Development of a Semisolid Baobab-based Protein Snack.

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Development of a Semisolid, Baobab-based Protein Snack

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Popular Scientific Abstract

Baobab is a fruit from Sub-Saharan Africa, which has been consumed in local diets for as long as anyone can remember. It has a hard shell and hard seeds surrounded by a white fruit pulp. This fruit pulp is acidic (meaning it has a low pH, around 3,0), and contains a lot of pectin. Pectin is a type of molecule that makes liquids turn into gels, which can be seen in marmalade for example. Not only does Baobab have a unique and pleasant taste, it is also very nutritious, as it contains a lot of vitamins and minerals.

In order to create a more sustainable future for the food industry, Arwa Foodtech AB wants to use the baobab fruit to replace animal-based ingredients, since it has the potential to do so, but is far better for the environment. In this particular project, Baobab has been used to create a base for a plant-based, spoonable protein snack, similar to a dairy-based quarg. Baobab has two main functions in the product:

1. The low pH of the Baobab along with a touch of salt helps the plant proteins added to the mix become solubilized. This makes the product taste smooth rather than mealy.
2. The pectin in the Baobab makes the product thicker and makes it more like the quarg it aims to mimic.

In addition to Baobab, the product also contains Water, Oat protein concentrate, Pea protein concentrate, Apple juice concentrate, Coconut oil, and Salt.
That means that not only is it suitable for vegans, it is also free from additives and added sugar, which is becoming increasingly appreciated by consumers.

Thanks to careful optimization of the ratio of Oat to Pea protein, the product has a protein quality score (PDCAAS) at 100%, which is usually only achieved by animal proteins.

By tweaking the cooking temperature and the protein content, the recipe was made to mimic the gel behaviour of commercial quargs and plant-based alternatives, giving the consumer a similar eating experience as a “normal” quarg.

After almost six months of development, the base is now ready for the finishing touches, or in other words, some flavour and colour, and then the product will be ready for the supermarket shelves.

By sharing the discoveries on how different parameters influence the sensory and physiochemical properties of a plant-protein based formulation, we hope to make future developments of similar products easier and more efficient.

Abstract

This thesis was done in collaboration with the department of Food Technology at LTH, Aventure AB, and Arwa Foodtech AB. The aim of the project was to develop the base for a baobab based, spoonable protein snack, comparable to quarg but free from ingredients of animal origin.

At the time of writing, one of the biggest if not the biggest trend in the food industry is the replacement of animal-sourced proteins with plant-based proteins.
Another big trend is the "clean label" trend, i.e., consumers rejecting food additives and preferring less processed foods.
Arwa Foodtech AB is a company that specializes in the use of baobab fruit, an acidic, pectin-rich fruit hailing from sub-Saharan Africa. Since Arwa Foodtech is built around the baobab fruit, our formulation should be based on baobab as well.

Several plant protein powders from different suppliers were assessed for the project, and PDCAAS for all possible protein combinations between the powders were calculated. Pea protein and Oat protein was selected as the best combination, with a PDCAAS over 100% when combined at a ratio of 44% Oat and 56% Pea.

It quickly became clear that the biggest challenge when working with plant proteins was the mealy mouthfeel caused by protein insolubility. To fight this, the influence of pH and salt concentration on mealiness was investigated, and it was found that low pH and high salt content resulted in a smoother mouthfeel. However, this led to the formulation having an unacceptably tart taste. This was balanced by adding sweetness in the form of apple juice concentrate.

The influence of protein concentration and cooking temperature on the gel strength of the product was investigated as well, and was compared to the gel strength of several competing products. The results showed that our formulation had very similar yield- and breakpoints to commercial products, indicating similar consumer experience from the product.

After further sensory analysis, a final formulation was decided, containing baobab fruit, pea protein concentrate, oat protein concentrate, apple juice concentrate, water, coconut oil, and salt. The formulation has no E-numbered additives, has a protein content of 7,8 grams per 100g, 20,6 E% from protein, and has a PDCAAS score of 100%.

This base is now ready for further development at Arwa Foodtech in order to colour and flavour the base, thus creating nutritious and sustainable products ready for the supermarket shelves.

Preface

This thesis would not have been possible without all of the wonderful people at Aventure AB, who were an overall joy to work with, and who volunteered as participants in our sensory panels even when the formulations were far from palatable.

A special thanks goes out to Arwa Mustafa, for initiating the project and for pushing us to leave the theory and explore reality in the lab instead.

A special thanks also goes out to Shohreh Askaripour, for being the godmother of the development lab, for always doing more than what could be reasonably expected from her, and for making us and all the other master students feel at home.

We want to thank Olof Böök for giving us the opportunity to join Aventure AB for this project, and making us feel included at the company.

We also want to thank Yvonne Granfeldt, our supervisor who always made time for us when we were confused or felt that we were on deep water.

Last but not least we want to thank each other for great teamwork, a fun spring, and many thrilling games of table tennis during lunch.

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1 List of abbreviations

Abbreviation	**Explanation**
SAA	Sulphur-containing Amino Acids
AAA	Aromatic Amino Acids
PDCAAS	Protein Digestibility Corrected Amino Acid Scores
LM pectin	Low-methoxylated pectin
HM pectin	High-methoxylated pectin
BP	Banana Puree
AP	Apple Puree
AC	Apple (juice) Concentrate
LBB	Liquid Baobab Base
OPC	Oat Protein Concentrate
PPC	Pea Protein Concentrate
FBPC	Faba Bean Protein Concentrate
MPC	Mycoprotein Concentrate
PPI	Pea Protein Isolate

2 Introduction

Nowadays, many consumers are becoming aware of the effect the food industry has on the environment, regarding greenhouse gas emissions, water and land usage and its effect on biodiversity. This has started to affect people's consumer habits. More and more people have started to incorporate alternatives to meat and dairy into their diets, both to reduce their footprint on the environment but also to live a healthier lifestyle. Alternatives which are plant based and with locally produced ingredients are getting more and more popular among the consumers.

The industry for meat and dairy alternatives has been growing significantly over the last couple of years, especially the market of alternatives to dairy which has an annual growth in Sweden of around 17% (Malm, 2020). In the industry for dairy alternatives, many alternative products have been released, ranging from milk, to yoghurt, to ice cream. For most of the traditional dairy products, there are several alternatives to choose from, but for quarg, there is a shortage of alternatives.

Quarg is created with lactic acid fermentation which helps creating its creamy texture and its acidic taste. Quarg usually contains a lower amount of fat compared to other dairy products, but high fat versions do exist. The fat content usually ranges between 0.05-10%. Quarg also has high protein content with about 12-14% of protein (Fox et al., 2017). Being low in fat and high in protein, the marketing of quarg usually targets people who live healthier lifestyles, especially people who exercise frequently and are especially interested in the protein content (Gregow, 2019).

In this project, in collaboration with Arwa Foodtech AB and Aventure AB, the aim was to create and develop a base for a spoonable, baobab-based, protein-rich snack, which should be similar to quarg, but free from ingredients of animal origin. This base should have nutritional similarities to quarg, especially when it comes to protein. Several plant-based protein sources were investigated and combined to ensure a high-quality protein for the product. Local products were used as much as possible to reduce the environmental impact even further. The product had no added sugar nor additives in order to achieve a clean label.

The base of the product was baobab fruit, grown on the tree *A. digitata L* which can be found in sub-Saharan Africa. Baobab fruit has an acidic taste, which can resemble the acidity of quarg, but it also contains high concentrations of pectin, which has functional properties (Council, 2008). These functional properties were believed to be beneficial for the texture of the product. Baobab is also the core of Arwa Foodtech AB's company identity, making use of the fruit key for marketability.

3 Aims

The aim of this project was to develop a base for a high protein, spoonable snack free from ingredients of animal origin, based on Arwa Foodtech AB's proprietary liquid baobab base (LBB). The term "base" is of importance, as the aim was not to develop a final product ready for the market, but rather a baseline formulation that gives the product its nutritional and textural characteristics. When this base formulation has been developed, other ingredients may be added to achieve different tastes, aromas, and colours to develop a range of products based on this single base formulation.

The desired properties of the formulation were as follows:

- It should be rich in protein, preferably over 20% of the caloric content should come from protein.
- It should have as high protein quality as possible.
- It should be free from ingredients of animal origin.
- It should contain baobab fruit.
- It should not contain E-numbered additives.
- It should contain locally produced ingredients when possible.
- It should have rheological properties similar to relevant competitors. Relevant competitors refers to dairy based quarg and plant-based alternatives.
    - This means it should have semisolid, gel-like characteristics.
    - This means it should have yield- and breakpoints in the same range as relevant competitors.
- It should have as smooth a mouthfeel as possible.
- It should have an acceptable taste.
- It should have an acceptable aroma.

To be able to develop a base that fulfils all these criteria, some subgoals needed to be met.

First, protein quality must be assessed, and a protein mix selected.

Second, the parameters that impact the textural and rheological properties of the formulation must be identified.

Third, the sensory properties of the formulation must be tuned such that the base is acceptable to consumers, and ready for further development.

4 Theory and Background

Throughout this project many different ingredients have been used and investigated to enable. The ingredients used are listed in section 5.1, Materials. Some of the ingredients will be discussed in this section, as understanding of their functional properties is key for understanding this project.

4.1 Baobab

Baobab is a part of a genus of trees which mainly grow in the mainland of Africa, in Madagascar and in Australia (Council, 2008). Its scientific name is *Adansonia digitata L,* and it is native to the African continent. *A. digitata L* thrives in dryer climates with soil mainly made up of sand in areas such as savannas. The baobab tree has traditionally been a valuable source of food, water, shelter, medical uses etc. Not only the fruit, but many parts of the tree provide important functions for the consumers.

The white chalky fruit pulp can be used to create a porridge or milk with a high nutritional value, also the husk of the fruit can be used to provide meals (Council, 2008). Even the leaves are edible and can be considered as a source of protein. They also contain plenty of micronutrients (Gebauer et al., 2002). The seeds are a rich source of energy, protein and micronutrients which can be prepared for consumption in several ways such as raw, roasted, fermented, or made into a porridge etc (Council, 2008). The trunk of the tree can be used as a source of water as one single baobab trunk can store as much as 10 000 litres of water. The tree also has several uses with medical purpose. Different parts of the tree can be used to help with back- and stomach pain, diarrhoea, kidney- and bladder disease and many more issues.

The fruits of the baobab tree are a valuable food source with good nutritional value which is shown below in Table 1 and Table 2:

Table 1: Nutritional value of baobab fruit pulp, macronutrients (Stadlmayr et al., 2010).

Per 100g	**Energy [kcal]**	**Water [g]**	**Protein [g]**	**Fat [g]**	**Carbohydrates [g]**	**Fibre [g]**
Baobab fruit pulp	337	10.7	2.7	0.7	76.7	6.8

Table 2: Nutritional value of baobab fruit pulp, micronutrients (Stadlmayr et al., 2010)

Per 100g	**Ca [mg]**	**Fe [mg]**	**K [mg]**	**Vit A [µg]**	**β-carotene [µg]**	**Vit C [mg]**
Baobab fruit pulp	251	8.4	2010	16	70	222

The pulp from the baobab contains polysaccharides such as pectin which provides beneficial gelling properties (Ndabikunze et al., 2011). The percentage of pectin in the pulp of the baobab has been measured at 2.56% in fruit pulp. In dried baobab fruit pulp, the pectin levels are drastically higher and ranges between 23.4-33.8 % (Asogwa et al., 2021).

4.2 Quarg

The goal of this project is to develop an alternative to quarg, similar in nutrition and texture but free from ingredients of animal origin. In order to do this, it is of importance to have a good understanding of quarg, from several perspectives.

Quarg is a type of fresh cheese with a creamy mouthfeel and semisolid characteristics, similar to a Greek-style yoghurt. The texture of quarg is achieved by addition of small amounts of rennet and lactic acid bacteria fermentation (Yadav et al., 2019). The fermentation lowers the pH of the product to around 4.6 which causes the protein to aggregate and form a 3-dimensional network. The fermentation also gives the product an acidic flavour. Unflavoured quarg is white in colour, but flavoured versions are commonly found in Swedish supermarkets. These can vary in colour from white to purple, depending on the flavouring.

Quarg is often consumed as breakfast or as a snack in packages of the size range between 150-200g. It is eaten as a yoghurt and can be bought at most supermarkets and at other locations as well, such as the gym as it targets the consumer group who lives a healthier lifestyle. The companies that sell quarg usually focus on putting the protein and fat content on their packaging as it is appreciated by their targeted consumer group.

Quarg usually comes in two types, low fat, and full fat. A low fat quarg has a high moisture content at 82 % and protein content at 12.5-13.5% (Fox et al., 2004). Quarg usually contains lower amounts of fat and carbohydrates at 0.05% and 3-4%. For full fat quarg, the moisture content is lower at 76% but with equal amount of protein at 12.5-13.5%. The fat and carbohydrate content are usually at 9.6% and 2.5-3.5%. Quarg also contains less calcium compared to other dairy products since the calcium is removed with the excess liquid during production (Yadav et al., 2019). During this separation, most of the soluble whey is also removed with the liquid. Quarg has quite a high lactose content which usually makes up a major part of the carbohydrates content.

The flow behaviour of quarg is described as shear thinning, the viscosity is lowered as the shear rate increases. This behaviour is more noticeable at temperatures below 4ºC (Fox et al., 2017). As the fat content increases, the product acts more as a fluid and less shear rate is needed for the shear thinning properties. Quarg can be described as a weak viscoelastic gel since the storage modulus G’ is larger than the loss modulus G’’ at low shear stress (Tunick, 2000).

4.3 Protein quality

The FAO and WHO have long acknowledged that protein intake has a significant impact on human health (World Health Organization, 2007). Protein intake is needed for normal body function and for upholding the nitrogen balance in the human body. Protein intake is also important for body growth, and for temporal increase in metabolic requirements, such as pregnancy or lactation.
However, it is not only the amount of protein that matters. The ratios of the amino acids that make up the proteins are also of importance, particularly the nine essential amino acids: Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine. These amino acids are called essential because the human body cannot synthesize them (at least not at high enough capacity), hence they need to be acquired through the diet.

While the nine essential amino acids are required from the diet, some other amino acids that can be synthesized in the body can be consumed partially in place of an essential amino acid (World Health Organization, 2007). This has made the FAO/WHO recommend the use of combined requirements for Sulphur-containing amino acids (SAA), referring to methionine and cysteine, and Aromatic amino acids (AAA), referring to Tryptophan, Phenylalanine, and Tyrosine.

While most foods contain all essential amino acids, most foods do not contain them in sufficient quantities (World Health Organization, 2007). Some foods do however, usually animal proteins like eggs and whey. This does not mean that people who do not consume animal proteins cannot easily obtain the essential amino acids they need, but it does mean that they should be more mindful about protein quality, something that is most commonly measured by calculating a food's Protein Digestibility Corrected Amino Acid Score, or PDCAAS for short.

4.3.1 PDCAAS

PDCAAS is the protein quality index recommended by the World Health Organisation. (World Health Organization, 2007) It is calculated by first measuring the digestibility of the protein source. This is usually done via *in vivo* studies where a known amount of a given protein is ingested, followed by measurement of nitrogen content in the faeces, corrected for baseline nitrogen excretion. The digestibility is then calculated as in equation 1 below:

$$Digestibility\ (\%) = \frac{I - (F - F_k) \times 100}{I} \qquad 1$$

Where I = Nitrogen intake, F = Faecal nitrogen loss on test diet, and F_k = Faecal nitrogen loss on a protein-free diet.

Then the amino acid score for each amino acid is determined. This is done by dividing the amount of a certain amino acid in 1 g of test protein by the mg/g value of the same amino acid in the WHO requirement reference pattern, as shown in equation 2 below.

$$Amino\ Acid\ Score = \frac{mg\ of\ Amino\ Acid\ in\ 1\ g\ test\ protein}{mg\ of\ Amino\ Acid\ in\ requirement\ pattern} \qquad 2$$

The requirement reference pattern depends on age, and details how much of a given EAA the body needs for normal function. Historically, the standard pattern has been that of preschool children, aged 1-2. However, since 2007 the WHO recommends using the pattern for 3-10 year olds for products aimed at non-infants. WHO's requirement reference patterns for all ages are shown below in Table 3.

Table 3: WHO Amino Acid Scoring pattern, 2007 (mg/g protein)

Age (years)	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
0,5	20	32	66	57	28	52	31	8,5	43
1-2	18	31	63	52	26	46	27	7,4	42
3-10	16	31	61	48	24	41	25	6,6	40
11-14	16	30	60	48	23	41	25	6,5	40
15-18	16	30	60	47	23	40	24	6,3	40
18 +	15	30	59	45	22	38	23	6,0	39

After all amino acid scores have been calculated, the amino acid with the lowest score is identified as the limiting amino acid. The limiting amino acid score is then multiplied by the digestibility of the food to get the PDCAAS index of the food, as shown below in equation 3:

$$PDCAAS\ (\%) = Limiting\ Amino\ Acid\ Score\ \times Digestibility\ \times 100 \qquad 3$$

If the protein is of very high quality, it is possible to get a PDCAAS index greater than 100%, however if this occurs the value is truncated to 100% (World Health Organization, 2007).

PDCAAS can be calculated for foods composed of several protein sources if the amino acid composition and digestibility of each protein source is known (World Health Organization, 2007). This is done by using equation 4 below, where *X* denotes a specific amino acid, n is the number of protein sources, *i* denotes a specific protein source, and *AA* is short for amino acid.

$$Amino\ Acid\ Score_{Amino\ acid\ X} = \frac{\sum_{i=0}^{n}(Digestibility_i \times Ratio_i \times mg\ AAX_i)}{mg\ AAX_{reference\ pattern}} \qquad 4$$

When all amino acid scores have been calculated, the PDCAAS is simply the lowest amino acid score multiplied by 100.

4.4 Nutritional claims

Today, it is common knowledge that diet, and health have a strong connection. By following and consuming a healthy diet, the risk of non-communicable diseases (such as diabetes, heart diseases and many more) are drastically reduced, which helps people live a longer and happier life.

The European union has an many regulations for food products to ensure the safety of the food and to communicate to the consumers which product contribute to a healthy diet. The EU defines nutritional claims as an indication that the product has nutritional benefits based on the calories content or the nutrients in a product according to the Council directive 1924/2006 (2014).

For protein, there are two types of claims which may be achieved, “source of protein” and “high protein”. To reach the claim of “source of protein”, 12% of the energy of the product needs come from protein and for “high protein” that amount is 20%.

Fat has four categories. “Low fat” and “fat free” cannot contain more than 3g and 0.5g of fat per 100g, respectively. “Low saturated-fat” and “saturated fat-free” have their limits of saturated fat at 1.5g and 0.1g per 100g, respectively.

4.5 Protein solubility

Protein solubility is a functional property which is important for food formulation, as it is closely related to other functions such as emulsions, foams, and gels (Zayas, 1997). Protein solubility is affected by both internal and external properties. The internal properties which affect the solubility of a protein is the structure/confirmation, amino acid sequence, molecular weight and the number of amino acids which are polar or non-polar present in the protein. The external properties which influence the solubility of proteins include solvent-protein interaction, temperature, pH, and ionic strength.

4.5.1 pH

The solubility of a protein is closely tied to its isoelectric point (Li and Xiong, 2020). When the pH is at a protein’s isoelectric point, the surface charge of the protein is neutralized, which

causes a severe reduction in the interaction between the protein and the water, since the protein is effectively non-polar. This increases the possibility of protein aggregation, which leads to decreased solubility. This is due to the fact that electric repulsion between the proteins is decreased due to the neutral charge at the protein surface (Lam et al., 2018). When the pH is higher than the isoelectric point, the surface of the protein has a negative charge which increases the interactions of the hydrogen end of the water molecules (Li and Xiong, 2020). If the pH is lower than the isoelectric point, the charge of the protein surface is positive and causes increased interaction the oxygen in the water molecule.

4.5.2 Salt content

At lower salt concentrations, the protein solubility may increase depending on the specific protein (Zayas, 1997). This can be called "salting in" and is dependent on the ionic strength of the salt. The ionic strength can be calculated with equation 5 below:

$$\mu = 0.5 * \sum C_i Z_i^2 \quad (5)$$

Where μ represents the ionic strength, C_i is the molar concentration of the ion i and Z is the valence of the ion i. The solubility of proteins can be calculated with equation 6 below:

$$Log(S) = Log(S_0) - K\mu \quad (6)$$

Where S is the protein solubility and S_0 is the protein solubility when there is no ionic strength (μ=0), and the K is a constant specific to the system in question.

The mechanism of this can be described by the double-layer theory. For proteins, or any colloidal particles, it can be generally said that when the concentration of salt is close to zero, solubility is generally high, due to the electrostatic repulsion between the proteins. (Pashley, 2004) When a small amount of salt is added, a double layer is formed around the proteins, due to the attractive forces between the protein surface and the dissociated counter ions. This double layer is a cloud of ions swarming around the protein, attracted by the charged surface. Since the concentration of ions around the proteins is much higher than in the bulk fluid, when two protein particles approach each other the ion concentration in the space between them increases sharply. This leads to osmotic repulsion between the particles, increasing further the closer they approach one another. The screening length of this counter ion cloud, also called the Debye-Hückel length, is dependent on the valency and the concentration of the counter ions. The higher the concentration and valency, the shorter the screening length, and the greater the risk of flocculation. (Pashley, 2004) This is because flocculation usually occurs when particles approach one another with enough force to overcome the osmotic/electrostatic repulsion, leading to Van Der Waals interaction between the particles. The shorter the repulsion length, the lower the amount of force needed to get close enough for Van Der Waals bonding.

