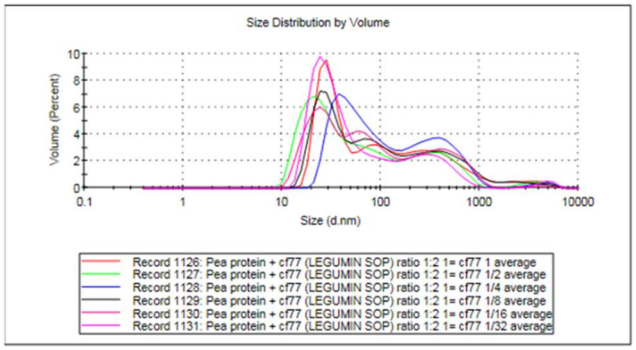


C

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 157.5	Peak 1:	30.51	42.7	8.426
PdI: 0.360	Peak 2:	96.94	24.3	35.26
Intercept: 0.932	Peak 3:	456.2	29.1	317.3

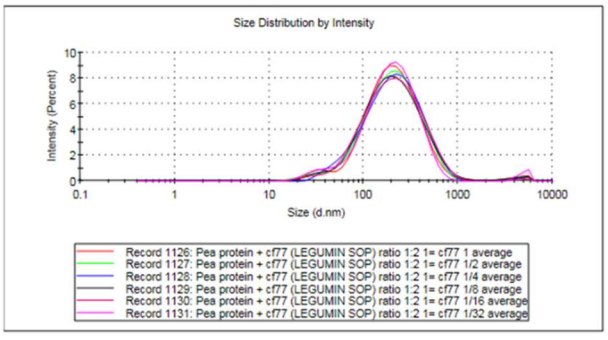
Result quality Refer to quality report



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 157.5	Peak 1:	245.1	94.3	172.5
PdI: 0.360	Peak 2:	33.51	3.9	6.925
Intercept: 0.932	Peak 3:	4208	1.8	1064

Result quality Refer to quality report

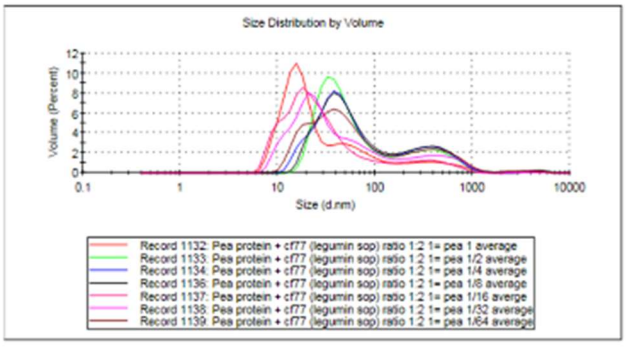


D

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 136.0	Peak 1:	16.69	63.9	6.066
PdI: 0.425	Peak 2:	68.84	23.8	34.75
Intercept: 0.941	Peak 3:	454.1	12.0	281.8

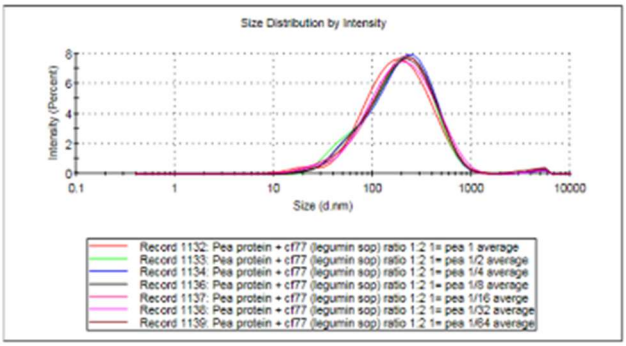
Result quality Good



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 136.0	Peak 1:	227.1	96.5	170.0
PdI: 0.425	Peak 2:	19.79	2.5	5.518
Intercept: 0.941	Peak 3:	4696	1.1	794.7

Result quality Good

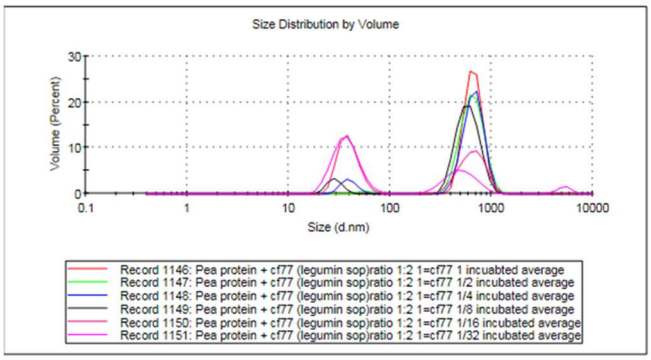


E

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 791.2	Peak 1:	666.9	100.0	134.9
PdI: 0.251	Peak 2:	0.000	0.0	0.000
Intercept: 0.932	Peak 3:	0.000	0.0	0.000