The Debye-Hückel length is not only dependent on the valency and concentration of the counter ions, but also the surface potential, which, in the case of proteins, varies with pH as the degree of protonation at the surface groups varies. (Pashley, 2004)

4.5.3 Temperature dependence and denaturation

Protein solubility is also dependent on the system temperature (Bogahawaththa et al., 2019). Higher temperature usually enhances the solubility until the temperature reaches high enough temperatures to denature the protein. Many proteins start to denature around 50°C, but this varies between proteins. When proteins denature, the three-dimensional structure of the protein unfolds, and interior groups of the protein, which are usually hydrophobic, are exposed to the solvent (Nick Pace et al., 2004). This usually results in a lower solubility even at the isoelectric point, assuming that the protein was soluble in native form. To increase the solubility, a larger change in pH or higher change in salt concentration is needed than for the native protein. Flocculation can occur when denatured proteins interact with other denatured protein via the hydrophobic effect, or cross-linkages (salt-bridges, hydrogen bonds etc). Flocculation leads to larger particles of protein forming, which further decreases solubility.

4.5.4 Oat Protein solubility

There are many different types of proteins in oats, but the majority of them are 12S globulins, a protein that has a relatively high solubility in salt solutions (Li and Xiong, 2020). However, even though the majority of the proteins are 12s globulins, the overall protein makeup will vary depending on the specific oat strain, growing conditions etc. Therefore, an exact isoelectric point for oat proteins overall cannot be determined, as it varies with protein composition. However, what can be said is that the average isoelectric point or region seems to be around 4.5 (Li and Xiong, 2020).

According to Li and Xiong (2020), solubility of oat protein with low molar concentrations of NaCl is highest below pH 4, preferably around pH 3 or lower. This is shown below in Figure 1. The solubility of oat protein is also increased for high NaCl concentration at higher pH (5-7,5), but this is usually not applicable in food formulations as the high salt concentration would result in an unacceptable taste.

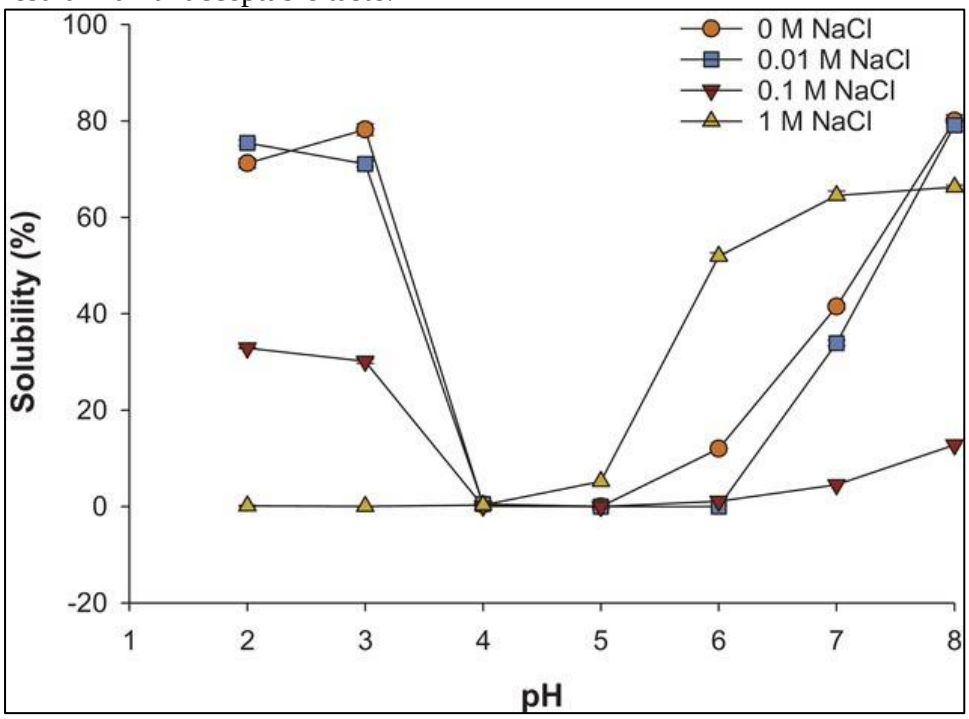

Figure 1: Effect on the oat protein solubility at different NaCl concentrations and pH (Li and Xiong, 2020)

4.5.5 Pea protein solubility

The main component (55-65%) of the pea protein is, as in many other plants, globulin. Globulins usually have high solubility in salt solutions (Lu et al., 2020). Albumin makes up around 18-25% of the protein in peas and is water-soluble. This combination of proteins gives an average of the isoelectric point around pH 4.3 (Doan and Ghosh, 2019).

Pea protein has its lowest solubility around its isoelectric point (Lam et al., 2018). To increase the protein solubility, the pH should be outside the range of 4-6 pH. A study with 5 different pea proteins was investigated regarding their solubility indicated that lower pH (around pH 2) has a solubility between 66-77%. Higher pH at pH 9, indicated an increased solubility at 70–95% and at pH 5 the solubility was only at 2–4%.

The addition of salt will increase the ionic strength which could increase the solubility (Lam et al., 2018). This is due to the ions from the salt contributing to an electric double-layer around the proteins. This increases the electrostatic repulsions and prevents aggregation which could lead to protein precipitation.

4.6 Hydrocolloids

Hydrocolloids are large molecules or particles that are typically used in food formulations to regulate macroscopic behaviour such as viscosity, or to induce gelling. In this project the two main hydrocolloids of interest are pectins and starches.

4.6.1 Pectin in general

Pectin is a group of naturally occurring polysaccharides that are commonly found in plant tissue (Axelos and Thibault, 1991). It is a thickener and gelling agent that forms viscoelastic gels, commonly seen in fruit jellies and fruit candies. Pectin is composed mainly of galacturonic acid residues linked α-(1-4). The regions where only galacturonic acid residues are linked together is called the homogalacturonan domains and forms the backbone of the pectin molecules. However, pectin is a diverse group of molecules, owing mainly to the composition and nature of the side chains attached to the homogalacturonan backbone. These side chains may contain different saccharides, but rhamnose is the most common.

Pectins are classified as either high-methoxylated (HM) or low-methoxylated (LM) (Axelos and Thibault, 1991). This distinction is made based on the degree of methoxylation on the homogalacturonic backbone, specifically on C6 which is normally a carboxyl group. If less than 45% of the galacturonic acid residues are methoxylated, the pectin is considered a LM pectin, if it is higher, it is considered a HM pectin. In Figure 2 below, LM pectin is shown as A, and HM pectin as B. (Belkheiri et al., 2021)

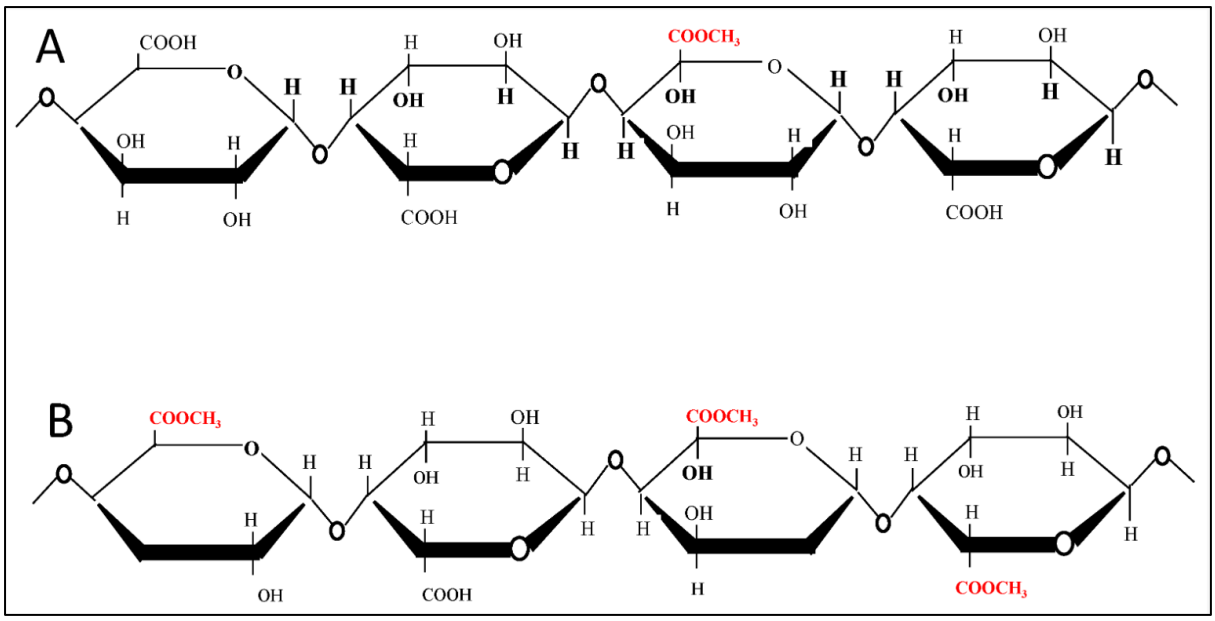

Figure 2: LM pectin (A) and HM pectin (B). source: Belkheiri et al. 2021

Baobab fruit pectin has a methoxylation degree of ~20% according to a 2021 study by Dimopoulou et. al, making it an LM pectin source. (Dimopoulou et al., 2021)

4.6.2 LM Pectin

LM pectin's ability to form gels, and the strength of the gel formed, is dependent on the following parameters: The molecular weight of the pectin, the charge distribution over the molecule, the degree of acetylation, the degree of amidation, the pectin concentration in the system, the calcium ion concentration in the continuous phase, the pH of the continuous phase, the ionic strength of the continuous phase, the sugar content of the continuous phase, and the temperature of the system (Axelos and Thibault, 1991).

Of these parameters, the most important and the easiest to control are the concentration of the pectin, the concentration of calcium ions, the pH, and the temperature (Axelos and Thibault, 1991). The mechanism of LM pectin gelation is most commonly described with the "egg-box"-model, which argues that the formation of a 3D network of pectin molecules occurs due to local association between molecules at "junction sites". In addition to these junction sites, the molecules also have regions that do not aggregate with other molecules, instead they exist as random coils, which connect the junction sites with each other.

The mechanism of the association is ion bridging, two chains of homogalacturonan lie close together, and between each chain lie calcium ions. These ions stabilize the associations due to electron sharing with the COO^- groups of two galacturonic acid residues, one in each chain. Without calcium ions, no bridging can occur, and no gel will form. However, if the calcium concentration is too high, syneresis will occur, so care must be taken not to add too much.

LM pectin is not overly sensitive to pH, but if the pH is low enough there will not be enough dissociated COO^- groups in the backbone available for ion bridging, and the junction sites will not form.

LM pectin gels are thermally reversible, with a melting temperature around 70 °C. Gelation does not occur immediately below 70 °C, but at the gelation temperature, which is determined by a range of factors and differs greatly.

Lastly, the gel strength of a LM pectin gel is dependent on the pectin concentration. Below a certain concentration, often referred to as C_0, the pectin molecules are too far apart to form a network, and can only exist as a sol. At concentrations higher than C_0 gel strength increases with pectin concentration. C_0 is a characteristic property of a particular pectin, and varies with source and co-solutes. (Axelos and Thibault, 1991)

4.6.3 Starches

Starch is present in most plant cells, existing as granules within the cells (Copeland et al., 2009). These granules vary in shape and size, from spherical to polygonal, from 1µm to 100µm. Starch is composed of two main molecules, amylose, and amylopectin. Amylose is a linear, unbranched polyglucan linked α-(1-4), and amylopectin is a branched polyglucan with chains of α-(1-4) linked glucose units interspaced with α-(1-6) branching sites. The ratio between amylose and amylopectin, as well as the structure of the amylopectin, greatly impact the crystallinity, shape, and functional properties of a starch. (Copeland et al., 2009)

An important phenomenon for starch functionality is gelatinization. Gelatinization is the process of de-crystallization of the starch granules, where water penetrates the granules and starts dissolving the crystalline structures. (Ratnayake et al., 2002) This leads to an increase in granule size and leaching of amylose into the continuous phase. The consequence is an increase in system viscosity. However, if the starch is heated past the gelatinization temperature, enough amylose is leached out into the continuous phase for another phenomenon to take place. This phenomenon is retrogradation, which occurs during cooling. During retrogradation the amylose molecules aggregate via hydrogen bonding, leading to the formation of an elastic gel. Over time this gel becomes firmer, which is thought to be due to partial amylopectin recrystallization. (Ratnayake et al., 2002)

4.6.3.1 Pea starch

There are many varieties of pea, or *Pisum sativum* as they are more accurately named (Ratnayake et al., 2002). Most pea varieties have peak starch gelatinization temperatures around 64 °C, however gelatinization is not complete until over 80 °C. According to Ratnayake et al, pea starches form firmer retrogradation gels than maize, wheat and potato, both immediately upon cooling and after storage.

4.6.3.2 Oat starch

Oat, also called *Avena sativa*, has a gelatinization peak temperature of around 66 °C, but gelatinization is not complete until around 73 °C (Punia et al., 2020). While pea starch forms very firm retrogradation gels, oat forms less firm but more elastic gels than most other cereals.

4.7 Rheology

Rheology can be described as the science of flow of matter in gas, liquid, and soft solid states, as well as the deformation of materials when force is applied (Schowalter, 1978). It can also be described as the behaviour of materials when strain or stress is applied (Steffe, 1996). In this study, rheology is of importance since the flow and viscoelastic properties of the product is important for the consumers acceptance of the product.

4.7.1 Viscometry

Measuring the viscosity of a liquid is usually done by using a rotational viscometer, which operates by submerging a probe in the liquid, and spinning the probe at a certain speed while measuring the force required to spin the probe. (Steffe, 1996). Liquids with complex compositions usually show complex flow behaviour as well, one example of this is thixotropic properties, where prolonged stirring of a liquid reduces the viscosity. Viscosity is also dependent on temperature, and the amount of stress applied to the liquid. This makes rotational viscometry quite limited in its applications. However, by using the exact same protocol for each sample, comparisons between the samples can be made. (Steffe, 1996)

4.7.2 Oscillatory rheometry

Viscoelasticity is a property that can be observed in certain materials which exhibit both viscous and elastic behaviours (Meyers and Chawla, 2008). The definition of viscosity can be determined by the ability to resist deformation when stress is applied, over time. Elasticity can be described as the ability a material has to return to its former shape and volume when stress has been applied (Steffe, 1996).

Oscillatory testing is commonly used when investigating the viscoelastic properties of materials (Steffe, 1996). Oscillatory testing can use tension, shear, and compression. A common test is parallel plate testing, which uses shear to investigate the viscoelasticity of the material. To perform a parallel plate test, the sample (in liquid, semifluid, gel or solid from) is placed between two plates. Then one of the plates starts to move (usually by rotation) with certain amplitude and frequency, while the force needed for this movement is registered. With this, the shear stress and strain can be calculated as the shear strain is a function of the radius of the plate and the length travelled. The shear strain depends on the distance to the centre of the plate. At the centre, where r=0, the strain is zero, but at the edge of the plate, where r=R, one can measure and calculate the maximum shear strain (γ_0) according to equation 7.
The variables for this type of test are the distance between the plates *h* (mm), the speed of the rotations or frequency ω (rad/s) and rotations amplitude or the number of radians the plate rotates each direction Ψ (radians). More details are shown in the Figure 3.

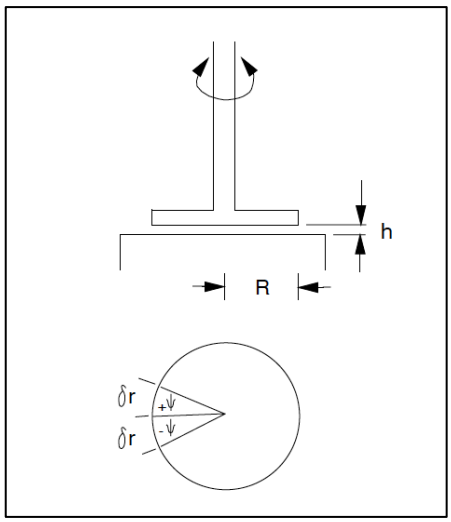

Figure 3: Parallel plate setup for oscillatory testing (Steffe, 1996).

The maximum shear strain can be calculated with the following equation.

$$\gamma_0 = \frac{R\psi}{h} \quad (7)$$

The maximum shear strain is used when calculating the shear strain (γ) at any given time (t) or frequency (ω) with equation 8 below.

$$\gamma = \gamma_0 \omega \cos(\omega t) \quad (8)$$

By having the amplitude (Ψ) or the frequency (ω) as the variable while the other one is constant, we can either perform a stress or strain sweep.

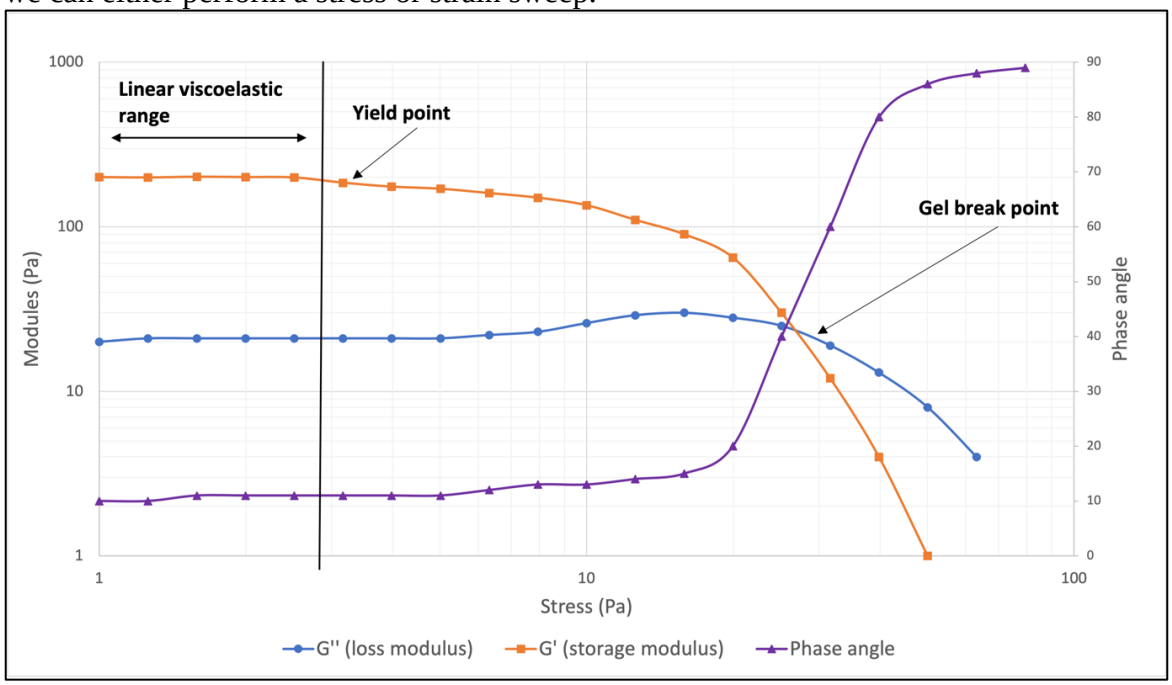

Figure 4: Shear stress sweep with increasing amplitude over time represented in storage and loss modulus and phase angle.

Figure 4 above represents the behaviour of a viscoelastic material when shear stress is applied. It shows the storage modulus (often denoted G') and loss modulus (often denoted G'') (Joyner, 2019). Storage modulus can be described as the energy which is stored in the elastic structure and represents the elastic behaviour. The loss modulus represents the viscous behaviour, the energy which is lost when stress is applied. Figure 4 also represents the phase angle.

The phase angle describes if a material has a viscus or elastic behaviour, and the phase angle ranges between 0 to 90 degrees (Steffe, 1996). If the phase angel is below 45 degrees, the indicates more solid-like behaviour which stores more energy and when the phase angle is over 45 degrees, the sample act more like a liquid which store very little energy (or none at all). When the phase angle is exact 45 degrees, it indicates the same result as when the storage and loss modules intercept, or in other words, when G'=G'', (as seen in Figure 4), it indicates the gel break point of the material.

Yield and gel break point are two measurements which are often used to describe the viscoelastic behaviour of semi-solids (Mezger, 2011, Joyner, 2019). Yield stress can be described as the amount of stress which can be applied without change in the internal structure

which is reversible to the structure. The yield point may be calculated in different ways. One option to calculate the yield point is to first establish the linear viscoelastic range and when the linear viscoelastic range decreases with 1-10% (exact percentage needs to be defined before experiment), the yield point is found. The linear viscoelastic range is determined by the plateau of the first measurements according to Figure 4.
The gel break point or the flow point can be defined as the amount of applied shear stress needed to break the internal structure, leading to a change from elastic, solid-like behaviour to a liquid-like behaviour where the loss modulus surpasses the storage modulus. This deforms and distributes the material, or in other words, causes the sample to flow.

4.8 Sensory analysis

Sensory analysis is the measurement of product attributes as we humans perceive them with our senses. (Lundgren, 1981) These attributes can be taste, smell, appearance, mouthfeel, or any other perception. The importance of sensory attributes should not be underestimated since the success of a food product on the market depends on people's perceptions of it.

4.8.1 Descriptive test

A descriptive sensory test is often used to identify and receive data on the intensity of different characteristics of the samples (Lawless and Heymann, 2010). It gives information about how intense a certain sensory aspect of a product is. When performing a descriptive test, it is preferrable to use a trained panel.

Before performing a descriptive test, one should carefully decide which aspects of the product the participants should assess and the objectives of the test (Lundgren, 1981). The sensory aspects should be ranked on a scale, with clear definitions for the different levels. The samples need to have a code, usually a randomized 3-digit code, when presented to the panellists. When the samples are presented, it should be in a randomised order. The panellists should be provided something to cleanse and refresh their palate between samples, such as water and white bread or crackers. All the participants should receive clear information about the test itself and what they should do.

4.8.2 Hedonic test

A hedonic or affective test is used to determine how much the participants like or dislike a product (Lawless and Heymann, 2010). It can be used to determine which sample is more preferred, and to investigate the level of acceptance of a product. Hedonic tests do not require a trained panel but should have more participants for more secure results. The most common practice is that the panellists rank the product on a 9-point scale, where 1 represents "dislike extremely", 9 represents "like extremely", and 5 represents "neither like nor dislike".

Before a hedonic test, one needs to decide which sensory aspects of the product which the panellists need to assess, such as *taste, aroma, texture* etc (Lundgren, 1981). The samples need to have a code, usually a randomized 3-digit code, when presented to the panellists. When the samples are presented, it should be in a randomised order. The panellists should be provided something to cleanse and refresh their palate, such as water and white bread or crackers. All the participants should receive clear information about the test itself and what they should do.

5 Materials and methods

Throughout this project, many different experiment and investigations has been performed. This has resulted in many different materials and methods in different steps. That's why this section has been divided into the section *material* and *methods*, which is further divided into sections *analytical methods* and *sample preparation methods* and *product development steps, in chronological order*. This was done to distinguish the actual methods used and the work process for this project.

5.1 Materials

The materials used are divided into two subgroups, ingredients, and equipment. In this section, the material is only presented and summarised, for further details when the ingredient and equipment was used, see section Product development steps, in chronological order, 5.3.

5.1.1 Ingredients

- LBB – Liquid baobab base – Made in house
    - The dry matter of the LBB varies throughout the project which result in an adjustment in some of the formulation so the total solid of baobab is content in all formulations. When the LBB content varies between formulations, it was due to adjust to the different dry matters or adjust the tartness of the formulation.
- Sodium chloride (NaCl), Iodised - JOZO
- Calcium chloride ($CaCl_2$) - in house at Aventure
- Tricalcium citrate ($Ca_3(C_6H_5O_7)_2$) – Cladic *food service and retail*
- Banana puree (BP) – in house at Aventure
- Apple puree (AP) - in house at Aventure
- Apple juice concentrate (AC) - Kivik
- Rapeseed oil - Garant
- Coconut oil – Kung Markatta
- Pea protein isolate – Supplier not disclosed due to confidentiality
- Pea protein concentrate – Supplier not disclosed due to confidentiality
- Oat protein concentrate – Supplier not disclosed due to confidentiality
- Faba bean protein concentrate – Supplier not disclosed due to confidentiality

A summary of the nutritional profiles of the ingredients used are found below in Table 4.

Table 4: Nutrient content per 100g of ingredients used

Nutritional values	**Energy [kcal]**	**Protein [g]**	**Fat [g]**	***Saturated fat [g]***	**Carbohydrates [g]**	***Sugar [g]***	**Fibre [g]**
LBB (15.8% DM)	58.0	1.38	0.50	-	10.5	*5.04*	2.70
Pea protein isolate	422	84.0	9.50	*2.30*	0.20	*0*	2.30
Pea protein concentrate	355	55.0	3.00	*0.45*	26.0	*0*	2.00
Oat protein concentrate	423	53.0	13.0	*2.60*	21.0	*0.70*	4.60

Faba bean protein concentrate	338	60.0	3.80	*0.70*	11.5	*1.60*	13.6
Apple puree[A]	52.0	0.27	0.16	*0*	12.3	*9.66*	2.64
Banana puree[B]	98	0.74	0.74	*0*	23	*21.7*	1.3
Rapeseed oil	824	0	99.6	*7.50*	0	*0*	0
Coconut oil[D]	900	0	100	*93*	0	*0*	0
Apple juice concentrate[E]	225	0	2.50	*0*	50.0	*50.0*	3.50

A: (USDA, 2022), B:(USDA, 2020), C:(Zeta), D:(KungMarkatta, 2022), E: (Granatskafferiet).

Several different protein sources were used and/or assessed throughout the project. These proteins can be found in Table 5 below.

Table 5: Protein powders assessed with Protein Quality Optimization Program, source: supplier companies

Protein Source	Product type	Acronym	Protein content	Country of origin
Oat	Concentrate	OPC	55%	Sweden
Pea	Concentrate	PPC	55%	Norway
Faba bean	Concentrate	FBPC	60%	Canada
Mycoprotein	Concentrate	MPC	60%	Sweden
Pea	Isolate	PPI	85%	Israel

The amino acid composition of each protein powder was obtained from the different supplier companies. The amino acid composition of each protein source is presented in Table 6.

Table 6: Amino Acid concentrations, mg/g protein in protein powders, source: supplier companies

Product	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
OPC	20	37	70	30	36	101	27	13	48
PPC	26.6	43.9	76.6	76.6	21	94.4	37.8	10.0	48.0
FBPC	26.3	42.1	75.4	66.7	17.5	86.0	35.1	10.5	45.6
MPC	17.1	33.6	51.9	50.5	23.6	71.4	32.0	12.9	38.5
PPI	26.2	47.6	82.1	75.0	19.0	104.8	38.1	8.3	51.2

Some suppliers had conducted digestibility studies on the specific products, but most had not. However, a 2015 study conducted by Rutherford et. al. showed that faecal digestibility usually varies between 88% to 99% for plant protein concentrates. For the products with unknown digestibility, 88% was therefore assumed as a conservative estimate (Rutherfurd et al., 2015).

5.2 Methods

Several different methods were used in the pursuit of a formulation that satisfies all the criteria in the aims section. In this section they are all listed and described.

5.2.1 Calculations

Two main methods for calculation were used, one was a general approach to calculating the nutritional profile of the product, and one was a python script that calculates the PDCAAS scores of all possible combinations between a set of proteins, and outputs the combinations that scored the highest.

5.2.1.1 Protein Quality Optimization Program

As stated in the section Aims, 3, an aim of this project was to find a protein combination that resulted in as high protein quality as possible. PDCAAS was chosen as the index for protein quality, as it is recommended by the WHO. In order to calculate the PDCAAS of different protein mixes, and find the mixes resulting in the highest PDCAAS, a computer program was developed in python. The program takes the names, digestibility, and amino acid profile of different protein concentrates/isolates as inputs. It also takes the wanted number of "top candidates" based on PDCAAS, and the "resolution percentage", i.e. the smallest allowed percentage unit. If resolution was set to 1, ratios like 51:49 was allowed, while if the resolution was set to 5, only ratios like 55:45 was allowed.

The program then creates a vector for each protein, from 1 to 100, with the steps in between depending on the resolution percentage input. If the resolution was set to 1, the vector would be [1,2,3…10]. The program then calculates the cartesian product of all of these vectors. However, only the combinations that add up to 100 (as in 100%) was of interest, hence these combinations was saved in a new vector, called the ratios vector.

The program then runs through all the possible ratios and calculates the PDCAAS for the given mix. For every mix, the amino acid scores for each amino acid was calculated according to equation 4 in the theory section.

Since digestibility has already been factored in, the program simply finds the limiting amino acid in the mix and multiplies its score by 100, which gives the PDCAAS index for the mix.

The program then sorts the protein mixes by PDCAAS score and outputs the mixes resulting in the highest scores. It also outputs which amino acid was the limiting one.

5.2.1.2 Nutritional profile calculations

The nutritional values for the different formulations was calculated based on the values in Table 4. The percentage of each ingredient was multiplied with one specific measurement, energy for example, and was then added together. Equation 9 below shows how each component is calculated.

$$c_{final} = \sum \frac{(c_i * X_i)}{100} \qquad 9$$

Where c_{final} is the final concentration [% w/w] of a nutritional component such as, *energy, fat, protein etc.* c_i is the concentration of the nutritional component in ingredient *i*. X_i represents the percentage at which ingredient *i* is present in the formulation.

5.2.2 Analytical methods

A number of analytical methods were applied to measure properties of the formulations and ingredients in many steps of the process. These methods are listed below.

5.2.2.1 pH

The pH was measured using Mettler Toledo FiveEasy Plus (Zürich, Switzerland). The instrument uses an electrode which was lowered into the sample until it reads a steady pH level. The electrode needs to be rinsed with distilled water before and after each sample and dried off gently with absorbent paper. The electrode was stored in KCl between samples and when not in use. If needed a 2-point or 3-point calibration was performed based on the manual for the instrument (Mettler-Toledo, 2006).

This method was used in section, Effect of NaCl and pH on protein solubility for different protein combinations, 5.3.3 and Increasing sweetness of the product, 5.3.5.

5.2.2.2 Dry matter content

The instrument Mettler Toledo HB43-S Halogen was used to measure dry matter. First, an aluminium sample pan was tared in the instrument. Then around 2.6-3.5g of the sample was placed on the sample pan. The sample was then heated at 105°C until the moisture loss was no longer continuing. The instrument then presents the amount of water lost from the sample as a percentage ((Mettler-Toledo, 2011). This procedure was replicated 3 times.

Dry matter was measured after each time a new batch LBB was made, which would alter the amount of LBB needed for the formulation so the amount of total solids of baobab would remain the same.

5.2.2.3 Relative viscosity

The relative viscosity of the samples was measured with a Brookefield DV1 Viscometer (Middleboro, USA). The instrument was autozeroed before each usage. A suitable spindle was chosen. During these investigations, the spindle 63 and 64 was used. The spindle was attached to the instrument and lowered into the cylinder containing around 100ml of the sample. The main settings which need to be set was speed, which was measured in RPM, the stress range in pascal and total time for the test in minutes (Brookfield).

This method was used in section, Effect of NaCl and pH on protein solubility for different protein combinations, 5.3.3 and Increasing sweetness of the product, 5.3.5.

5.2.2.4 Gel Yield- and Break point

The gel yield and break points were measured with a rheometer of model Malvern Kinexus Pro+ (Worcestershire, UK). The Rheometer was connected to a computer where the software rSpace was used to measure and analyse the results. The geometry used was serrated parallel plates. The test performed was a stress-controlled amplitude sweep. The settings used for each sample was the same, except for the initial and end shear stress, according to Table 7. The reason for the different start and end shear stresses was due to large differences in sample gel strength.

Table 7: Settings used for the amplitude sweep stress control

Settings	Gap [mm]	Amount [ml]	Temperature [°C]	Frequency [Hz]	Decades	Initial shear stress [Pa]	End shear stress [Pa]
	1	1.38	10	1	15	0.01-1	300-500

The initial and end stress varies depending on the texture of the sample, if the sample was less viscous it would require a lower initial and end shear stress setting. The gap indicates the gap between the parallel plates and the amount was the recommended sample amount from the machine. The samples was usually placed on the bottom plate, then the two plates was pressed together, excess samples being pressed out was scraped off and removed. The plates can adjust their temperature to fit the desired measuring temperature. The purpose with the amplitude stress test is that the frequency is constant, but the amplitude increases which increases the shear stress (Malvern, 2014).

This method was used in Effect of protein concentration and cooking temperature on gel strength, and comparison to existing products, 5.3.6.

5.2.2.4.1 Gel yield and break point calculations

Based on the data from the measurements of the rheometer, the yield and the gel break point can be calculated.

For the yield point, first the plateau in the linear viscoelastic range needs to be determined, as can be seen in Figure 4. This was done be identifying the maximum value of the storage modulus G'' and using the 4 closest values to the maximum to determine the plateau value. This was done by taking the average of the maximum value plus the 4 closest values. The yield value was defined as 95% of the plateau value, and linear interpolation between the data points was used to find the shear stress resulting in the yield value.

The calculations of the gel break point were done by linear interpolation between the datapoints, in order to find the shear stress value that resulted in a phase angle of 45 degrees.

While linear interpolation is not the most exact method of finding the right value, it is a fair estimate since the data points was clustered quite closely.

5.2.2.5 Sensory analysis

Sensory panels were used several times in the project to identify preferred formulations, since the most important parameters for the product development were usually not measurable properties such as dry matter content or pH, but rather the mouthfeel, taste, or aroma. As recommended by Lundgren (Lundgren, 1981) a 9-point scale was used in the sensory trials. The sensory data was mean centered and standardized to reduce data noise due to individual preferences. All sensory data was analysed in Matlab R2020a.

5.2.2.5.1 Descriptive tests

A sensory analysis was conducted where the samples were tested based on the following criteria, mealiness, tartness and saltness. The results from this investigation provided information regarding which direction, regarding LBB and salt concentration, which needed further investigation when it came to mouthfeel, which is related to protein solubility. The test

used was a descriptive test, where each panellist got to rank each sample on a scale (1-9) for each criterion. For the mealiness, the panellists were provided reference samples of "drick-kvarg", which represents a 1 one the scale and a mixture of cold water, protein concentrate (10% w/w) and LBB (10% w/w) which represents a 9 on the scale.
The panellist was provided with clear instructions of the procedure. They were provided water and white crackers which they was instructed to use between each sample to cleanse the palate. Each panellist received the samples in a randomized order and each sample had a randomized 3-digit code.

5.2.2.5.2 Hedonic tests

The panellists were given a sample of each and needed to rank the samples according to a range between 1-9, where 1 was "*extremely dislike"* and 9 was *"extremely like"* on a form. The areas which the panellists were judging the samples was *texture*, *mouthfeel*, *taste*, *aroma.*
The panellists were given water and crackers to use between samples to clear their palates. All the samples were encoded with a random 3-digit code and each participant receive the samples in a randomized order.

5.2.3 Sample preparation methods

The different methods used to produce the protein snack samples was outlined in this section. During the course of the project, shortcomings in the mixing methods were identified, and correction were made to rectify this, resulting in three different preparation methods for product samples.

5.2.3.1 Method 1 for product preparation

All the ingredients were weighed to the predetermined amount. All the ingredients were mixed manually until the all the ingredients had mixed with each other in a glass beaker. The glass beaker was placed upon a hot plate at the temperature of 175°C and the mixture was mixed with a magnetic stirrer. The mixture was stirred until it reached a predetermined temperature which was measured with an electric thermometer. When the mixture reached the desired temperature, the beaker was removed from the hotplate and mixer. Afterwards the mixture was placed in new plastic containers and placed in the fridge to cool down.

5.2.3.2 Method 2 for product preparation

The instruction steps for mixing method 2 was the same as in 5.2.3.1, but instead of using a magnetic stirrer, a mechanical paddle stirrer was used. A layer of aluminium foil was also placed over the glass beaker to prevent water loss.

5.2.3.3 Method 3 for product preparation

The instruction steps for mixing method 2 was the same as in 5.2.3.2 but instead of 1 layer of aluminium placed over the glass beaker, 4 layers of aluminium layers was used.

5.2.3.4 Liquid baobab base (LBB)

LBB was produced according to the internal company procedure for liquid baobab.

5.2.3.5 Milling pea protein concentrate

The pea protein concentrate was milled with Perten lab mill 120 (Hägersten, Sweden) at the lowest setting at 0.5 mm. Then the powder was strained with a strainer with the measurement of 0.155 mm. The powder with particles with higher particle size than the strainer was ground further with a mortar and pestle until desired particle size was achieved. The strainer, Retsch

Vibratory Sieve Shaker AS 200 Control (Haan, Germany), sieved the milled powder at the lowest strainer size at 0.150 mm.

5.2.4 Statistical methods

The most common type of data in this project was score patterns from sensory analyses, however, physiochemical data such as pH, Viscosity etc. was also processed. Data was generally considered normally distributed, which meant that using the student-t distribution for confidence intervals and ANOVA for spotting significant differences was valid. Matlab R2020a was used for data processing.

5.2.4.1 Mean centering data

Mean centering was applied to sensory data in order to remove individual preference bias. A person who finds acidic foods unpalatable in general would always rate an acidic food with low scores, while a person who enjoys acidic foods would rate the same food with high scores. When the point of interest was to find differences between samples rather than an absolute grade of a sample, mean centering helps give clearer resolution in the data.

Mean centering was done by first taking the mean of the scores given for all samples on a given characteristic, by a specific individual, as shown in equation 10. In equation 10 below, *n* is the number of samples set before the participant, *i* denotes a specific sample, and *X* denotes a specific individual.

$$Mean\ Taste\ score\ for\ person\ X = \frac{\sum_{i=0}^{n}(Score_{i,Taste})}{n} \qquad 10$$

Once the mean was calculated, it was subtracted from all scores given by this individual in this characteristic.

5.2.4.2 Standardizing data

Standardization was also performed on sensory data. Some individuals may use the whole scoring spectrum, while others may use only a small section of it. When the result of interest is not the absolute scores, but rather the differences between the samples, standardizing the data may remove the difference in scoring width, and increase the resolution of the data.

Standardizing is done by calculating the standard deviation of the scores given for all samples on a given characteristic, by a specific individual. The scores given by this individual in this characteristic was then divided by the calculated standard deviation.

5.2.4.3 ANOVA

Both one- and multi-way ANOVAs were used to determine whether results were statistically significant or not. The confidence level used was always 95%.

5.3 Product development steps, in chronological order

The product development workflow is a highly iterative process, and the steps taken in this project were decided by the results of the previous steps. Every step-in process was study aiming to solve a problem that prevented the criteria in the aims section from being reached.

For a clear overview of the steps and thy they were performed in the order described in the section, see Figure 5.

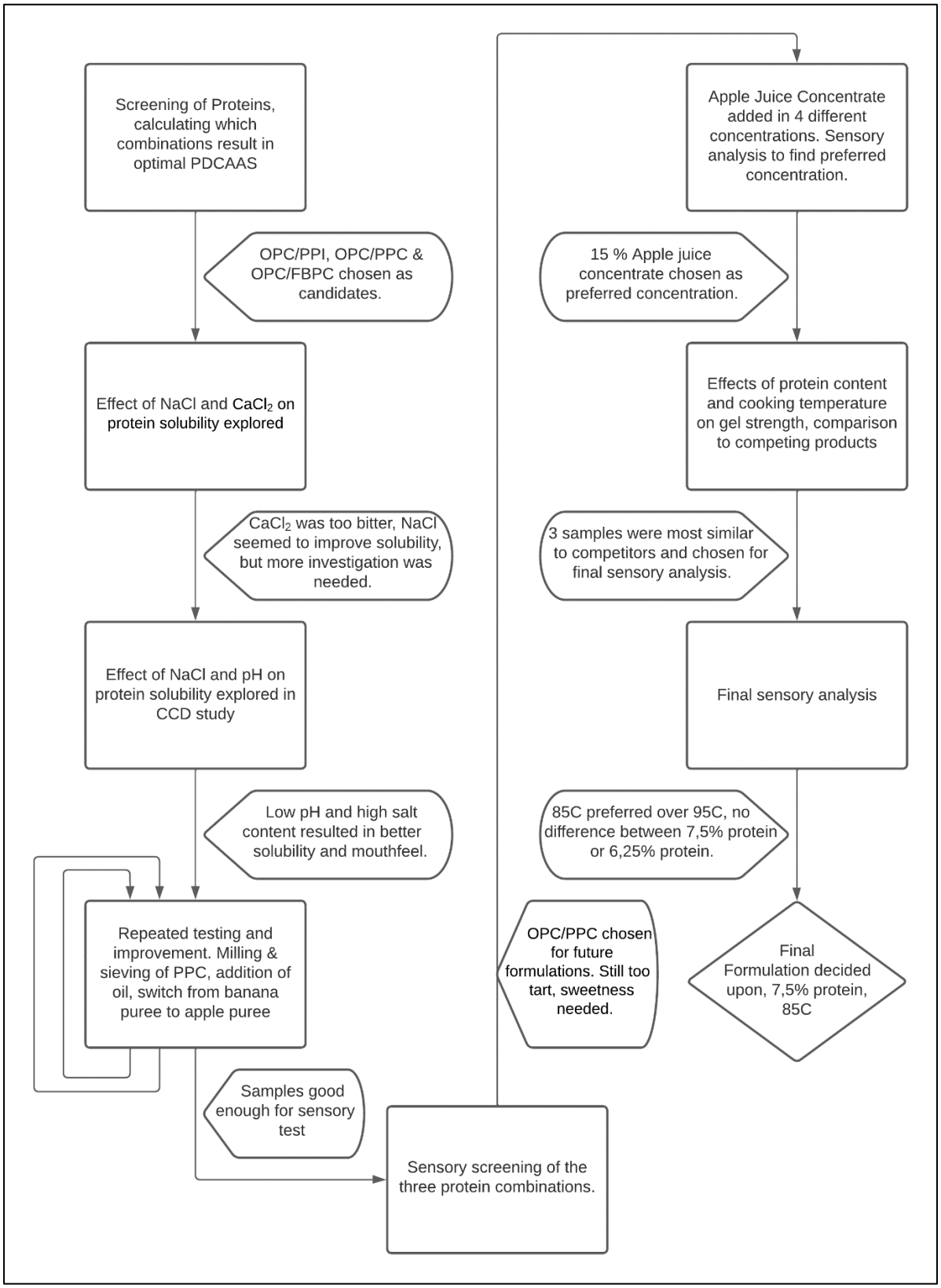

Figure 5: Flowchart of the Product Development process, showing a summary of activities, results, and decisions.

5.3.1 Calculation of PDCAAS for different protein sources and combinations

The criteria for protein powder decision were as follows: The protein should be free from ingredients of animal origin, be as high in protein as possible, be produced in or as close to Sweden as possible, not have too strong of a taste, and be readily available for purchase.