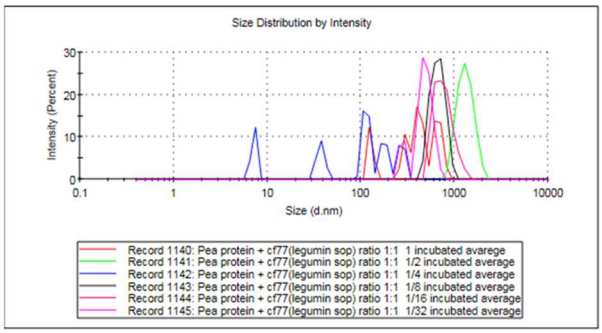
Result quality Refer to quality report



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 2942	Peak 1:	419.1	36.5	52.02
PdI: 0.918	Peak 2:	664.6	30.4	77.05
Intercept: 0.679	Peak 3:	305.2	17.9	28.68

Result quality Refer to quality report

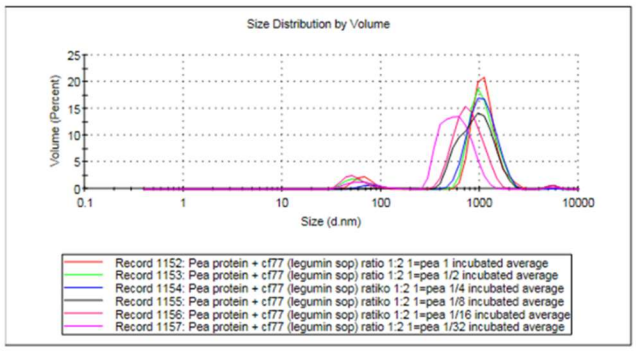


F

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 832.8	Peak 1:	67.54	7.9	23.77
PdI: 0.648	Peak 2:	596.3	91.3	207.3
Intercept: 1.01	Peak 3:	5424	0.8	645.3

Result quality Refer to quality report



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 832.8	Peak 1:	618.7	57.5	154.1
PdI: 0.648	Peak 2:	405.4	36.6	45.60
Intercept: 1.01	Peak 3:	86.13	5.1	27.56

Result quality Refer to quality report

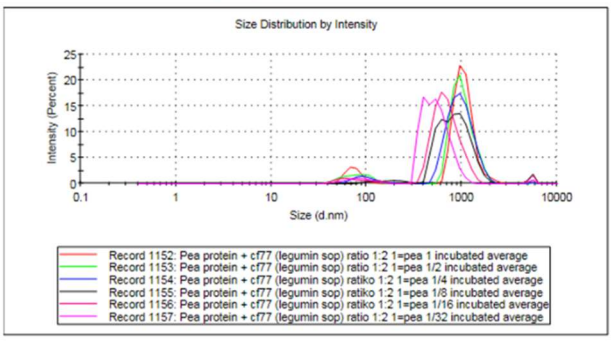


Figure 7: Showing the graphs obtained via DLS, the graphs are depicting volume vs size on the left and intensity vs size on the right. A) two graphs representing DLS of cf77 and Pea protein in a ratio of 1:1. B) two graphs representing DLS of incubated cf77 and Pea protein in a ratio of 1:1. C) two graphs representing DLS of cf77 and Pea protein in a ratio of 1:2 with lower ratio representing CF77. D) two graphs representing DLS of cf77 and Pea protein in a ratio of 1:2 with lower ratio representing pea protein. E) two graphs representing DLS of incubated cf77 and Pea protein in a ratio of 1:2 with lower ratio representing CF77. F) two graphs representing DLS of incubated cf77 and Pea protein in a ratio of 1:1 with lower ratio representing pea protein. G) two graphs representing DLS of incubated cf77 and Pea protein in a ratio of 1:3 with lower ratio representing CF77. H) two graphs representing DLS of incubated cf77 and Pea protein in a ratio of 1:3 with lower ratio representing pea protein. All measurements were done at 20°C.

3.3 Cooked vs uncooked Interaction study

3.3.1 Samples used

Buffer used	Buffer used (ml)	Pea protein (µL)	CF77 Starch (mg)	Cooked	Final Concentration (mg/ml)
PBS pH7.8	10	50	0	yes	0.88
PBS pH7.8	10	50	0	yes	0.94
PBS pH7.8	10	50	0	yes	0.47
PBS pH7.8	10	0	50	yes	3.55
PBS pH7.8	10	0	50	yes	2.57
PBS pH7.8	10	0	50	yes	3.25
PBS pH7.8	10	25	25	yes	1.78
PBS pH7.8	10	25	25	yes	1.56
PBS pH7.8	10	25	25	yes	1.41
PBS pH7.8	10	25	25	no	1.56
PBS pH7.8	10	25	25	no	1.41

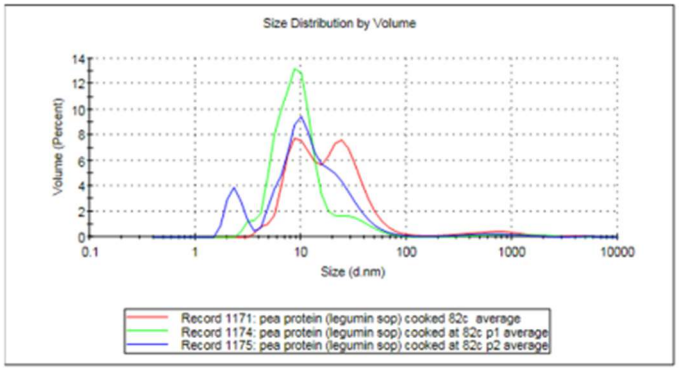
Table 3: showing the composition, techniques used, and final concentration of the samples used. The last two samples shown on the table were taken from the two samples above before the water bath.

The samples shown in table 3 are split into 3 replicates. The Pure pea samples average the final concentration of 0.76mg/ml. The average final concentration of pure cf77 starch is 3.12mg/ml, while the final average concentration of 1:1 ratioed substance is 1.58mg/ml. The lower concentration of the samples, and therefore lower dissolution of the samples in the buffer can

A

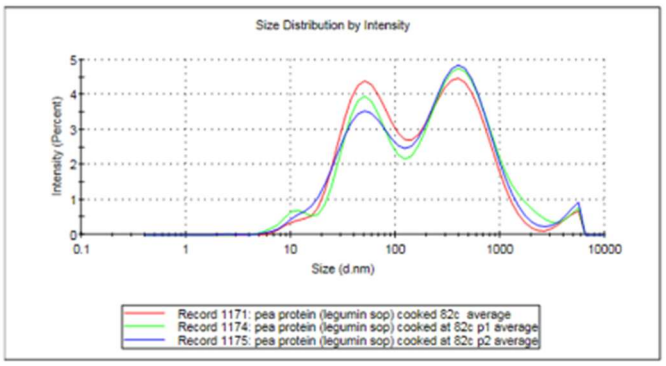
Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 96.49	Peak 1:	11.87	96.8	10.63
PdI: 0.911	Peak 2:	749.8	2.2	381.0
Intercept: 0.916	Peak 3:	2144	0.8	668.2
Result quality: Refer to quality report				



Results

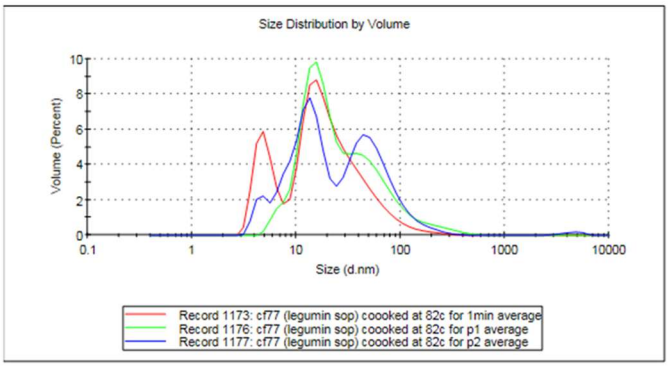
		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 96.49	Peak 1:	587.6	58.7	544.5
PdI: 0.911	Peak 2:	58.74	35.7	28.24
Intercept: 0.916	Peak 3:	11.08	3.5	2.924
Result quality: Refer to quality report				



B

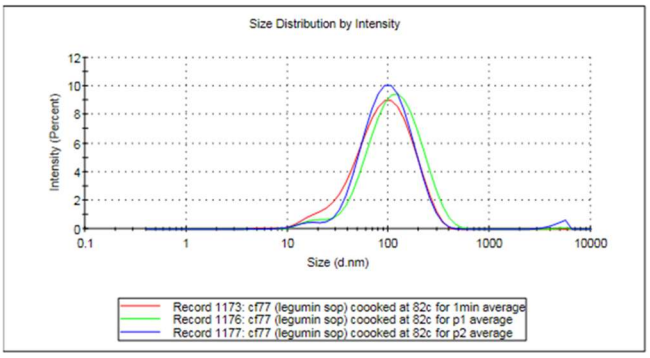
Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 91.02	Peak 1:	77.86	34.7	66.83
PdI: 0.295	Peak 2:	17.23	65.2	6.997
Intercept: 0.917	Peak 3:	4990	0.0	818.6
Result quality: Good				