5 protein powders were selected for screening, (see Table 5) as they were free from ingredients of animal origin, readily available, and had a protein content over 40%. Due to confidentiality the product and company names have been omitted from the report. General characteristics of the powders are shown in Table 5 in section ingredients 5.1.1, Amino acid profiles are shown in Table 6. All data used is supplied by the supplier companies. The PDCAAS was calculated and optimized as described in section 5.2.1.1

5.3.2 Effect of salts on protein solubility

An investigation was performed with the aim to investigate the effects different type of salts have on protein solubility. The salts used were $CaCl_2$ and NaCl, and the protein source was oat protein concentrate. The composition of the samples was according to Table 8. The sample was mixed according to *section Mixing method 1* until it reached 85°C. The salts were added as they should improve the protein solubility. Another expected effect of $CaCl_2$ was increased viscosity, as LM pectin gelation increases with calcium content.

Table 8: Composition of trails for samples with different (or none) salt content.

	No salt Weight [%]	**$CaCl_2$ Weight [%]**	**NaCl Weight [%]**
Oat protein concentrate	10.0	10.0	10.0
LBB	10.0	10.0	10.0
Water	80.0	79.0	79.0
$CaCl_2$	-	1.00	-
Sea salt	-	-	1.00

5.3.3 Effect of NaCl and pH on protein solubility for different protein combinations

An investigation of the effect of salt and pH was performed for two different combinations of protein. The first combination was with an oat protein concentrate/pea protein isolate with a ratio of 46:54 and the second protein combination with oat protein concentrate/faba bean protein concentrate had a ratio of 41:59. These ratios were chosen due to the protein quality optimization program designating them as the best ratios. Banana puree was also added to this formulation to add sweetness and flavour to the samples.
The experiment was designed as two central composite designs, one for each protein combination. The varying parameters were salt percentage and LBB percentage, and five replicates of the central point were used. The experimental design is shown in Figure 6 below:

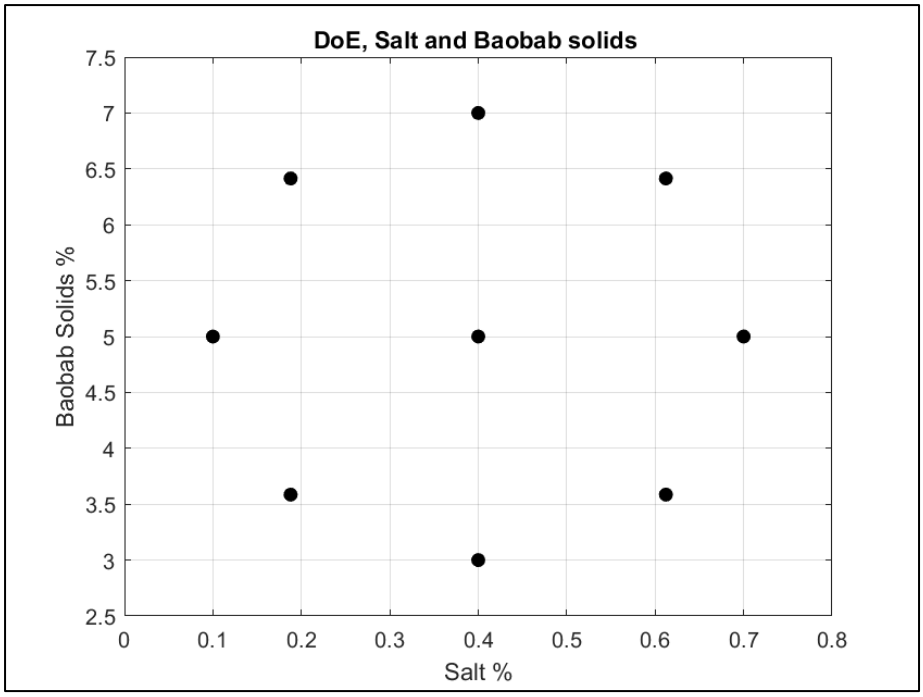

Figure 6: Design of Experiment: Salt and baobab solids content effect on protein solubility, 5 replicates of center point.

Samples of each of these protein combinations were made according to Table 9.
The preparation of each sample started with weighing of all ingredients (according to Table 9) and manual mixing in a 400 ml glass beaker. The beaker was then placed on a hotplate and stirred with a magnetic stirrer until it reached 85 °C. The product was then placed in 200ml plastic containers and stored in the refrigerator.

Table 9: Composition of samples containing oat protein concentrate and pea protein isolate or faba bean protein concentrate with the variables of salt and LBB concentration.

Samples	Salt [%]	LBB [%]	Water [%]	Banana pure [%]	Protein powder [%]
1	0.40	30.8	48.8	10.0	10.0
2	0.40	18.4	61.5	10.0	10.0
3	0.19	39.4	40.4	10.0	10.0
4	0.10	30.8	49.1	10.0	10.0
5	0.40	30.8	48.8	10.0	10.0
6	0.61	39.4	39.9	10.0	10.0
7	0.40	30.8	48.8	10.0	10.0
8	0.40	43.1	36.5	10.0	10.0
9	0.40	30.8	48.8	10.0	10.0
10	0.19	22.1	57.8	10.0	10.0
11	0.70	30.8	48.5	10.0	10.0
12	0.61	22.1	57.3	10.0	10.0
13	0.40	30.8	48.8	10.0	10.0

The different values of salt consent were decided based an observation of competing products on the market where the salt concentration ranged between 0.1-0.5%. The water content between samples did vary when LBB was increased or decreased, and the rest was compensated with water.

Viscosity and pH were measured for all the samples, more details can be found in sections *5.2.2.1* and 5.2.2.3 measurements *5.2.2.1* and relative viscosity measurements *5.2.2.3*. The settings for the viscometer were set at:

- Spindle: 63
- Speed: 30 RMP
- Range: 4-40 Pa
- Time: 3 min

A descriptive test was performed according to section Descriptive tests *5.2.2.5.1*. The sensory analysis had 5 panellists which tested 26 samples in total throughout six occasions, three for each protein combination. As there were 13 samples for each protein combination, the number of samples for the two first sensory occasion was 4, and for the last occasion there was 5 samples. For each occasion, mealiness reference samples were provided, see 5.2.2.5.1. The occasions were divided throughout 2 days, with three occasions per day.

5.3.4 Deciding final protein combination

The decision of which protein combination would be most suitable for the formulation took several sub-experiments before the final decision. These experiments are described below.

5.3.4.1 *Effect of different protein combinations with higher protein concentration has on sensory perception.*

Three protein combinations were prepared and tested to enable a decision of which combination of protein source which should be used. The three different formulations were the same except for the source of protein, as can be seen in Table 10. All three protein combinations did contain oat protein concentrate, but the second protein source would vary. The three protein sources were faba bean protein concentrate, pea protein isolate and pea protein concentrate. The ration and amount of each protein did vary depending on how much protein was needed to ensure that 20% of the total energy of the formulation would come from proteins.

Table 10: Composition of each sample with different protein sources and combinations.

	FBPC / OPC [%]	**PPI / OPC [%]**	**PPC / OPC [%]**
Oat protein concentrate	3.00	2.70	3.64
Pea protein isolate	-	3.20	-
Faba bean protein concentrate	4.48	-	-
Pea protein concentrate	-	-	4.61
LBB (16.75 % DW)	60.0	60.0	60.0
Salt	0.60	0.60	0.60
Banana puree	10.0	10.0	10.0
Water	21.9	23.5	21.2

An internal sensory analysis was conducted with an analytical test.

5.3.4.2 *Effect of lowered salt and LBB concentration and addition of tricalcium citrate.*

An adjusted formulation was developed, were the salt concentration and the amount of LBB was lowered and the banana pure was increased. These new parameters were tested on one of the protein combinations, as can be seen in Table 11. Tricalcium citrate was added as it may enhance the gelling properties of pectin and increase the protein solubility.

Table 11: Composition of adjusted salt and LBB concentration.

Ingredient	**Weight [%]**
LBB	55.0
Oat protein concentrate	2.70
Pea protein isolate	3.20
Salt or Tricalcium citrate	0.40 or 0.41
Banana puree	20.0
Water	19.1

5.3.4.3 *Effect of rapeseed oil and particle size of pea protein concentrate on sensory perception.*

The pea concentrate had a large fraction of big granules. These were strained with a 0,355mm mesh. The granules which did not pass were removed for further milling with a blade grinder and then strained again.

Table 12: Composition of samples of the protein combinations pea protein concentrate/ oat protein concentrate and faba bean protein concentrate / oat protein concentrate with added rapeseed oil and apple pure.

Ingredients	**Weight [%] (PPC/OPC)**	**Weight [%] (FBPC/OPC)**
LBB	55.0	55.0
Oat protein	3.24	2.70
Pea protein concentrate (fine grit)	4.15	-
Faba bean protein concentrate	-	4.00
Salt	0.40	0.40
Apple puree	20.0	20.0
Water	14.3	14.9
Rapeseed oil	3.00	3.00

The sample was then ground to a finer particle size with a mortar and pestle and was then sieved through a sieve with the pore size of 0.150 mm.
The formulation was identical as in Table 12, but with a lower particle size of the pea protein concentrate.

Apple puree was used instead of banana since it is a product which can be grown locally and could contribute with more sweetness. The rapeseed oil was added to dampen the tartness and provide better mouthfeel.

Further along, an investigation was made on the effect different types of oil have on the texture and taste on the product with two different protein combinations, oat protein concentrate mixed with pea protein concentrate or faba bean protein concentrate. The formulation of this investigation was identical to Table 12, except that the rapeseed oil was replaced with coconut oil. Coconut oil was added instead of rapeseed oil since it could mask the strong pea flavour and contribute to better texture of the sample.

5.3.4.4 *Protein screening, sensory analysis*

Three compositions were selected based on previous trials to decide which combination of proteins would continue to future trials. The formulations are shown in Table 13. A hedonic sensory analysis was performed with 12 panellists, according to Hedonic tests, 5.2.2.5.2. The panellists were given a sample of each and asked to rate different aspects of the samples on a scale from 1-9, where 1 is "*extremely dislike"* and 9 is *"extremely like".* The aspects which the panellists were testing was *texture*, *mouthfeel*, *taste,* and *aroma.*

Table 13: Composition for samples for final decision of protein combination.

Ingredients	**Weight [%] (FBPC/OPC)**	**Weight [%] (PPI/OPC)**	**Weight [%] (PPC/OPC)**
LBB	59.0	59.0	59.0
Salt	0.40	0.40	0.40
Coconut oil	3.00	3.00	3.00
Apple puree	20.0	20.0	20.0
Oat protein concentrate	2.70	2.35	3.24
Faba bean protein concentrate	4.00	-	-
Pea protein isolate	-	2.95	-
Pea protein concentrate	-	-	4.15
Water	10.9	12.3	10.2

5.3.5 Increasing sweetness of the product

This experiment aimed to find a new source of sweetness for the product. Apple pure was replaced or combined with apple juice concentrate according to Table 14. Apple juice concentrate is used to increase the sweetness of the sample.

Table 14: Composition of samples with the source of sweetness from apple pure, apple juice concentrate or both.

Ingredients	**AC weight [%]**	**AC & AP weight [%]**	**AP weight [%]**
LBB	55.0	55.0	60.0
Salt	0.40	0.40	0.40
Coconut oil	3.00	3.00	3.00
Apple puree	-	10.0	20.0
Apple concentrate	20.0	10.0	-
Oat protein concentrate	5.00	5.0	5.00
Pea protein concentrate	6.35	6.35	6.35
Water	9.25	9.25	5.25

Further investigations were conducted to decide which concentration of apple juice concentrate would be ideal, according to Table 15. The samples were prepared according to Method 1 for product preparation, 5.2.3.1 and a hedonic sensory analysis was performed according to section Hedonic tests, 5.2.2.5.2. pH was also analysed as described in 4.5.1.

Table 15: Investigation of different concentrations of apple juice concentrate in the formulation.

Ingredients	**AC 5%**	**AC 10%**	**AC 15%**	**AC 20%**
LBB	51.0	51.0	51.0	51.0
Salt	0.40	0.40	0.40	0.40
Coconut oil	3.00	3.00	3.00	3.00
Apple concentrate	5.00	10.0	15.0	20.0
Oat protein concentrate	3.24	3.24	3.24	3.24
Pea protein concentrate	4.15	4.15	4.15	4.15
Water	32.2	28.2	23.2	18.2

5.3.6 Effect of protein concentration and cooking temperature on gel strength, and comparison to existing products

A full factorial design was used when preparing the samples for the oscillatory rheometry. The variables for this investigation were 3 different added protein concentrations, and 3 different cooking temperatures which was 85, 90 and 95°C. The samples were only heated to the desired temperature and was immediately taken off when this temperature was reached. The sample was weighed to see how much water had been lost, water was added to compensate, and then a portion of the sample was poured out of the beaker. The beaker was then placed back on the heat, and the remaining sample heated to the next temperature. The formulations are shown in Table 16Table 16.

Table 16: Composition of the samples prepared for the rheology study

Ingredients	**Added protein 5%**	**Added protein 6.25%**	**Added protein 7.5%**
LBB	46.4	46.4	46.4
Salt	0.40	0.40	0.40
Coconut oil	3.00	3.00	3.00
Apple concentrate	15.0	15.0	15.0
Oat protein concentrate	4.15	5.19	6.23
Pea protein concentrate	5.09	6.36	7.64
Water	26.0	23.7	21.4

Throughout the heating process, the system experiences a water loss. The water loss is adjusted by weighing the sample before and after heating and adding water to the product equal to the amount of water which was evaporated.

The samples were analysed with a Malvern Kinexus Pro+, according to the section Gel Yield- and Break point , 5.2.2.4. Serrated parallel plates were used to perform amplitude sweep tests, with amplitude increasing, and frequency constant at 1 Hz. The temperature was set to 10 °C, to mimic the situation where a sample has been recently taken out of the fridge to be consumed. Two store-bought quargs was tested, “Lindalhs vanilj kvarg” and “Valio vanilj kvarg”. Two

plant-based options to quargs were also tested, “Alpro greek-style passion” and “Valio Oddly-Good”. Each sample and competitor were analysed in triplicates.

5.3.7 Sensory analysis to determine final formulation

Three samples were prepared for the final formulation with different added protein concentration and heating temperatures, according to Table 17. The samples were analysed through a hedonic sensory test according to Hedonic tests, 5.2.2.5.2. 16 panellists participated in this study.

Table 17: Composition of the 3 potential final products.

Ingredients	**Added protein 6.25% Temp 85 °C**	**Added protein 7.5% Temp 85 °C**	**Added protein 7.5% Temp 95 °C**
LBB	46.4	46.4	46.4
Salt	0.40	0.40	0.40
Coconut oil	3.00	3.00	3.00
Apple concentrate	15.0	15.0	15.0
Oat protein concentrate	5.19	6.23	6.23
Pea protein concentrate	6.36	7.64	7.64
Water	23.7	21.4	21.4

6 Results & Discussion

6.1 Calculations of PDCAAS for different protein sources and combinations

The results from the optimization are presented in Table 18. The results showed that combinations between pea and oat protein resulted in the highest protein qualities, with many reaching >100%. However, the faba bean concentrate also showed good values, with a maximum at 94,6% PDCAAS. However the Mycoprotein showed little promise, as the maximum PDCAAS it could reach was 75,4%.

Table 18: Results from Optimization calculations

	A OPC	B PPC	C FBPC	D MPC	E PPI
A OPC	PDCAAS: 56,9 % Limiting AA: Lys				
B PPC	Best Ratio: A44 : B56 PDCAAS: 103,2 %	PDCAAS: 77,0% Limiting AA: SAA			
C FBPC	Best Ratio: A41 : C59 PDCAAS: 94,6 %	Best Ratio: B100 : C0 PDCAAS: 77,0 %	PDCAAS: 64,2 % Limiting AA: SAA		
D MPC	Best Ratio: A25 : D75 PDCAAS: 75,8 %	Best Ratio: B100 : D0 PDCAAS: 77,0 %	Best Ratio: C19 : D81 PDCAAS: 74,3 %	PDCAAS: 66,4 % Limiting AA: Leu	
E PPI	Best Ratio: A46 : B54 PDCAAS: 100,4 %	Best Ratio: B100 : E0 PDCAAS: 77,0 %	Best Ratio: C0 : E100 PDCAAS: 69,7 %	Best Ratio: D82 : E18 PDCAAS: 75,4 %	PDCAAS: 69,7 % Limiting AA: SAA

There were two samples with an PDCAAS which surpassed 100% and one sample which was close at 94,6%. The PDCAAS was calculated based on the essential amino acid requirements for children between 3-10 years old, which require higher amounts of amino acids compared to adults. This product is not targeted against younger children, rather teenagers and adults, which have less demanding amino acids requirements, which can be seen in Table 3. If the

PDCAAS was adjusted for the target consumers, the combination of FBPC and OPC would be closer to 100%. Based on these results, three combinations were chosen for future studies:

- Oat Protein Concentrate & Pea Protein Concentrate
- Oat Protein Concentrate & Faba Bean Protein Concentrate
- Oat Protein Concentrate & Pea Protein Isolate

These results were somewhat expected. When looking at Table 3, showing the WHO reference patterns, and Table 6 showing the amino acid profiles of the products tested, it is clear that OPC lacks lysine but is rich in SAA, while all pulse proteins (PPC, FBPC, and PPI) are low in SAA but rich in lysine. It is not strange then, that when combined they form well rounded combinations.

6.2 Effect of salts on protein solubility

The three different samples were tasted to assess whether the salts had an effect on protein solubility. Sensory panel assessment was deemed excessive since the difference in taste was obvious. The findings from the tasting are presented in Table 19 below:

Table 19: Results from salt addition experiment

	No salt Weight [%]	**$CaCl_2$ Weight [%]**	**NaCl Weight [%]**
Oat protein concentrate	10.0	10.0	10.0
LBB (19.67% DM)	10.0	10.0	10.0
Water	80.0	79.0	79.0
$CaCl_2$	-	1.00	-
Sea salt	-	-	1.00
Texture	Liquid, similar to sourmilk	Slightly thicker	Liquid, similar to sourmilk
Mouthfeel	Mealy/sandy sensation from the protein	Significantly smoother texture, slightly gelatinous mouthfeel, but still some mealiness	Much smoother than sample with no salt, but not as smooth as $CaCl_2$, some mealiness remaining.
Taste	Fruity, tart, oat flavours, quite mild. Needs sweetness to balance tartness.	Very bitter with mineral long, mineral-like aftertaste. Almost inedible.	Very salty, like seawater, hard to eat more than a small spoon.
Aroma	No differences	No differences	No differences

The results showed a clear effect from both salts, however the taste from the calcium dichloride was deemed unacceptable. It was decided that NaCl would be used in future formulations, while $CaCl_2$ would not be explored further. It was also noted that some sweetness was needed in the formulation to balance the prominent tartness of the baobab.

The sample with added $CaCl_2$ did show a thicker consistency, which was in line with the theory stating that pectin gelation increases with calcium content. However, while pleasant the effect was not drastic, and was overshadowed by the bitter taste. Both samples with added salt showed

improved mouthfeel, which was in line with the theory stating that oat protein solubility increases with ionic strength at pH above 4,5 (see Figure 1).

6.3 Effect of NaCl and pH on protein solubility for different protein combinations

Confidence intervals (95 %) were constructed from the five central points, and 3D plots were constructed with Salt percentage and pH as X and Y axes. pH was chosen instead of baobab solids content due to its relationship to the isoelectric point and protein solubility. The main purpose of this experiment was to see if salt and pH had any effect on the protein solubility, which causes a mealy mouthfeel when low. Mealiness was therefore plotted on the Z axis, resulting in Figure 7 and Figure 8.