Results

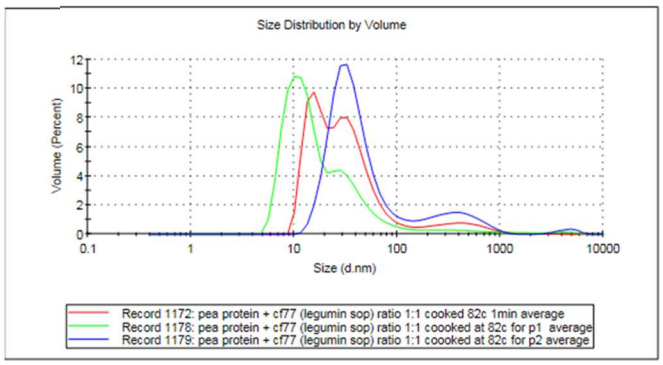
		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 91.02	Peak 1:	132.9	96.5	77.89
PdI: 0.295	Peak 2:	18.25	3.3	4.422
Intercept: 0.917	Peak 3:	5052	0.2	579.4
Result quality: Good				



C

Results

		Size (d.nm):	% Volume:	St Dev (d.nm):
Z-Average (d.nm): 107.4	Peak 1:	11.93	66.1	3.985
PdI: 0.481	Peak 2:	43.04	29.3	29.71
Intercept: 0.938	Peak 3:	664.7	3.7	534.7
Result quality: Good				



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 107.4	Peak 1:	228.1	94.7	266.2
PdI: 0.481	Peak 2:	4264	2.9	1040
Intercept: 0.938	Peak 3:	13.65	2.3	3.312
Result quality: Good				

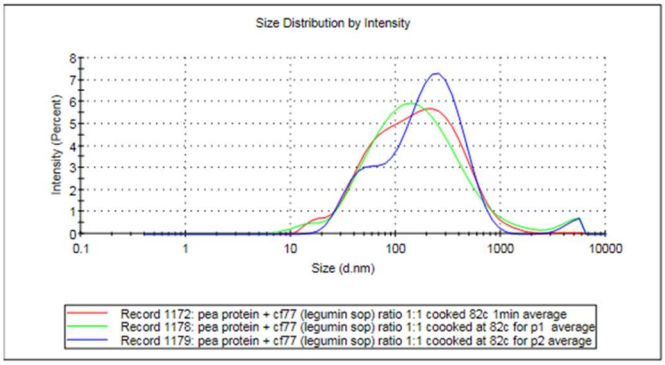


Figure 8: Showing the graphs obtained via DLS, the graphs are depicting volume vs size on the left and intensity vs size on the right. A) two graphs representing DLS of cooked pure Pea protein. B) two graphs representing DLS of cooked pure cf77. C) two graphs representing DLS of cooked cf77 and Pea protein in a ratio of 1:1. All measurements were done at 20°C.

be attributed to the lack of incubation. Water bath was used instead as shown in 2.3.1 which resulted in much lower concentration.