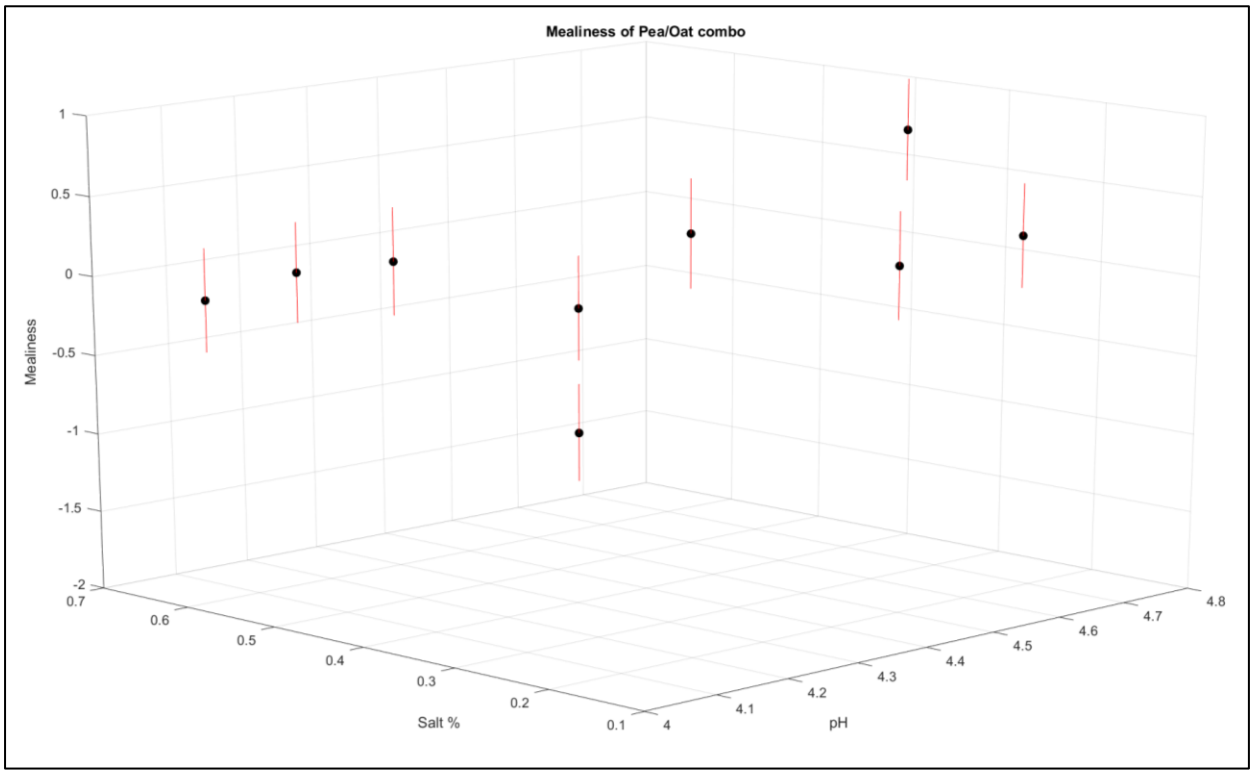

Figure 7: Centered and Standardized mealiness vs Salt % and pH for OPC / PPI combination

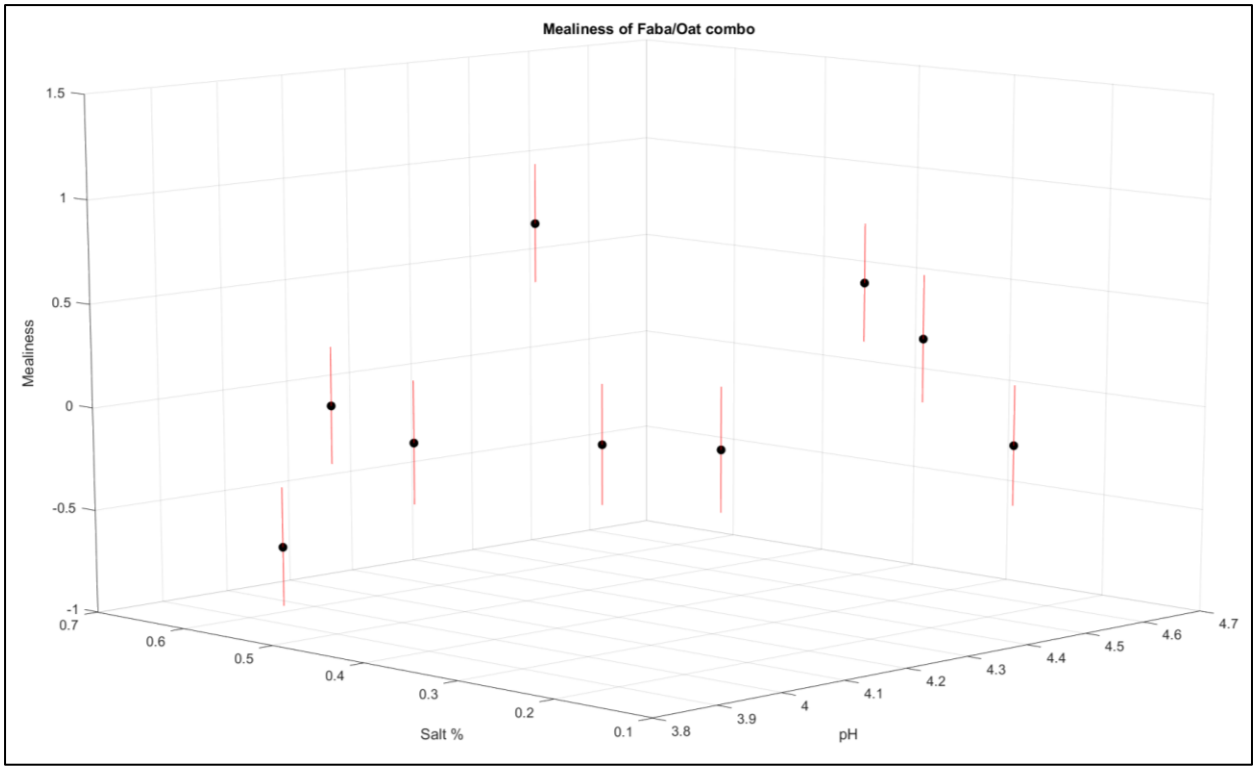

Figure 8: Centered and Standardized mealiness vs Salt % and pH for OPC / FBPC

While it may be difficult to see in 2D, the plots showed that mealiness decreased when salt % increased and pH decreased, for both OPC/PPI and OPC/FBPC.

The numerical results are shown below in Table 20 and Table 21

Table 20: Mealiness results for OPC/PPI

OPC / PPI Mealiness Results				
Baobab %	pH	Salt %	Mealiness (centered & standardized)	+/- 95% CI
3.00	4.79	0.40	0.67	0.33
3.59	4.66	0.19	0.27	0.33
3.59	4.58	0.61	-1.38	0.33
5.00	4.35	0.10	0.39	0.33
5.00	4.28	0.70	-0.18	0.33
6.41	4.18	0.19	0.62	0.33
6.41	4.05	0.61	-0.10	0.33
7.00	4.05	0.40	0.34	0.33

Table 21: Mealiness results for OPC/FBPC

OPC / FBPC Mealiness Results				
Baobab %	pH	Salt %	Mean Mealiness (centered & standardized)	+/- 95% CI
3.00	4.61	0.40	0.46	0.29
3.59	4.52	0.19	-0.16	0.29
3.59	4.38	0.61	0.72	0.29
5.00	4.22	0.10	0.53	0.29
5.00	4.17	0.70	-0.16	0.29
6.41	4.04	0.19	0.04	0.29
6.41	3.97	0.61	-0.70	0.29
7.00	3.88	0.40	0.00	0.29

While increasing the salt further would likely lead to an unacceptably salty taste, it was decided that pH would be decreased further by increasing the baobab solids content in the formulation. Not only would this decrease pH, possibly improving solubility further, it would likely increase viscosity, as viscosity was seen to be heavily associated with baobab solids content, as shown below in Figure 9.

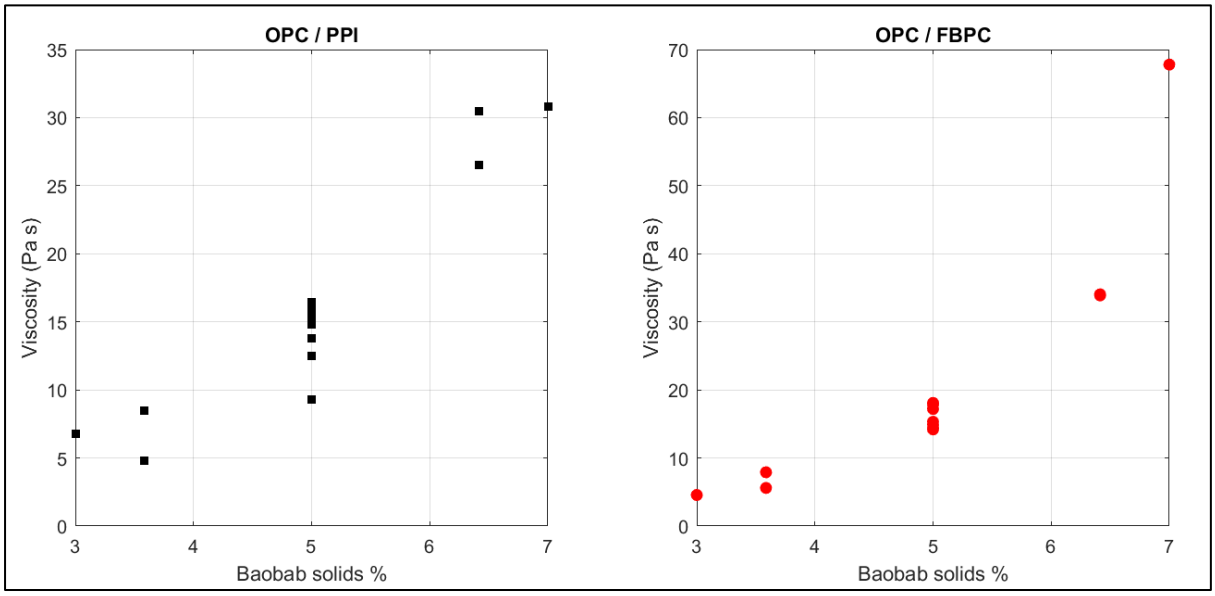

Figure 9: Viscosity vs Baobab solids content

The results from this investigation were somewhat expected. The result indicates that the pH had a clear effect on the protein solubility. As can be seen in the theory section, the isoelectric point of oat protein is around pH 4.5 while pea protein is around pH 4.3. When the pH is close to the isoelectric point, the protein surface charge is neutralised, and its solubility is decreased. The lowest solubility for both oat and pea protein has been measured at pH between 4-6, this correlates with our results where the participants of the sensory evaluation perceived that most

of the samples had a mealy mouthfeel. One could though observe a trend that the perceived mealiness was reduced when the pH was lowered, which correlates with the theory.

The result indicates that the salt content influences the protein solubility. This result correlates with the literature, that addition of salt (NaCl) can increase the solubility of the proteins. Both oat and pea protein are mainly made up of globulins which has higher solubility when salt is present. The salt increases the electric repulsion as it contributes to the electric double-layer around the protein which prevents interactions and aggregation.

According to the literature, the solubility of oat protein varies with the ionic strength. In this case, the exact ionic strength of the formulation was difficult to calculate as the samples creates a complex matrix of ingredients. The matrixes did contain several other ions (as can be seen in Table 2) and weak acids form the other ingredients in which may affect the ionic strength.

To get an estimation of what the ionic strength of the samples may be, equation 4 from *section Salt content, 4.5.2* is used. The assumption for this calculation is that only the salt which was added will affect the ionic strength. The C_i in the equation represents the molar concentration of NaCl. To calculate this, the total amount of water ($m_{w,tot}$) needs to be calculated in the sample first. The values used for the calculations were based around the composition of sample 11 in Table 9.

$$m_{w,tot} = w_{LBB} * m_{LBB} + w_{bp} * m_{bp} + m_{water} = 0.834 * 30.8g + 0.753 * 10.0g + 48.5g = 81.7g$$

Where w_{LBB} is the water content in the LBB, w_{bp} is the water content in the banana puree and m is the mass of the different ingredients. An assumption was used that the result 81.7g of water equals 81.7 ml of water. The molar concentration of the salt was calculated with the equation below.

$$C_{NaCl} = \frac{n}{v} = \frac{\frac{m}{M}}{v} = \frac{\frac{0.7\ g}{58.44\ g/mol}}{0.0817\ l} = 0.147\ mol/l$$

Where *n* represents the amount of mole of the NaCl, and *v* is the volume of the water. *M* was the molar mass for NaCl, and *m* was the mass of NaCl, which was the highest amount of salt used in this investigation.

$$\mu = 0.5 * \sum C_i Z_i^2 = 0.5 * (0.147 * 1^2 + 0.147 * -1^2) = 0.147\ mol/l$$

According to Li and Xiong, at this ionic strength, the protein solubility is suboptimal and is substantially lower than when the ionic strength is very low or when there is none. This does not correlate with the results from this investigation, where the sample with the highest ionic strength had the best mouthfeel. The reason why this result may not correlate with this literature is the composition of the samples. Our samples had many different ingredients which may

affect the ionic strength in different ways, whereas Li and Xiong used only a combination of distilled water, salt, oat protein isolate and HCl to adjust the pH.

One other thing which may have affected the result was the structure of the protein concentrates, before and during the process. The oat protein concentrate and pea protein isolate was already denatured which affects their properties which would lead to a more unpredictable behaviour, depending on how their structure after extraction.

One other factor which could affect the protein solubility is that the NaCl may not only react with the proteins. The samples was a matrix of many different polymers which the salt could interact with instead of the proteins. This could reduce the solubility and that more salt could be added to counteract this problem, but the increased salt level could lead to issues regarding taste.

6.4 Deciding final protein combinations

6.4.1 Effect of different protein combinations with higher protein concentration has on sensory perception

The formulation had been adjusted based on the previous results, with increased amount of salt and LBB. The three samples with different protein sources (faba bean/oat concentrate, pea isolate/oat concentrate, and pea/oat concentrate) was tested with an internal sensory evaluation. The result for each sample is stated below:

Faba bean / oat concentrate: Had a very tart taste, especially the aftertaste. The mealiness was significantly reduced compared to previous investigations, but the mealiness was still more compared to quarg. The texture was worse compared to the other samples, it was less viscus and did not form a 3D-structure. This made it unsuitable as a snack which should be compared to quarg.

Pea isolate / oat concentrate: Had a stronger, unpleasant tartness compared to the other samples. The mealiness was greatly reduced compared to previous samples, but the texture was worse. The sample had low viscosity which made it unsuitable as a snack which should be compared to quarg. The sample had an unpleasant aftertaste, almost soapy, probably from the isolate since it is not experienced in the other samples.

Pea / oat concentrate: Had a prominent tartness. Not very homogenous in neither appearance not texture, the protein has granules about 0,5-1 mm in diameter. The granules were the only issue with the mouthfeel, not the mealiness of the actual protein. The sample had a clear characteristic flavour and aroma of pea. The texture indicated to be viscoelastic and very close to real quarg, as it exhibited such a strong gel that the container could be turned sideways and upside down without the gel moving.

The overall result was that the saltiness and tartness was intolerably high, so the LBB and salt needed to be reduced. The increased salt levels and reduced pH (as LBB was increased) probably resulted in an improved protein solubility as it did influence the mouthfeel, which was expected as it was in line with the theory. The particle size of the pea protein concentrate was too large though, which gave it an unpleasant mouthfeel. Pea protein concentrate seemed most promising, so further investigation regarding the pea protein concentrate needed to be done.

6.4.2 Effect of lowered salt and LBB concentration and addition of tricalcium citrate.

The reason why tricalcium citrate was added to the formulation was that, according to the theory, an increased calcium concentration can aid the gelation of LM pectin which would improve the texture to a more desired state. Also, the citrate ions was supposed to contribute to an improvement of the protein solubility of samples.

After an internal sensory evaluation, there was no significant difference between the samples when it came to mouthfeel (protein solubility), taste, aroma, or texture. Both samples had a more pleasant taste when it came to salt and tartness compared to previous samples. The samples both still had a strange and unpleasant aftertaste, which was experienced in the previous investigation, see 6.4.2.

The outcome of this investigation was that calcium citrate did not seem to be necessary to use to increase the protein solubility and improving the texture. The lowered content of salt and LBB provided pleasant results. The pea protein isolate has an off-putting aftertaste which is difficult to remove or conceal.

6.4.3 Effect of rapeseed oil and particle size of pea protein concentrate on sensory perception

The problem with both samples during this investigation was that the oil which was used was spoiled which was a dominating taste in both samples.
For the samples with the faba bean concentrate there was still a slight mealiness, according to an internal sensory evaluation. The balance between salt, tartness and sweetness was pleasant and provided a good flavour.

For the sample with pea concentrate, the balance between acid, tartness and salt was pleasant. The mouthfeel was improved as the granules was smaller. This resulted in a texture which was slightly sandy rather than mealy, probably due to the lower grit size. Problem was still not solved. A finer mesh of 0.150 mm was investigated and resulted in a better and pleasant mouthfeel.

During this investigation, the flavour of pea was stronger compared to previous trials. The reason behind this might be due to cooking temperature in this trial was 75°C instead of 85°C which was used be in previous trials. One possible reason why the pea flavour and aroma were more dominant when cooked to a lower temperature was that there might be volatile compounds in the pea protein concentrate which affect the pea taste, which usually would evaporate, remained in the sample. This time, the cooking time of 75°C was used to investigate if a lower temperature than 85°C was possible and still get the same result. 75°C was chosen to ensure that the pectin activates as the gelation temperature of pectin is 70°C.

In this investigation, apple puree was used instead for banana puree due to investigate if the flavour profile of apple would work better together with the baobab fruit. Apples can be grown and produced locally which would be more environmentally friendly as well as fulfilling the objective of using more locally produced ingredients. Apple also contain less kilocalories, which makes is easier to reach the claim “high in protein”.

The oil was added to the formulation as it has shown to reduce the tartness of the product based on previous studies at Aventure. Both rapeseed oil and coconut oil was tested, but coconut oil was chosen in the end. The coconut provided a flavour which complemented the test of the taste profile better than rapeseed oil and it also helped to reduce and mask the pea flavour from

the pea protein concentrate. As the coconut oil is mainly saturated fats, the oil is solid at fridge temperature (around 10°C), which could provide a more desired texture of the sample.

6.4.4 Protein screening, sensory analysis

A total of 12 panellists contributed to the panel, from different age groups and genders. The results are shown in Figure 10 below:

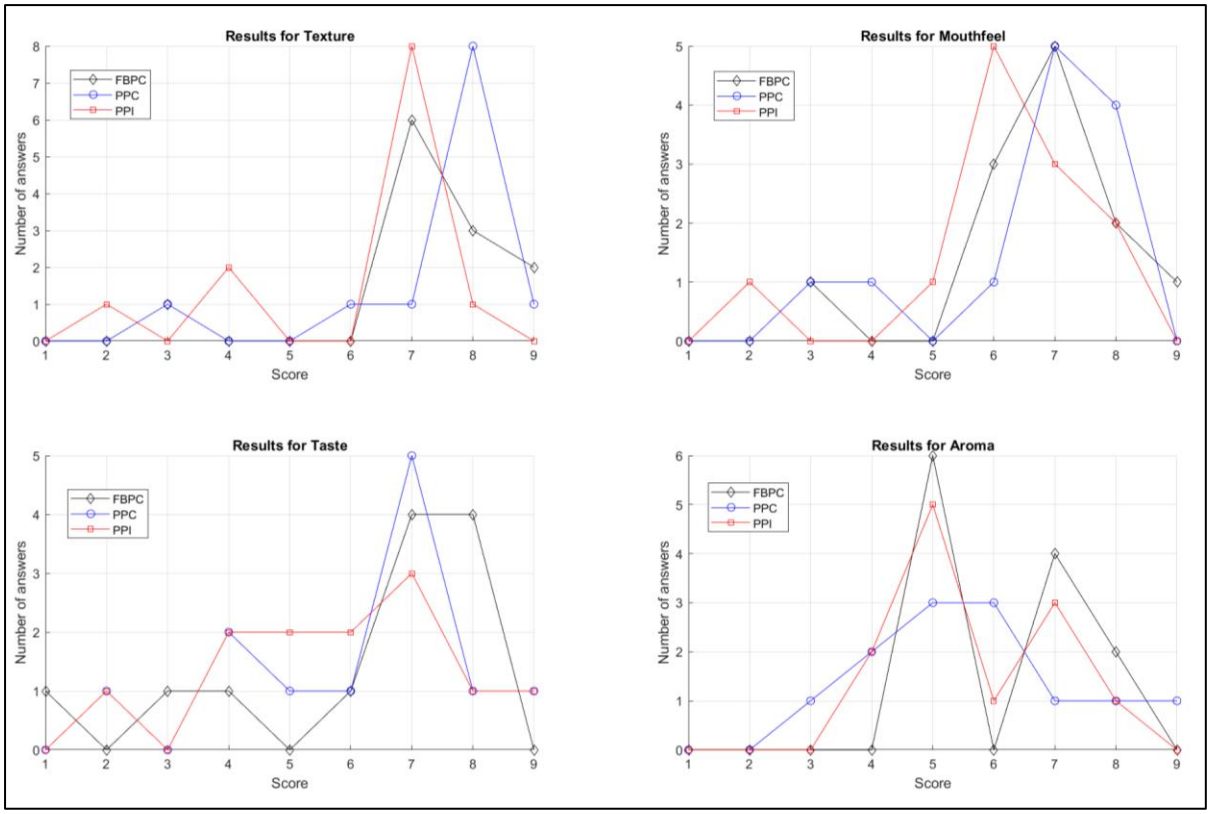

Figure 10: Score results from protein screening

To see whether there were any significant differences the results were first mean-centered and standardized by panelist, then one-way ANOVA's were performed on each aspect. The results are shown below in Figure 11. All three samples contain OPC in addition to the respective protein powder specified in the figure.

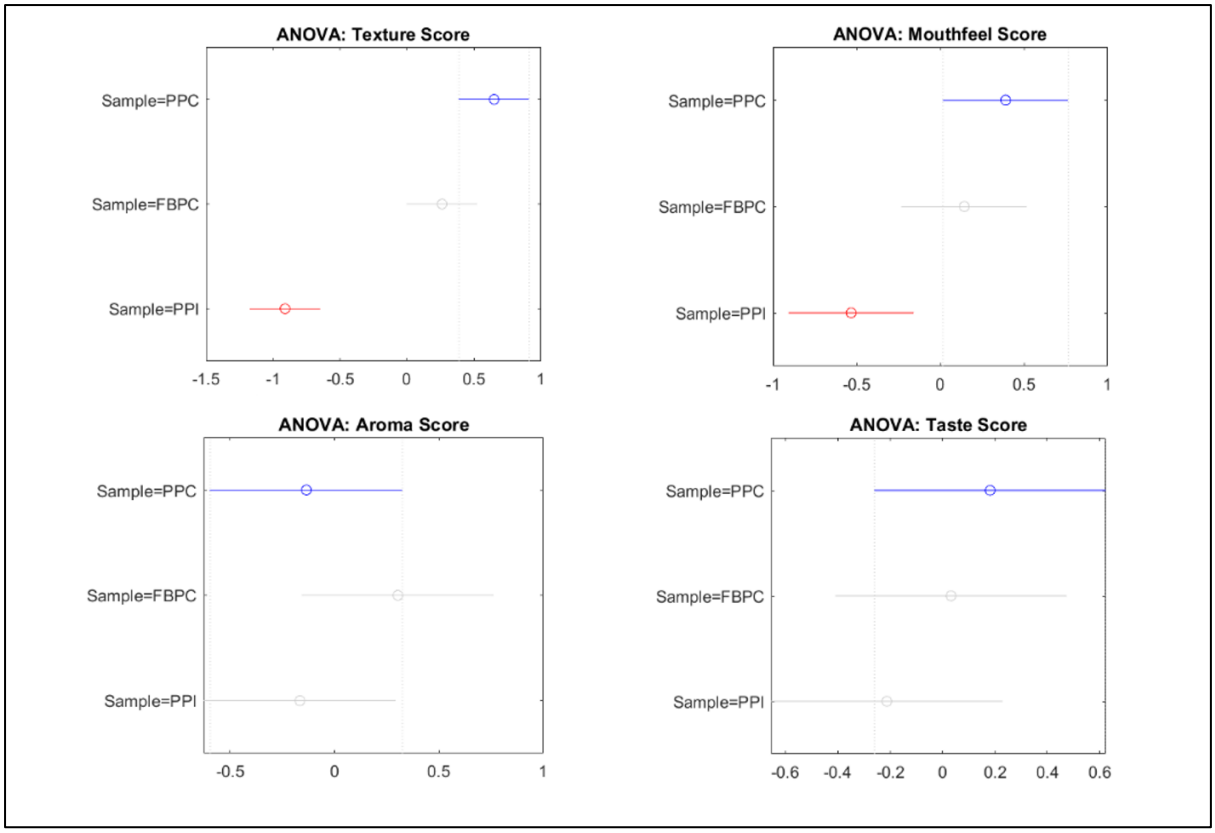

Figure 11: 95% Confidence ANOVA's for protein screening experiment. Chosen protein shown in blue, significantly different results shown in red.

As seen in Figure 11, the only statistically significant results are that PPI had significantly worse texture than both PPC and FBPC, and that PPI had significantly worse mouthfeel than PPC. While it is not a statistically significant difference (at 95 % confidence), there is an indication that PPC is more preferred than FBPC in all aspects but aroma. This trend, in combination with the fact that PPC is produced in Norway rather than Canada and results in a protein blend with higher PDCAAS made it the candidate of choice, and the protein source that will be used in future formulations.

6.4.5 Increasing sweetness of the product

As it was clear that the sweetness to acidity ratio needed improvement, apple juice concentrate was added to the formulation in four different ratios, as described in 5.2.2.5.2 a sensory analysis was performed, with 13 people participating in the panel.

The results from the sensory trial are shown in Figure 12 below:

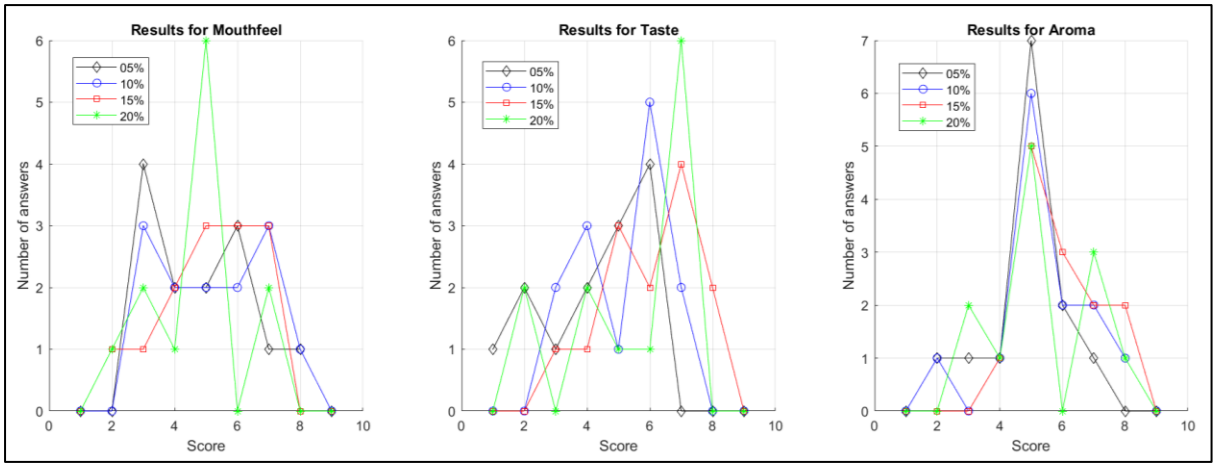

Figure 12: Score results from juice concentrate ratio study

To see whether there were any significant differences the results were first mean-centered and standardized by panelist, then one-way ANOVA's were performed. The results are shown below in Figure 13:

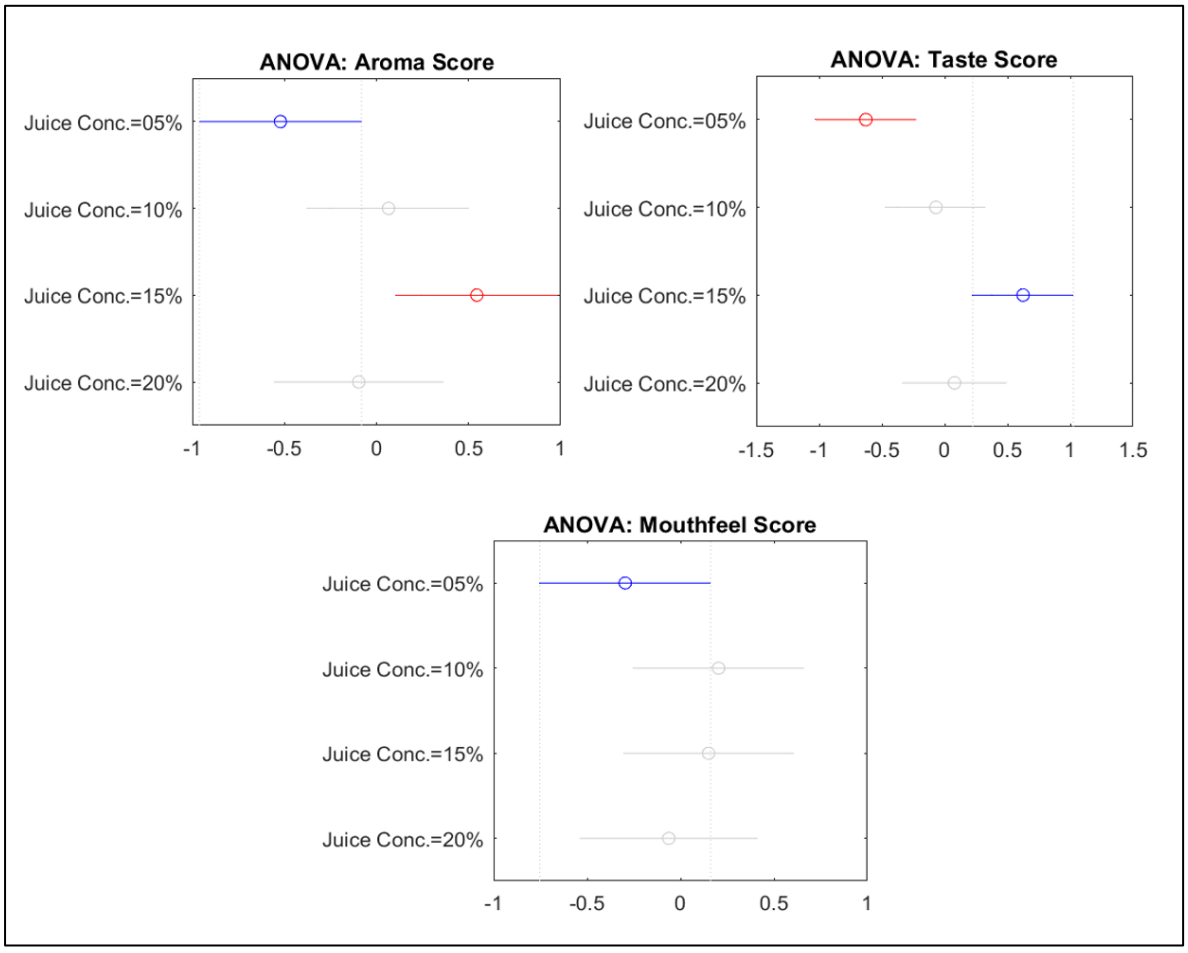

Figure 13: 95% Confidence ANOVA's for juice concentrate experiment

The results indicated that 15% juice concentrate was the most preferred ratio, even though statistically significant differences were only found between 5% and 15%. Therefore 15% apple juice concentrate was chosen for future formulations. For mouthfeel there were no significant differences at all, which is logical since salt levels are not changed, and pH does not vary significantly with AC concentration, as shown in Figure 14 below:

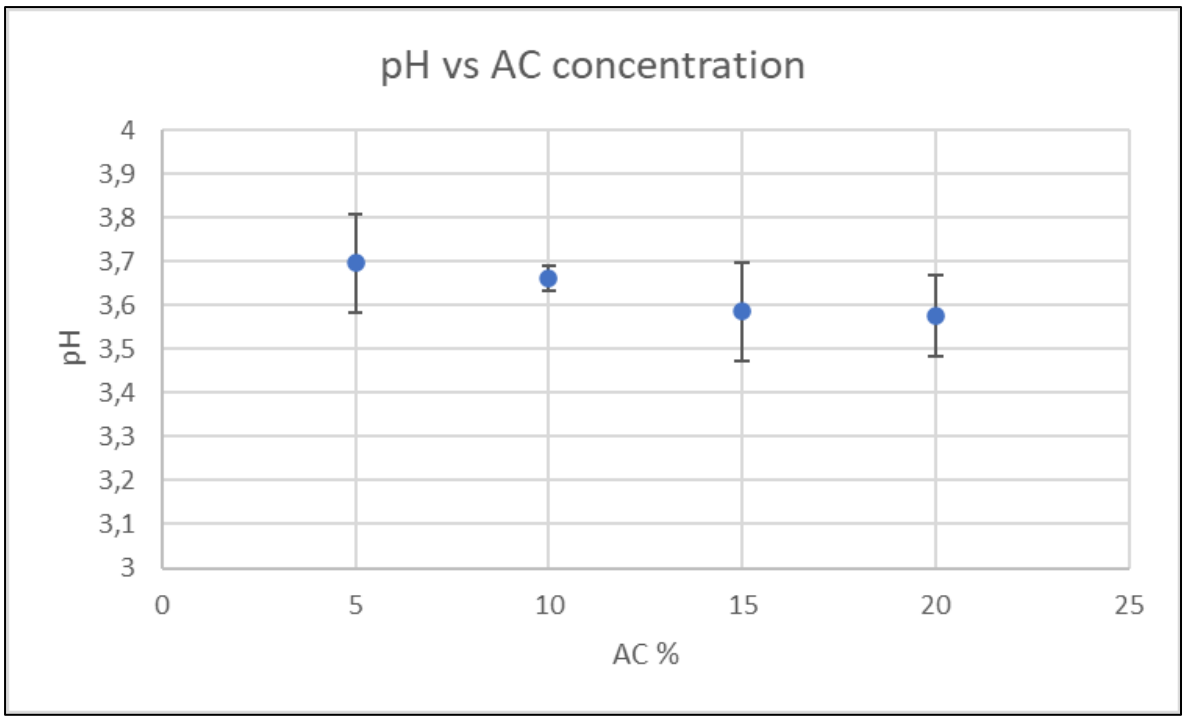

Figure 14: pH vs AC concentration, 95% CI

6.4.6 Effect of protein concentration and cooking temperature on gel strength, and comparison to existing products

Measurements were made according to 5.2.2.4. Full results can be found in appendix 9.2, but a summary is shown below in Table 22.

Table 22: Summary of Yield and Breakpoints of samples and competitors

Sample		Mean Yield Point	+/- (95% CI)	Mean Break Point	+/- (95% CI)
Protein %	Temp. °C				
5.0	85	0.12	0.12	3.28	3.22
5.0	90	0.10	0.03	2.01	0.77
5.0	95	0.11	0.06	1.28	1.32
6.25	85	4.79	0.92	127	20.4
6.25	90	1.87	0.15	45.4	5.16
6.25	95	3.52	2.88	85.6	57.8
7.5	85	9.87	1.40	268	22.4
7.5	90	4.82	1.78	155	36.0
7.5	95	7.96	1.85	249	35.7
Competitors					
Greek Style		13.8	8.99	206	32.2
Lindahls		8.32	10.4	96.8	47.2
Oddly Good		17.4	7.35	228	35.1
Valio		4.35	1.17	77.4	6.49

To make comparisons between samples and competitors easier, 3D plots were also constructed, these are shown in Figure 15 and Figure 16 below. Competitor values are plotted in the same field, but only as references. They do not have temperature or protein content values connected to them but are plotted along the Temperature axis for readability.

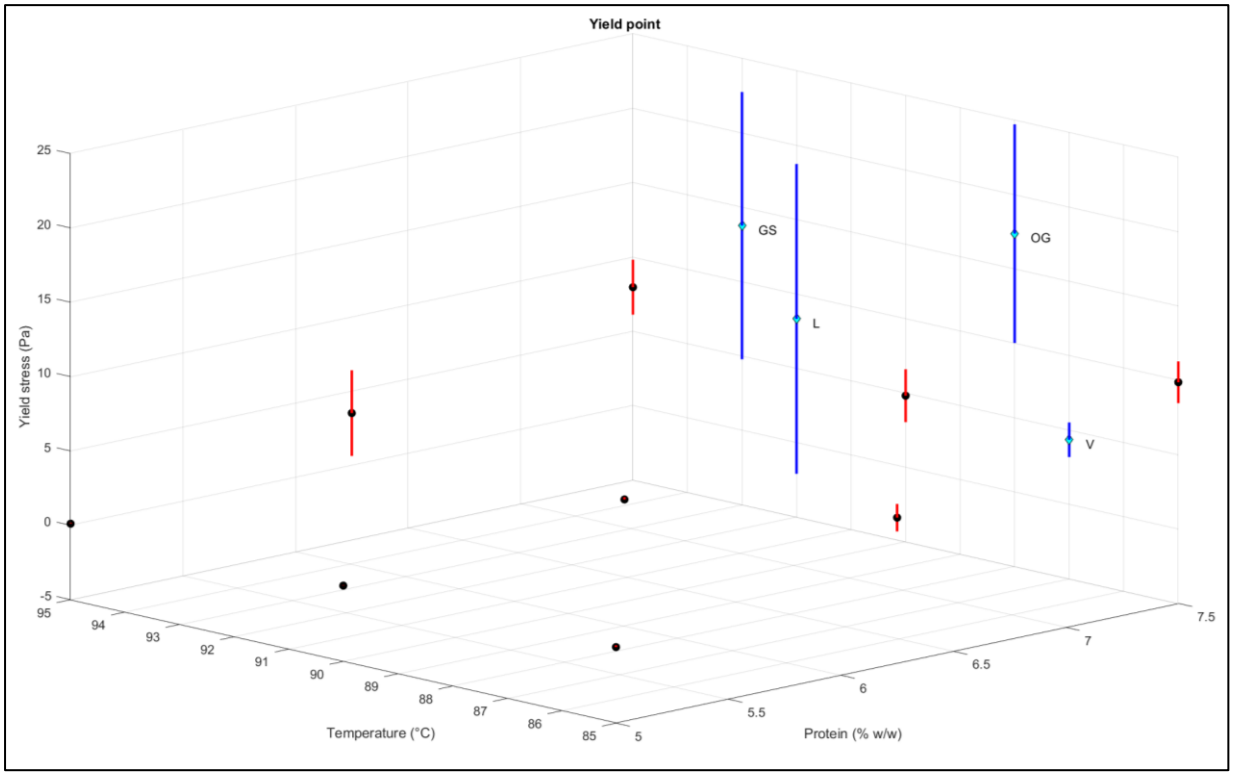

Figure 15: Yield stress vs Cooking temperature and Protein %. GS=Alpro Greek style, L=Lindahls kvarg, OG=Valio Oddly Good, V=Valio kvarg, Lines showing 95% confidence intervals

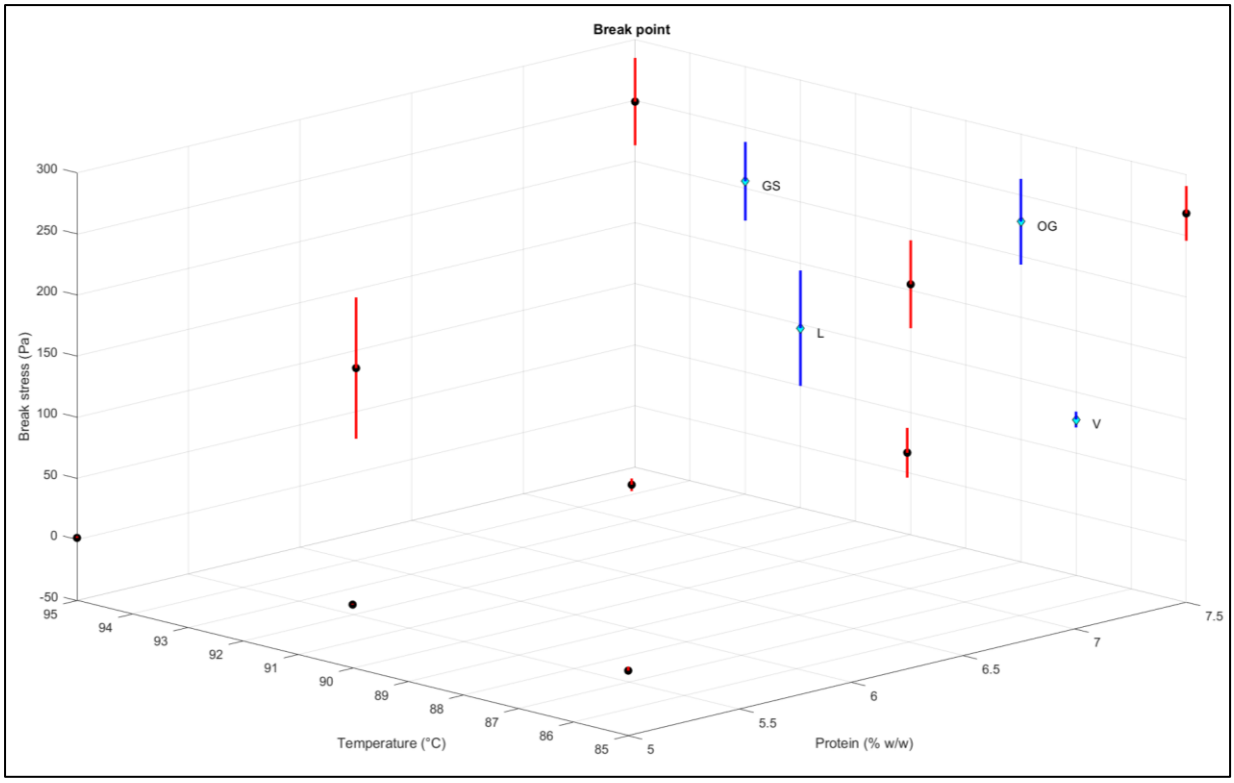

Figure 16: Break point vs Cooking temperature and Protein %. GS=Alpro Greek style, L=Lindahls kvarg, OG=Valio Oddly Good, V=Valio kvarg, Lines showing 95% confidence intervals

From these results it is clear that some samples show rheological behaviour similar to commercial products. A curious finding was that a V-shape can be observed for each protein % level, where 85 C and 95 C show higher yield and break points than 90° C. There is no obvious explanation to this phenomenon, and it is very possible that it is simply due to human error during sample preparation or analysis.

Based on the result from this investigation, it seems likely that the ingredient that affected the texture of the sample the most is not pectin as previously believed, but one or several of the components found in the protein concentrates. It is possible that the gel formation is largely due to the starches found in the protein concentrates, particularly the pea starch, as the literature states that it forms very strong retrogradation gels. This is based on that in all three of these samples, the pectin levels are the same, but they differ in protein concentration and gel strength. One reason why the pectin might not contribute to the texture is the low pH of the samples. According to the theory, when pH is low, there will not be enough of dissociated COO^- on the pectin for ion bridging. With no ion bridges, no gel will form.

Another reason why pectin might not form a strong enough gel which would contribute to the texture of the sample is that the total concentration of pectin might be too low. The pectin will not be able to form a gel if the concentration is too low. To counteract this, LBB content could be increased, but that might affect the tartness of the product to much.

Based on these results, three samples were chosen for a final sensory evaluation, in part based on their similarity to commercial products, but also to test how sensory properties were affected by protein content and cooking temperature. The samples were the following: Added protein 7,5% Temp 85 °C and 95 °C, and added protein 6,25% 85 °C.

6.4.7 Sensory analysis to determine final formulation

A total of 16 people participated in the panel, the results are presented below in Figure 17.