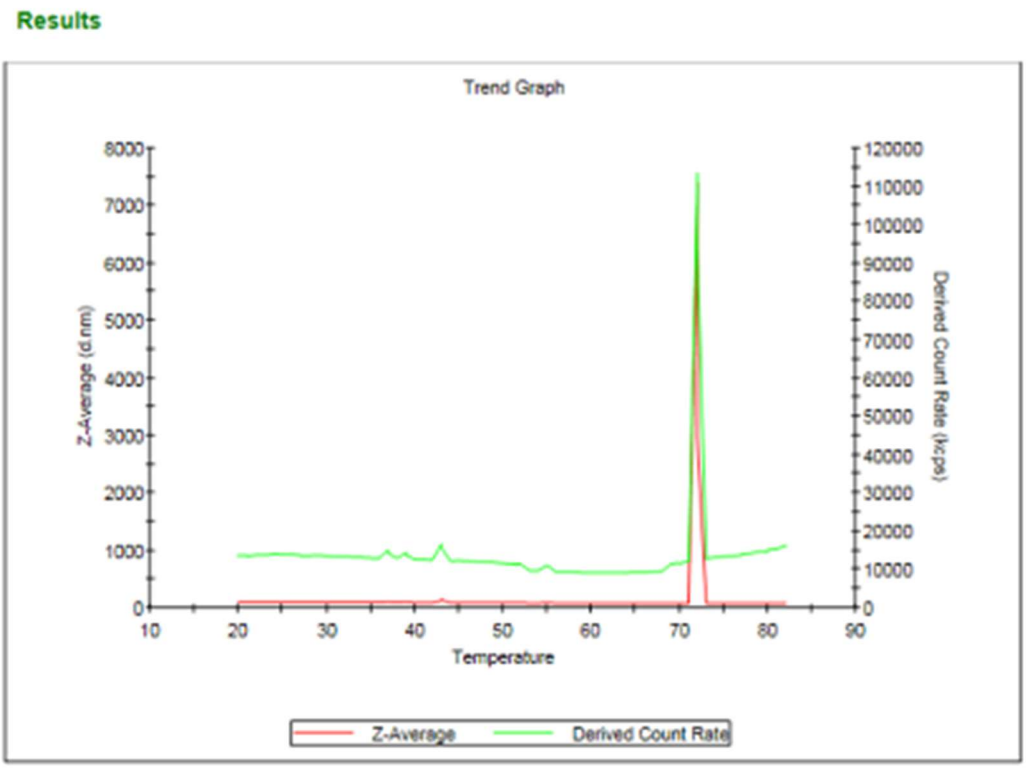
3.3.2 Dynamic light scattering

Samples 2A where show to have much steeper peaks in comparison to their uncooked counterparts, further whereas previously there was only one strong peak on the intensity graph now the said peak is much broader and is split into 3 smaller subpeaks ranging from 10 nm^2/s to 10000 nm^2/s. This suggests a strong aggregation.
Sample2 B again is different to its uncooked counterpart, the volume graph contains more peaks, and the overall peak is much broader again. Sample 2C again had much steeper peaks volume graph and the singular peak again was broken down to multiple peaks. All three sample types exhibit strong aggregation behaviours.

3.3.3 Dynamic light scattering Temperature trend

Sample 3A showed a large, short spike at 72°C
Which was expected as majority of the
reactions took place after the water bath which
denatured majority of molecules inside it.
Sample B also exhibited characteristic
Behaviour with the z average value going up
Due to aggregation and then spiking down due to denaturation,
With opposite happened to the derived count rate. The reverse of trends on both graphs happened around 60°C.