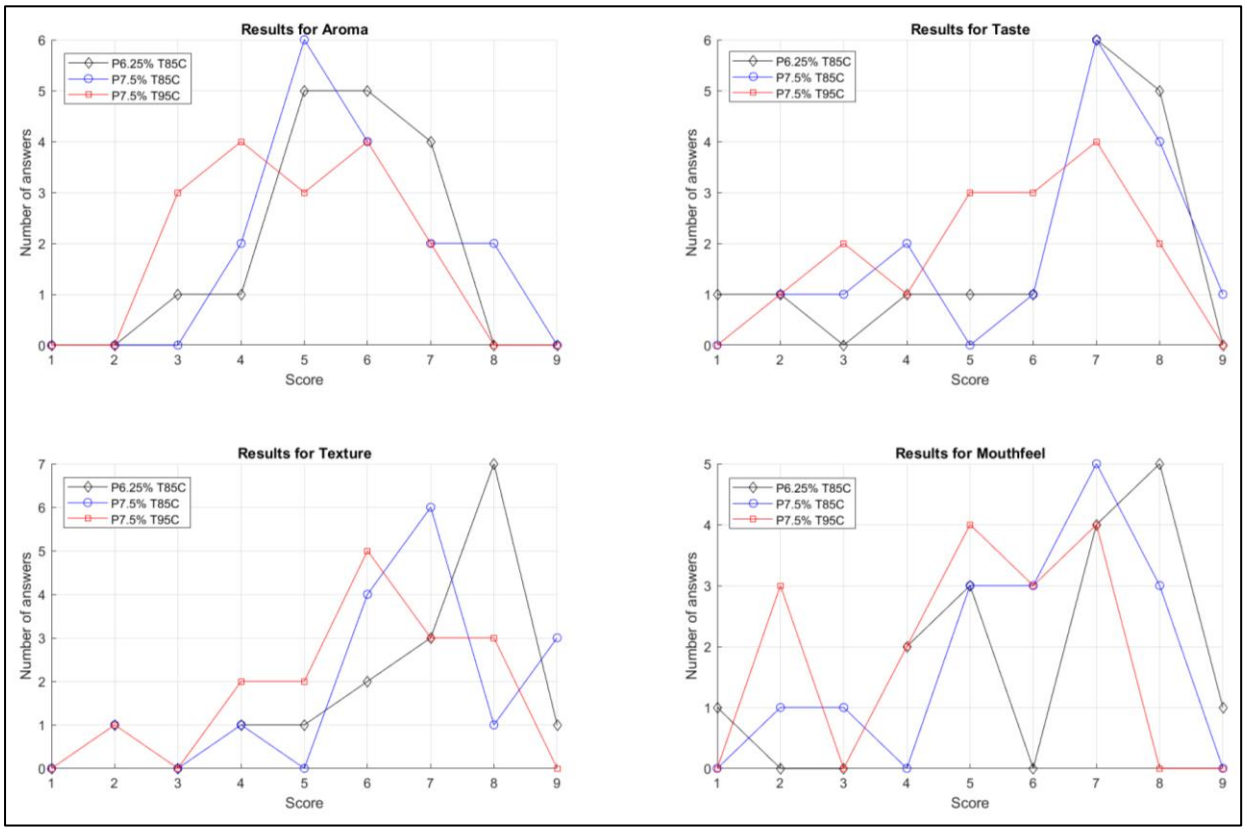

Figure 17: Score result from the sensory evaluation on the final formulations.

To see whether there were any significant differences the results were first mean-centered and standardized by panelist, then two-way ANOVA's were performed. The results are shown below in Figure 18.

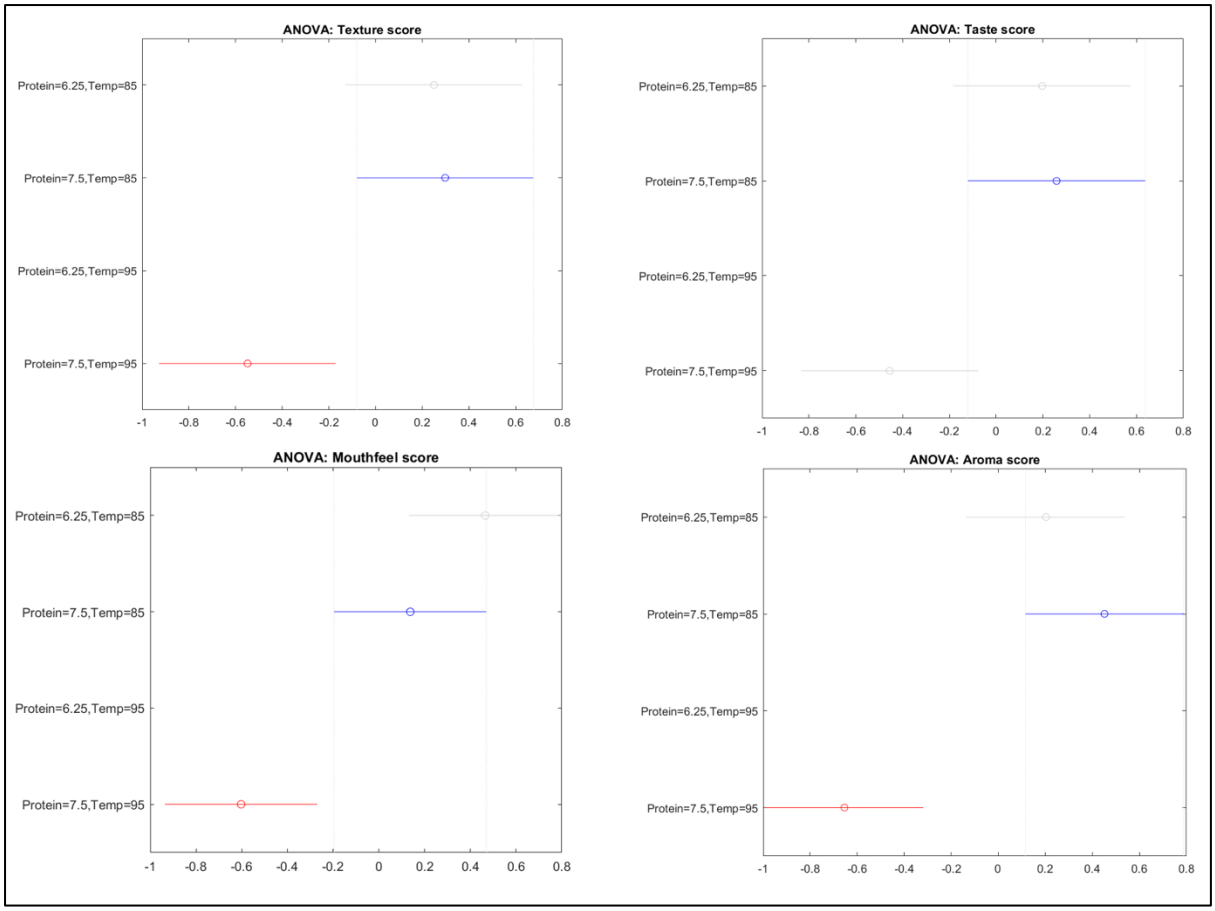

Figure 18: 95% Confidence ANOVA's for final formulation. Chosen formulation shown in blue, significantly different results shown in red.

As can be seen in Figure 18, there were no significant differences between the two samples cooked to 85 °C, however the sample cooked to 95 °C stands out as the least preferred sample in all aspects. It is clear then, that the sample cooked to 95 °C should not be considered as a candidate for the final formulation. The choice then stands between the samples cooked to 85 °C, one with 7,5 % protein and one with 6,25 % protein. Both were equally liked, however the formulation 7,5 % protein boasts a preferable nutritional profile, fulfilling the EU claim of "High in protein".

Another factor to consider is that a common serving size for similar products is 200g plastic cups. The amount of protein contained is usually presented in large print on the front of the package, usually as grams of protein per container. Choosing the 7,5% protein option would allow "15 grams of protein per container" to be printed on the front, which would be valuable from a marketing perspective.

Due to the reasons above, the formulation with 7,5% added protein and a cooking temperature of 85 °C was chosen as the final suggestion to Aventure AB for further development.

7 Conclusions

7.1 Final formulation

In this section, the final formulation is summarized and discussed.

7.1.1 Ingredients

7.1.1.1 Liquid Baobab Base (LBB)

Consisting of baobab fruit pulp and water, the LBB lowers the pH which improves protein solubility. It also provides pectin which contributes to viscosity and gel formation, and vitamins and minerals which contributes to nutritional value. It also gives the product a characteristic flavour.

7.1.1.2 Water

Hydrates the dry ingredients and dissolves the salt.

7.1.1.3 Apple Juice Concentrate

Provides sweetness and flavour while keeping the pH low.

7.1.1.4 Pea Protein Concentrate

Provides protein for nutritional value, provides high amounts of lysine which compensates for oat protein's lack thereof, provides pea fibres and starch for gel formation.

7.1.1.5 Oat Protein Concentrate

Provides protein for nutritional values, provides high amounts of sulphurated amino acids which compensates for pea protein's lack thereof, provides oat starch and fibre for gel formation.

7.1.1.6 Coconut oil

Reduces perceived tartness and masks the characteristic and unpleasant smell of pea protein.

7.1.1.7 NaCl

Improves mouthfeel and enhances flavour.

7.1.2 Nutritional profile

Table 23 represents the theoretical nutritional profile of the final formulation.

Table 23: Nutritional profile of the final formulation

Nutritional value of the percentage formulation	**/100g**	**/Serving (200g)**
Energy value [kcal]	152	304
Water content [g]	66.5	133
Protein [g]	7.80	15.6
Fat [g]	4.29	8.58
Saturated fat [g]	1.16	2.31
Carbohydrates [g]	19.4	38.7
Sugar [g]	7.54	15.1
Fibre [g]	1.73	3.45

Based on this nutritional profile, the product fulfils the criteria on some nutritional claims. The product can use the claim of "high in protein" due to the fact that 20.5% of the energy content comes from proteins. The product also reaches the claim of "low in saturated fat" as the limit need to below 1.5g/100g to fulfills this claim.

7.1.3 Discussion of aims

In the beginning of the project, a list of desired properties of the formulation was determined. In this section, each of those criteria are discussed.

- It should be rich in protein, preferably over 20% of the caloric content should come from protein.
    - This criteria is fulfilled, the caloric content coming from the protein is 20.5%
- It should have as high protein quality as possible.
    - This criteria is fulfilled, the PDCAAS of the formulation is 100%.
- It should be free from ingredients of animal origin.
    - This criteria is fulfilled.
- It should contain baobab fruit.
    - This criteria is fulfilled.
- It should not contain E-numbered additives.
    - This criteria is fulfilled, no ingredients that warrants the use of E-numbers in the table of ingredients was used.
- It should contain locally produced ingredients when possible.
    - This criteria is partially fulfilled, however Coconut oil is not a locally produced ingredient, and should be replaced in the future if a good enough replacement is found.
- It should have rheological properties similar to relevant competitors. Relevant competitors refers to dairy based quarg and plant-based alternatives.

    - This means it should have semisolid, gel-like characteristics.
        - This criteria is fulfilled, after being deformed it does not flow back even after several days.
    - This means it should have yield- and breakpoints in the same range as relevant competitors.
        - This criteria is fulfilled, see Figure 15 and Figure 16.
- It should have as smooth a mouthfeel as possible
    - While much effort has been spent on this, it is very possible that this can improve further.
- It should have an acceptable, but not necessarily appealing taste.
    - This criteria is fulfilled, the last sensory analysis confirmed that the chosen formulation was significantly more liked than disliked in this aspect.
- It should have an acceptable, but not necessarily appealing aroma.
    - This criteria is fulfilled, the last sensory analysis confirmed that the chosen formulation was significantly more liked than disliked in this aspect.

7.2 Suggestions for future work

Due to the time constraints of this project, there is much that remains to be done for this project to reach the supermarket shelves. In this section some of this work is outlined.

7.2.1 Time and Temperature

During the rheology study it became apparent that there were unexpected factors affecting the gel strength of the samples. Since 85 °C cooking temperature resulted in higher gel strength than 90 °C, it seems likely that cooking temperature might not be as important to the gel strength as theorized. Instead, it might be that storage time is an important parameter that was not properly investigated in this project. This is in line with the literature on pea starch, which states that pea amylose gels become firmer over time.
Since 85 °C emerged as the favoured cooking temperature, it should also be explored whether even lower cooking temperatures result in a better formulation.

7.2.2 Reduction of Baobab solids content

The rheology study showed that the difference between a strong gel and a weak gel was due to protein powder content rather than baobab solids content. Since Baobab is not a cheap ingredient, it might be of interest to investigate whether it is possible to reduce the baobab solids content and instead use an acid additive to keep pH low for protein solubility.

7.2.3 Finding a replacement for coconut oil

Coconut oil is relatively expensive and not possible to produce in the Scandinavian region. Therefore, it might be of interest to find another oil with similar characteristics to replace it.
While there are Nordic oils such as rapeseed oil that might be appealing, this was tested and rejected since it imparted an undesirable flavour. Further research is required to find a good substitute.

7.2.4 Homogenization

A process step that was considered but never explored was homogenization. A major challenge of the project was the reduction of mealiness in the formulation, an undesirable characteristic caused by low protein solubility. While this was solved with lowering of pH and addition of salt, it is not unthinkable that homogenization could improve this further by breaking up protein aggregates further and leading to a finer dispersion, leading to a smoother mouthfeel.

7.2.5 Salt concentration

While salt showed some impact on protein solubility in the pH range between 3,8 to 4,8, its impact was never explored in the final pH range of around 3,6. As seen in Figure 1, salt concentration has shown to have a far larger impact in this region for oat protein. Varying the salt content at this pH might lead to interesting findings, and perhaps improved mouthfeel.

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9 Appendices

9.1 Template, sensory evaluation

Protein screening hedonic test – 2022-03-XX

Before tasting the sample, please take a bite of the cracker and take a generous sip of water and swish it around in your mouth to cleanse your palate.

Before tasting the sample, please stir it with a clean spoon.

When tasting the sample, take a spoonful, and eat it like you would a spoonful of yoghurt. Hold the sample in your mouth for around 3 seconds, letting it coat your tongue, and then swallow. Wait 5 seconds before deciding on a score. Repeat procedure if needed.

Texture

Sample	*Dislike extremely*	*Dislike very much*	*Dislike moderately*	*Dislike slightly*	*Neither like nor dislike*	*Like slightly*	*Like moderately*	*Like very much*	*Like extremely*
472	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
196	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
887	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9

Mouthfeel

Sample	*Dislike extremely*	*Dislike very much*	*Dislike moderately*	*Dislike slightly*	*Neither like nor dislike*	*Like slightly*	*Like moderately*	*Like very much*	*Like extremely*
472	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
196	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
887	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9

Figure 19:Template for hedonic test, page 2

Taste

Sample	*Dislike extremely*	*Dislike very much*	*Dislike moderately*	*Dislike slightly*	*Neither like nor dislike*	*Like slightly*	*Like moderately*	*Like very much*	*Like extremely*
472	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
196	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
887	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9

Aroma

Sample	*Dislike extremely*	*Dislike very much*	*Dislike moderately*	*Dislike slightly*	*Neither like nor dislike*	*Like slightly*	*Like moderately*	*Like very much*	*Like extremely*
472	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
196	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9
887	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9

Comments or thoughts of the samples (voluntarily to fill in):

_____

_____

_____

_____

_____

Figure 20:Template for hedonic test, page 2

9.2 Effect of protein concentration and cooking temperature on gel strength, and comparison to existing products

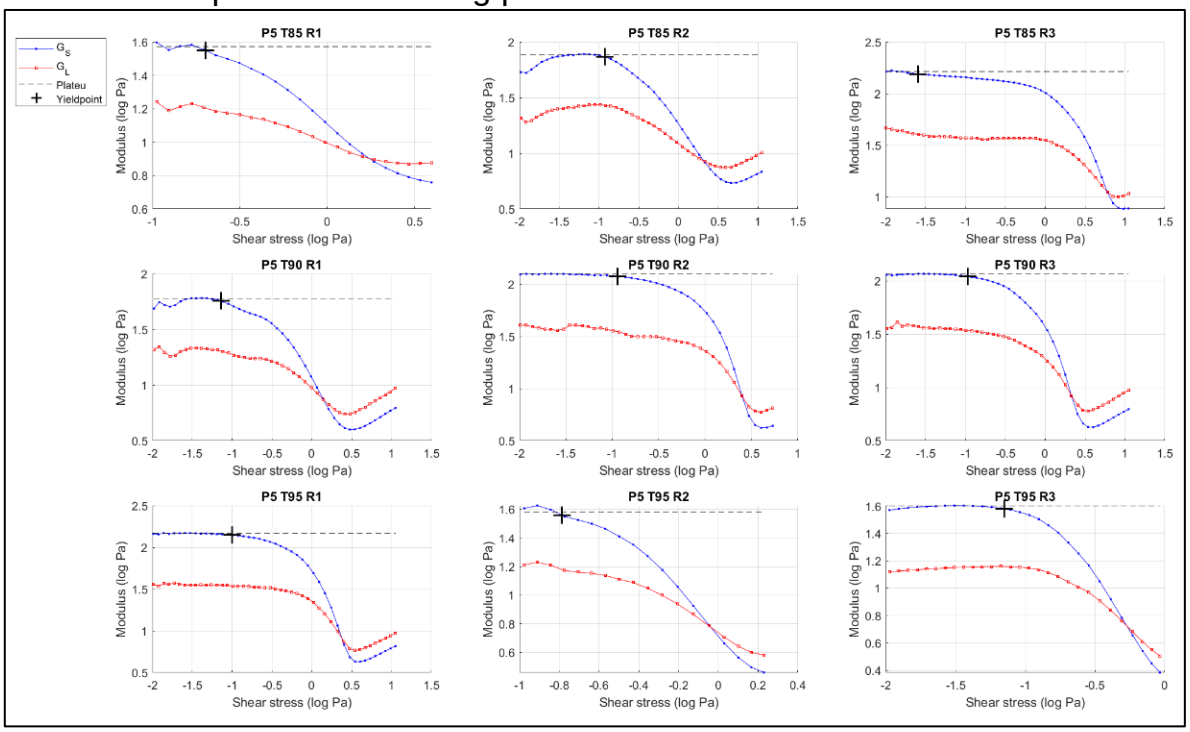

Figure 21: Results from amplitude sweep on 5% protein samples, subscript S for storage modulus, subscript L for loss modulus.

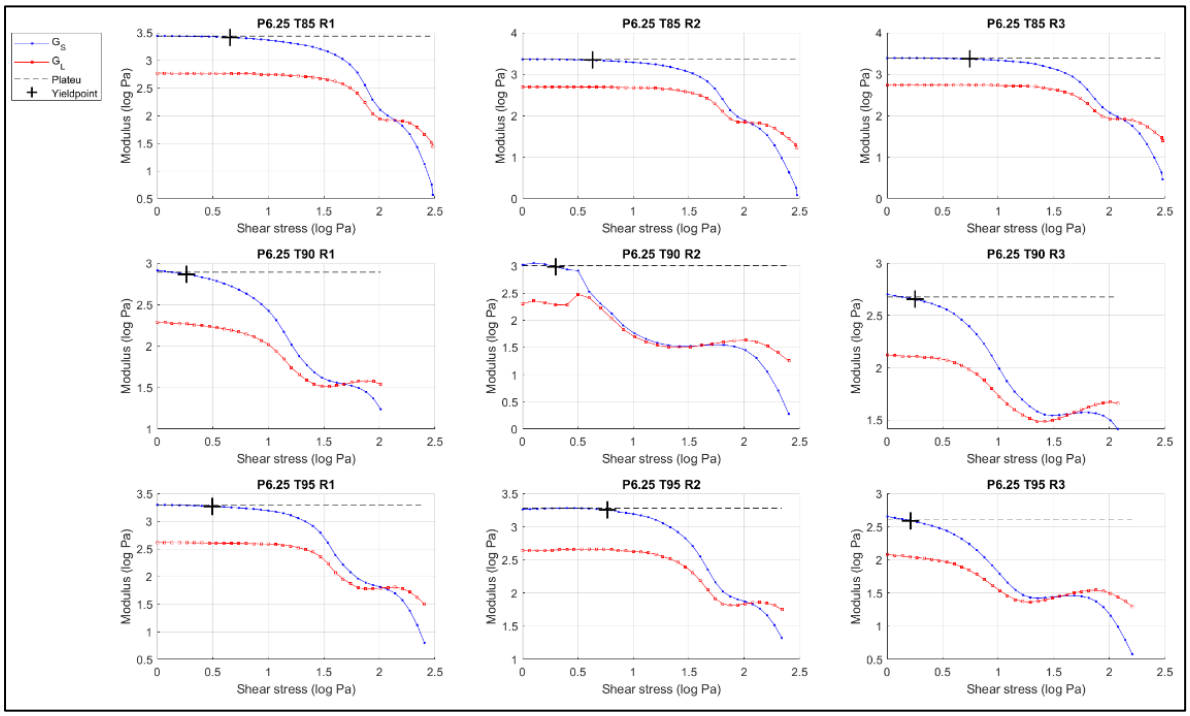

Figure 22: Results from amplitude sweep on 6,25% protein samples, subscript S for storage modulus, subscript L for loss modulus.

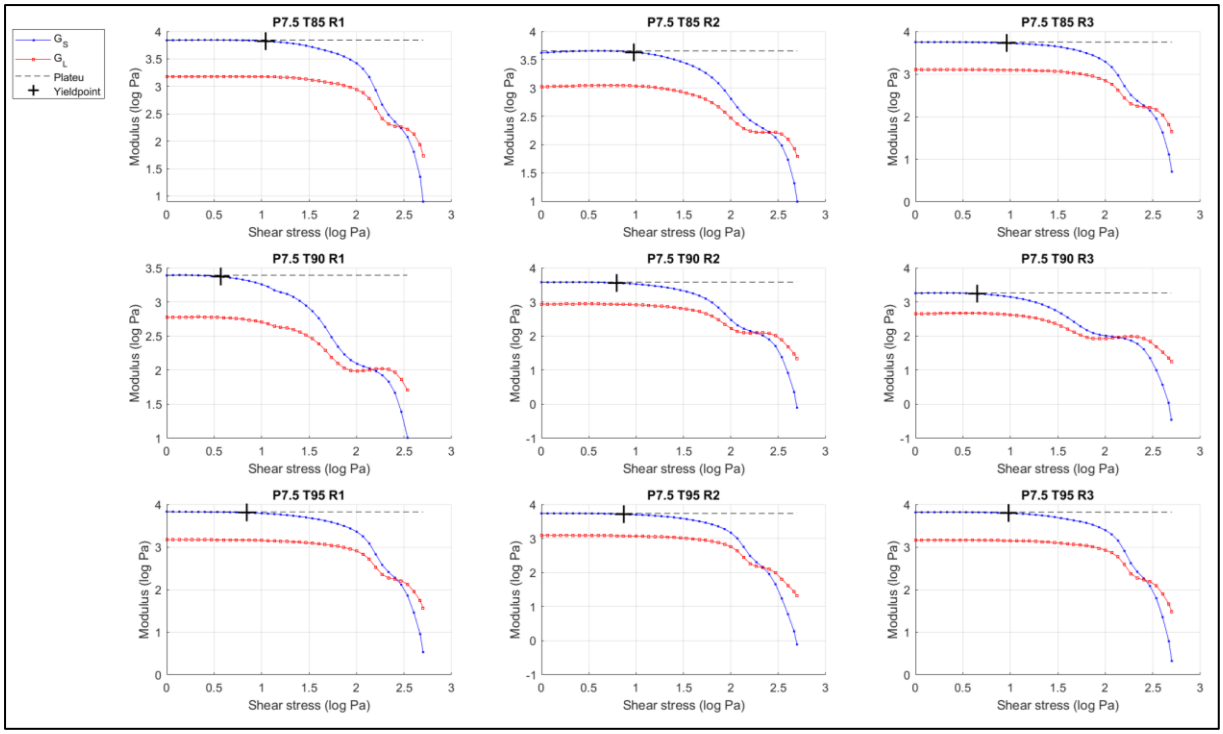

Figure 23: Results from amplitude sweep on 7,5% protein samples, subscript S for storage modulus, subscript L for loss modulus.