A



B

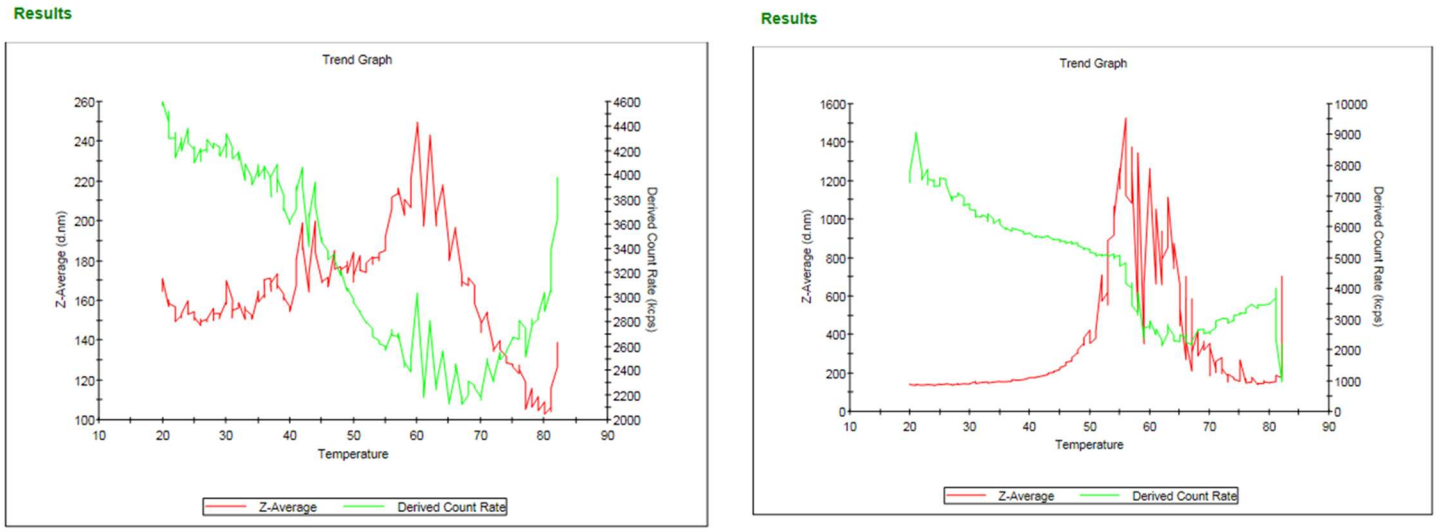


Figure 9: Showing the graphs obtained via DLS using a temperature rend SOP. A) Depicting the results of the DLS of a precooked mixture of cf77 starch and pea protein in a ratio of 1:1 In a (0.1M. pH7.8) PBS buffer, shown on a Z– average vs Temperature graph. B) Two repeats of the same experiment but using uncooked samples. All measurement were taken under 20°C.

3.3.4 Sedimentation Velocity

Results obtained via the analytical ultracentrifugation show us that the molecules inside the uncooked sample as shown in figure 11 had undergone interactions between themselves. There are some preserved peaks still visible like the 1.6S peak from Cf77 and 3.6S peak from Pea protein, but there are many very shallow continuous peaks at the bottom of the graph suggesting polydispersity. Sample from figure 12 behaves in similar manner with many small continues peaks being present, but all the preserved peaks from the two separate molecules have disappeared.

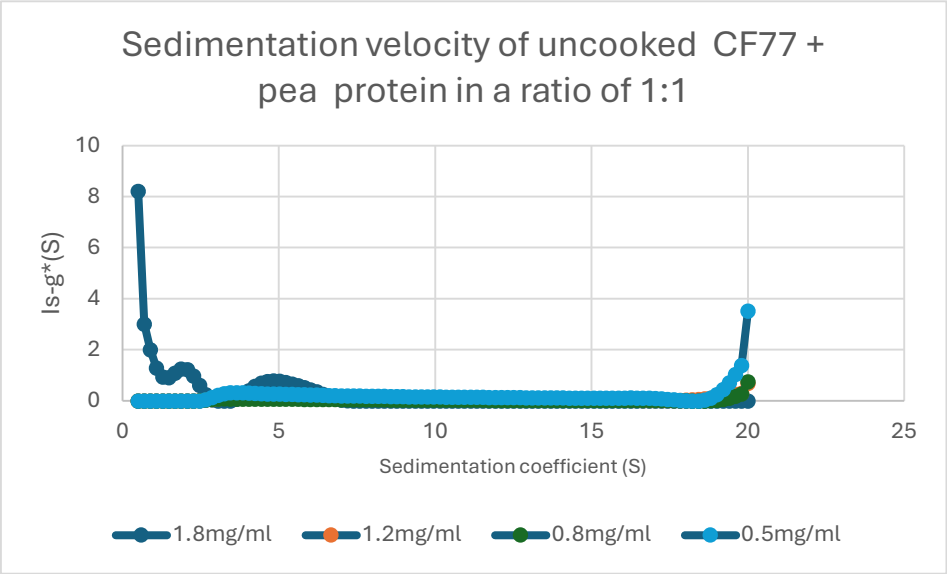


Figure 10: Showing the sedimentation velocity of un-cooked Ratio 1:1 mixture of pea protein an CF77 potato starch

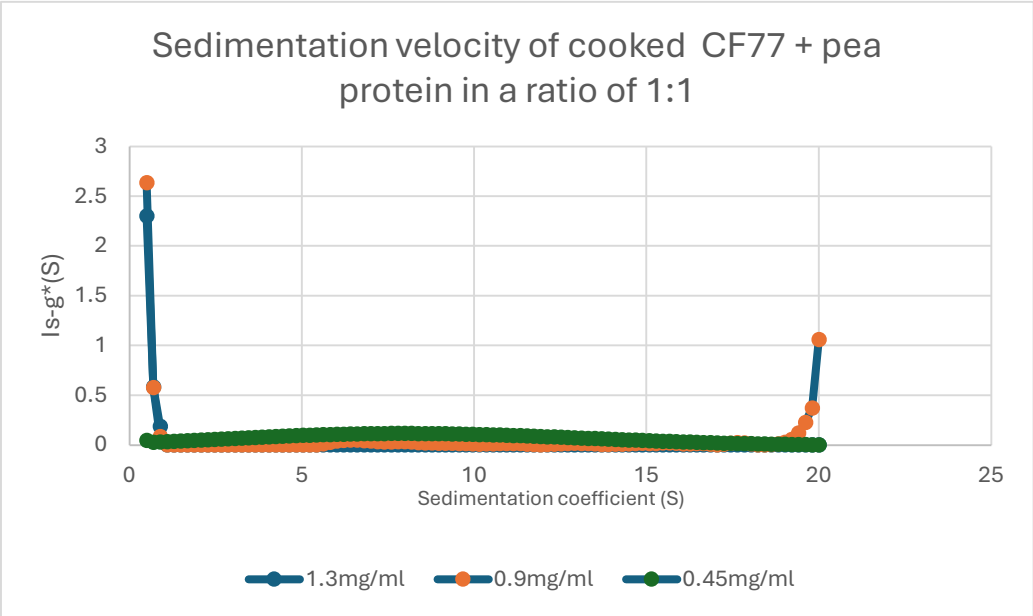


Figure 11: Showing the sedimentation velocity of precooked Ratio 1:1 mixture of pea protein an CF77 potato starch.

4. Discussion

This study investigated which characteristics of cf77 potato starch and Pea protein allow them to be good ingredients in vegan cheesemaking. To do so Dynamic light scattering was used to determine the polydispersity characteristics of the individual molecules and them how they would interact with each other under different ratios. Analytical ultracentrifugation was used to see if the molecules had any subspecies present in the sample, and how the heat treatment would affect the interactions between the said molecules. During the first phase of the study, using the Dynamic light scattering it was confirmed that the CF77 starch is in fact monodispersed, while the pea protein is not and depending on the sample preparation the polydispersity could be increased. This is a negative trait as industrial based food production needs to be stable, if the food component has high polydispersity the uniformity of the product could be lost, due to different taste and mouth feel of the product produced (Lucey, Johnson and Horne, 2003). Through the rigorous sample preparation planning especially when it comes to the pea protein, a method of dissolving the said protein in a widely available buffer was found. Analytical ultracentrifugation confirmed the make-up of the Cf77 starch and the purity of the pea protein.

During the second phase of the study the ratios of the materials used to make up the samples were studied in detail, and the results point towards ratios like 1:1 or 1:2 being less polydisperse, while still being able to interact with each other. Although the molecular weight of the cf77 is much larger than the ~400KDa pea protein (Mession et al., 2013) the DLS results showed that the ratios of 1:2, 2:1 did have much of an affect compared to ratios of 1:3 and 3:1. and that incubation of samples at a lower temperature still does the job instead of a very high as the thought was originally . During the last phase of the study the results showed that around 60 °C is where both molecules start to denature/ lose their structure, which will help in the usage of the said molecules in cheese making as that process requires high temperature.

Due to time constraints viscosity and molecular weight properties were not measured. Because of the heterogeneous nature of the system and low concentrations, conventional capillary or rolling ball viscometry would not be possible to get accurate results as we have shown, but a differential viscometer on-line to a separation column and light scattering detector (as in the “SEC-MALS” instrument(size exclusion chromatography coupled to lights scattering and a viscometer) instrument will prove useful in future work. In addition, more combination ratios can be tested, and more repeats of the original results can be acquired. Finally more legumin/ oil seed proteins can be tested, and, more importantly, actually making cheese with these molecules can then be done.

5. Conclusion

This study demonstrated both molecules are suitable for the usage in cheese making industry, and how much variety these molecules have if used correctly. With Cf77 starch being easy to use, stable, and monodisperse, while the pea protein being more varied, but still very viable due to its nutritious content, availability and as a better alternative to animal-based products. As previously mentioned, a more in depth look into the hydrodynamics of both of the molecules should be the next step along with a SEC-MALS to accurately determine the molecular weight distribution of the pea protein.

Acknowledgement

I sincerely would like to thank my supervisors Dr. Richard Gills and Prof Stephen Harding for their help in this project.

References:

ChemicalBook. (2025). Pea Protein | 222400-29-5. [online] Available at: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB11421258.htm

Craig, W.J., Mangels, A.R. and Brothers, C.J. (2022). Nutritional Profiles of Non-Dairy Plant-Based Cheese Alternatives. Nutrients, 14(6), p.1247. doi:https://doi.org/10.3390/nu14061247.

Dobson, S. and Marangoni, A.G. (2023). Methodology and development of a high-protein plant-based cheese alternative. Current Research in Food Science, 7, pp.100632–100632. doi:https://doi.org/10.1016/j.crfs.2023.100632.

Dupuis, J.H. and Liu, Q. (2019). Potato Starch: a Review of Physicochemical, Functional and Nutritional Properties. American Journal of Potato Research, 96(2), pp.127–138. doi:https://doi.org/10.1007/s12230-018-09696-2.