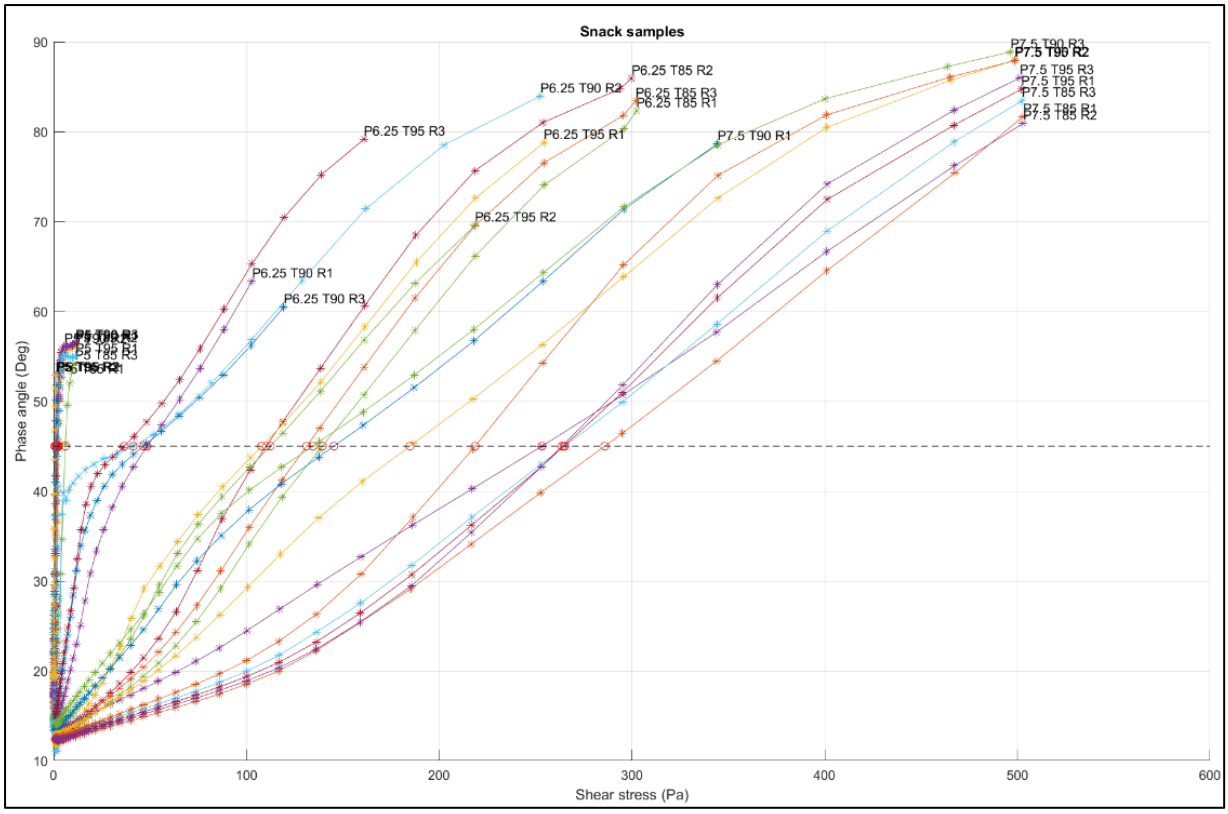

Figure 24: Results from amplitude sweeps on all snack samples, interpolated breakpoints shown as circles.

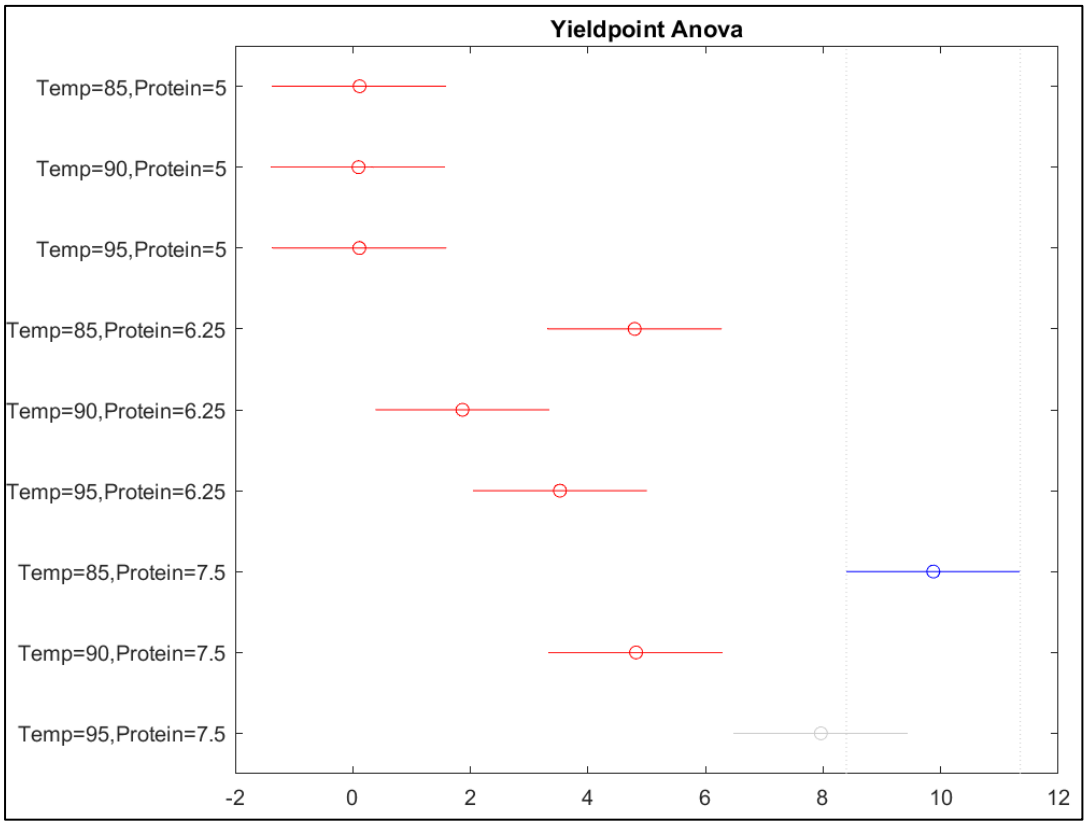

Figure 25: 2-way ANOVA on Yield point of snack samples

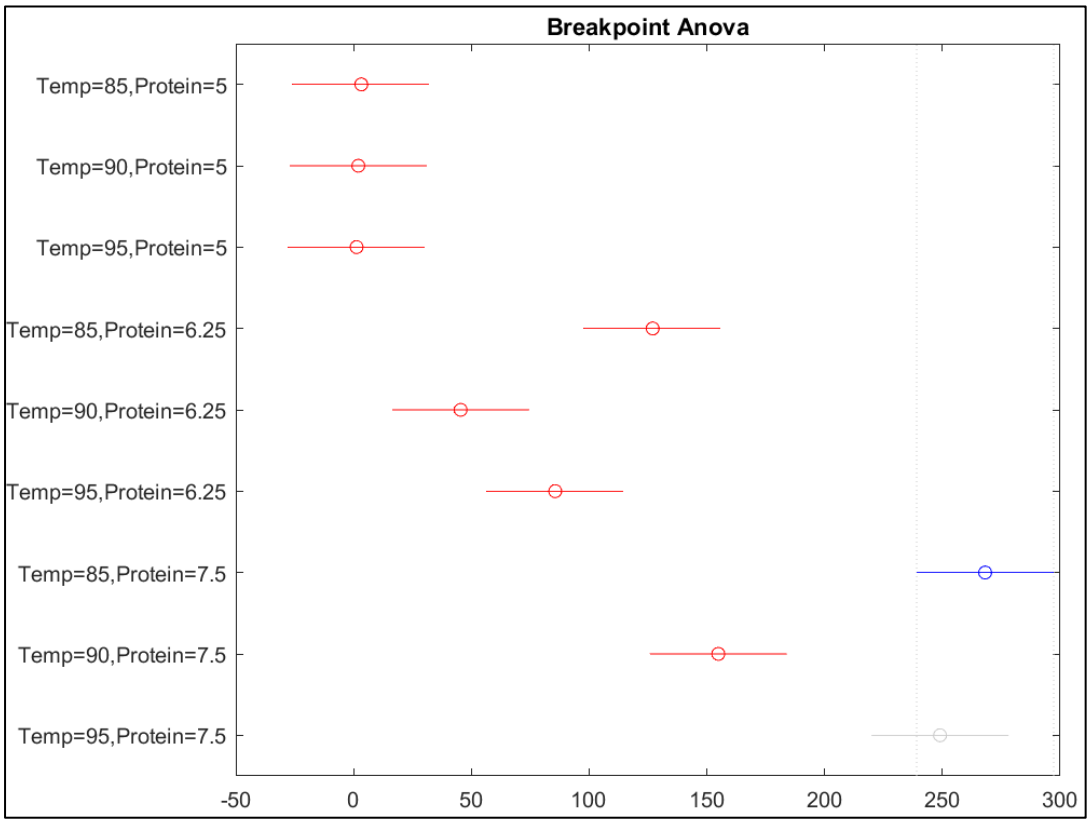

Figure 26: 2-way ANOVA on Break point of snack samples

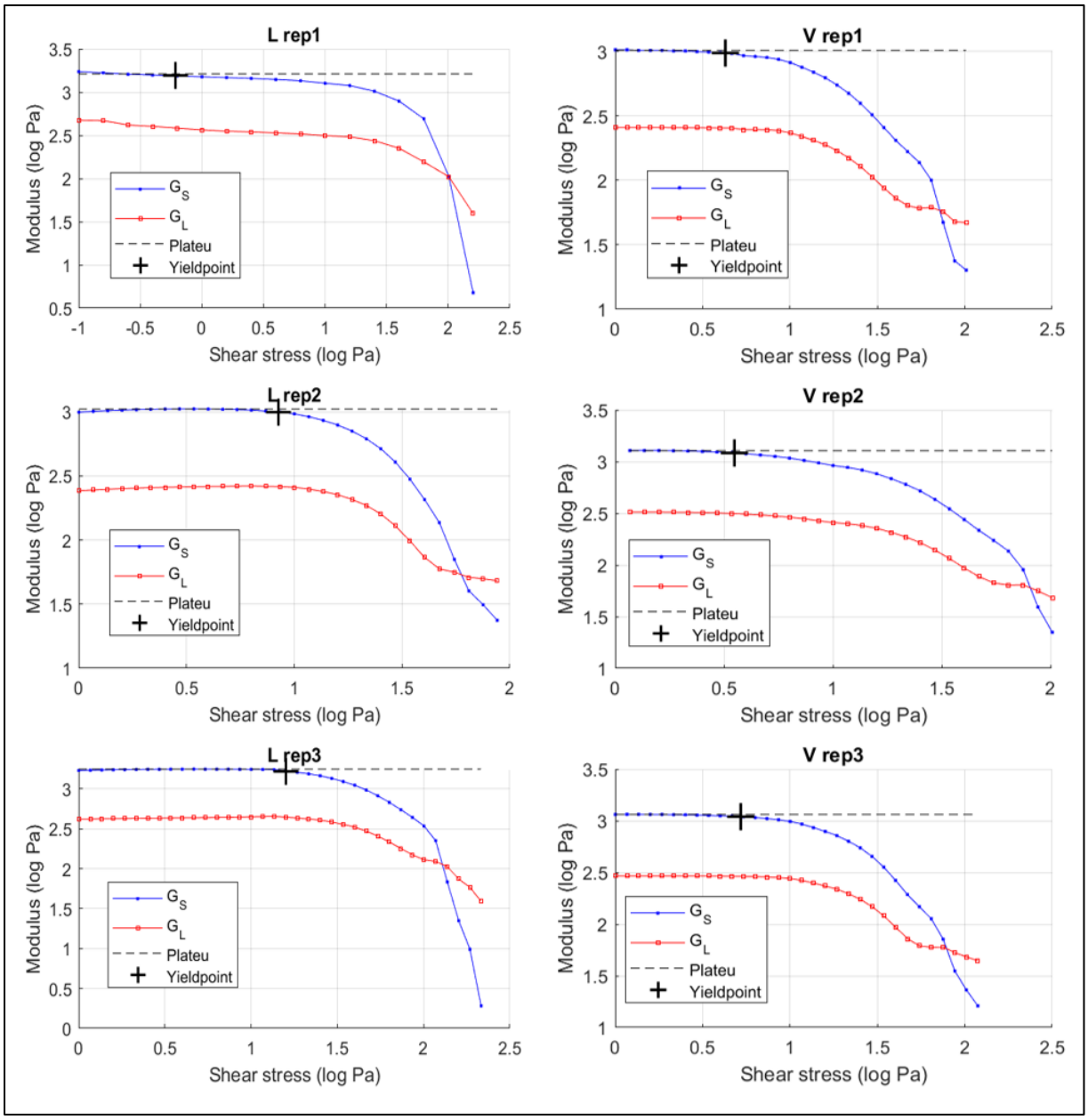

Figure 27: Amplitude sweep results for dairy based quarg samples, L for Lindahls, V for Valio.

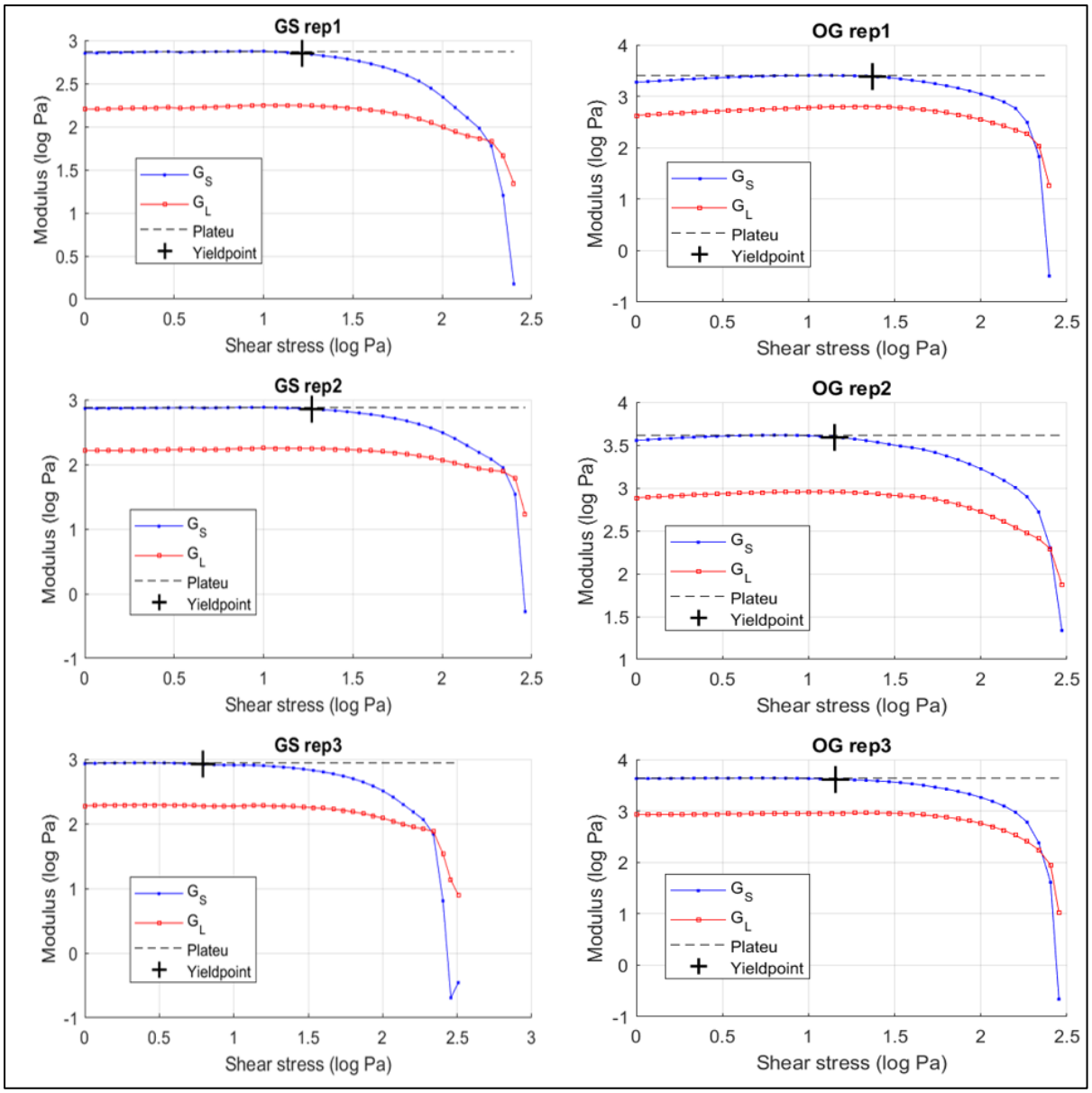

Figure 28 Amplitude sweep results for non-dairy alternative quarg samples, GS for Alpro Greek Style, OG for Valio Oddly Good.

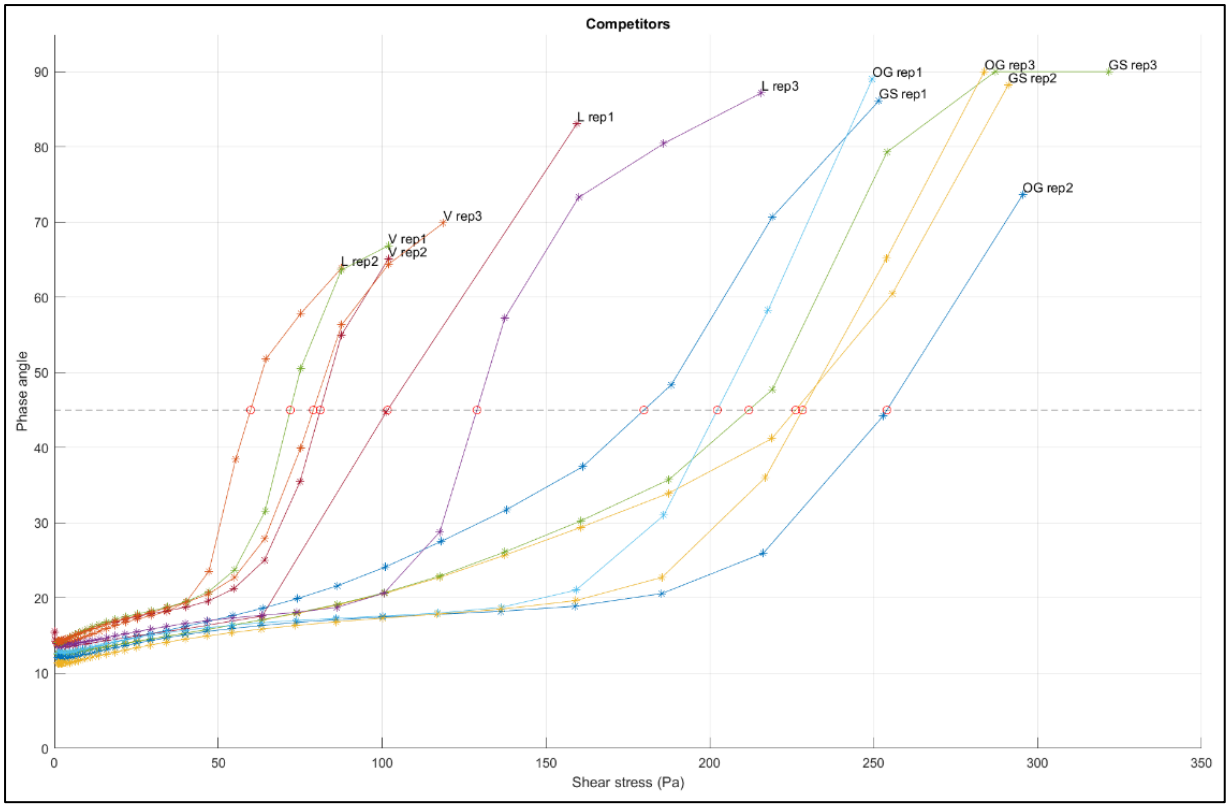

Figure 29: Amplitude sweep results for all competitor samples, showing interpolated breakpoints with circles