Lam, A.C.Y., Can Karaca, A., Tyler, R.T. and Nickerson, M.T. (2016). Pea protein isolates: Structure, extraction, and functionality. Food Reviews International, 34(2), pp.126–147. doi:https://doi.org/10.1080/87559129.2016.1242135.

Lucey, J.A., Johnson, M.E. and Horne, D.S. (2003). Invited Review: Perspectives on the Basis of the Rheology and Texture Properties of Cheese. Journal of Dairy Science, 86(9), pp.2725–2743. doi:https://doi.org/10.3168/jds.s0022-0302(03)73869-7.

Lu, Z.X., He, J.F., Zhang, Y.C. and Bing, D.J. (2019). Composition, physicochemical properties of pea protein and its application in functional foods. Critical Reviews in Food Science and Nutrition, 60(15), pp.2593–2605. doi:https://doi.org/10.1080/10408398.2019.1651248.

Mession, J.-L., Sok, N., Assifaoui, A. and Saurel, R. (2013). Thermal Denaturation of Pea Globulins (Pisum sativum L.)—Molecular Interactions Leading to Heat-Induced Protein Aggregation. Journal of Agricultural and Food Chemistry, 61(6), pp.1196–1204. doi:https://doi.org/10.1021/jf303739n.

Melissa Petruzzello (2019). Starch. In: Encyclopædia Britannica. [online] Available at: https://www.britannica.com/science/starch.

Moreau, E. (2024). What in the World is Vegan Cheese, Anyway? Can it Actually Replace 'Real' Cheese? [online] Organic Authority. Available at: https://www.organicauthority.com/recipes/what-is-vegan-cheese-made-of

Mortensen, A., Aguilar, F., Crebelli, R., Di Domenico, A., Dusemund, B., Frutos, M.J., Galtier, P., Gott, D., Gundert-Remy, U., Lambré , C., Leblanc, J., Lindtner, O., Moldeus, P., Mosesso, P., Parent-Massin, D., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Wright, M. and Younes, M. (2017). Re-evaluation of oxidised starch (E 1404), monostarch phosphate (E 1410), distarch phosphate (E 1412), phosphated distarch phosphate (E 1413), acetylated distarch phosphate (E 1414), acetylated starch (E 1420), acetylated distarch adipate (E 1422), hydroxypropyl starch (E 1440), hydroxypropyl distarch phosphate (E 1442), starch sodium

octenyl succinate (E 1450), acetylated oxidised starch (E 1451) and starch aluminium octenyl succinate (E 1452) as food additives. *EFSA Journal*, 15(10). doi:https://doi.org/10.2903/j.efsa.2017.4911.

Nadathur, S.R., Janitha P D Wanasundara and Scanlin, L. (2017). Sustainable protein sources. London Academic Press.

NguyenStarch (2023). Starch Sodium Octenyl Succinate (E/INS 1450): Properties, Uses, and Regulations - NguyenStarch. [online] NguyenStarch. Available at: https://nguyenstarch.com/starch-sodium-octenyl-succinate-ins-1450/

Quadragroup.store. (2025). Quadra. [online] Available at: https://www.quadragroup.store/products/cheesemaker-cf77

Real Vegan Cheese. (n.d.). *Real Vegan Cheese*. [online] Available at: https://www.realvegancheese.org/.

Ritchie, H. and Roser, M. (2023). Food production is responsible for one-quarter of the world's greenhouse gas emissions. Our World in Data. [online] Available at: https://ourworldindata.org/food-ghg-emissions#article-citation.

Science.ku.dk. (2025). Popular cooking cheese made with peas yields same taste and texture. [online] Available at: https://science.ku.dk/english/press/news/2025/popular-cooking-cheese-made-with-peas-yields-same-taste-and-texture/

Schuck, P., 2000. Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. Biophysical Journal, 78(3), pp. 1606-19..

Schuck, P., 2003. On the analysis of protein self-association by sedimentation velocity analytical ultracentrifugation. Analytical Biochemistry, 320(1), pp. 104-124.

Harding, S, E., Horton, J, C., Colfen, H,. (1997). The ELLIPS suite of macromolecular conformation algorithms. European Biophysics Journal, Volume 25, p. 347–359.

www.futuremarketinsights.com. (n.d.). *Vegan Cheese Market*. [online] Available at: https://www.futuremarketinsights.com/reports/vegan-cheese-market.

Xu, F., Zhang, L., Liu, W., Liu, Q., Wang, F., Zhang, H., Hu, H. and Blecker, C. (2021). Physicochemical and Structural Characterization of Potato Starch with Different Degrees of Gelatinization. Foods, [online] 10(5), p.1104. doi:https://doi.org/10.3390/foods10051104.