Development of a Vegan, Protein-Rich, Probiotic Beverage

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Master Thesis

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The market for plant-based products is increasing, causing a demand among consumers for new products to be developed. There are three main reasons for this increase in plant-based foods; sustainability, health, and animal welfare. Another increasing market is high protein products, and by combining these two trends together with probiotics, a vegan, protein-rich probiotic beverage is created.

In this project, the aim was to develop a plant-based, high-protein, probiotic fruit beverage. The application of different plant-based proteins and how they can react with other ingredients were studied. pH was considered as one of the crucial factors affecting proteins characteristics of the beverage. Several different plant-based proteins were examined, such as soy, potato, pea, and oat-protein. The main challenges with these proteins were their taste, texture and amino acid profile. Oat was the most preferred protein based on taste, and pea was the most preferred protein in terms of texture and amino acid profile. The blend of these two proteins, therefore, led to a combination that was more compatible with the object of the project which was to develop a smooth and nutritious high-protein drink. However, oat alone was still seen as a good option based on the taste.

The different protein mixtures were included in two different drinks, one based on strawberrypassionfruit, and one based on banana-tropical. Both tastes went well together with the earthy taste of the plant proteins. However, the primary concern was the texture that was perceived as sandy. Homogenization were performed on the proteins and it decreased the sandiness a lot both in the oat protein drinks and in the combination of pea and oat protein. However, some sandiness was still detectable, and a hydration step, where protein was mixed with water for one hour at 60°C, was therefore included in order to decrease the particle size of the proteins and thereby the sandiness. The hydration was performed on one oat protein sample and on one oat and pea protein sample. One oat and pea sample were not hydrated, and all three samples were analyzed, based on solubility, particle size and sensory analysis. Results from the analysis showed that the hydration step can be eliminated from the process since minor effects were observed from this step. The analysis also revealed that the combination of pea and oat protein were preferred over only oat proteins.

Two recipes have been developed that contain a good amino acid profile, solubility, and high protein content together with an acceptable taste, acidity and texture. The homogenization decreased the sandiness a lot, and the sensory panel considered the sandiness as acceptable. The development has thereby shown progress regarding the texture.

Abstract

This project aimed to develop a vegan high-protein probiotic drink with two different tastes. The protein used should have similar quality as whey, amino acid score (AAS) 1.0. One aim was also to detect necessary processing steps. The protein sources studied for this application were oat, pea, pea+rice, hydrolyzed pea, soy, and potato. The protein sources were evaluated based on descriptive sensory analysis, sedimentation, allergenicity and AAS. One processing step that was included was homogenization, and a hydration step for one hour at 60°C was evaluated. The final protein sources, hydrated oat, hydrated pea and oat protein, and not hydrated pea and oat protein, were included in one strawberry-passionfruit drink, and in one banana-tropical drink and was evaluated based on particle size, solubility, protein content, AAS and sensory analysis. Oat was preferred based on taste. However, the texture was not appealing due to sandiness, and the AAS was low. Pea had strong off-taste, but good texture and a better AAS. A combination of these protein sources provided a better product in terms of sandiness and AAS. The results from the analysis revealed that the hydration step did not make any significant difference on the texture of the final product and thus; the hydration step is not recommended. Two tastes of the new product were created, with a protein content of 4%. The protein sources were a combination of pea and oat protein, with an AAS of 0.81. A homogenization step was included.

Preface

This project did first come to our attention in October 2018. The project description immediately got our attention due to the exciting and educational task of developing a new product for the ProViva brand, that could open more doors within the food industry. After hearing about the main aim of the project which was to develop a vegan high protein probiotic drink, we were excited to contribute to the development of such a unique and interesting product. Most of the work was conducted at ProViva in Lunnarp, Sweden. Exceptions were one homogenization trial and a few analyses, including particle size measurement, protein content, and solubility, which took place at Kemicentrum, Lund, Sweden. This project took place during spring 2019.

During the development, we have received several inputs and feedback from our supervisor in Lunnarp, Karolina Göransson. We would like to thank her for help and guidance throughout the whole process of developing these products. Another thanks goes to Martin Antonsson the director at the R&I department and Marie Birger at the R&I at ProViva for their help and advice during our work. Yvonne Granfeldt, our supervisor at LTH has also provided us with a lot of directions especially regarding our report, and we would therefore thank her.

We also want to thank Hans Bolinsson for his help and patience when the homogenizer at Kemicentrum broke down several times due to the high viscosity and big particle size of our samples. A thank you also to all the participants in our sensory analysis, especially to the panel member who faced extra challenges on her tongue while tasting our products.

Finally a big thanks to our friends and families who have supported us during our master thesis.

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Abbreviation

CAGR: Compound annual growth rate L. plantarum: Lactobacillus plantarum AA: Amino acid EAA: Essential amino acid AAS: Amino acid score PDCAAS: Protein digestibility corrected amino acid score DIAAS: Digestible indispensable amino acid score pl: Isoelectric point DLVO: Derjaguin, Landau, Vervey, and Overbeek Arg: Arginine Glu: Glutamine Trp: Tryptophan Met: Methionin Cys: Cysteine Phe: Phenylalanine Tyr: Tyrosine His: Histidine Lys: lysine Val: Valine Ile: Isolosine Thr: Thyronine Leu: Leucine GhGs: Greenhouse gasses PCA: Principal component analysis

1. Introduction

1.1 Background

Nowadays, consumers are becoming more aware of the products they are consuming and this has led to growing demand in the food industry for healthier and more sustainable products (MarketWatch, 2019). Sweden had one of its largest decreases in consuming meat products for 30 years with a 2.6% drop in people eating meat in 2017 (Jordbruksverket.se, 2019). The growing demand for plant-based food products has led to a new form of communication between the consumers and the manufacturers to adapt more and more with the market and consumer's needs. Using plant-based protein in food products can promote sustainable lifestyle and reduce greenhouse gas emissions that arise from using animal products. However, using plant-based proteins instead of animal protein is one of the increasing trends and challenges that the industries are facing (Thøgersen, 2005). Consumer's demands towards healthy and sustainable beverages are leading towards the creation of a more advanced type of functional beverages with less sugar content and higher protein made with natural ingredients (Gao, Yu and Lee, 2013).

There has been an increasing growth in producing plant-based protein beverages among manufacturers (Reuters.com, 2019) and the consumers have accepted the fact that plantbased protein can also be a great source of protein. High protein diets are a new mainstream that is followed not only by athletes and bodybuilders but also by non-athletic consumers (De Backer and Hudders, 2015). There is clear evidence that a high protein diet can reduce energy intake and result in potential fat loss (Singh, 2015). While proteins are one of the essential compounds that structure our body tissues and muscles, it is also important that they cover the expected nutritional needs of the body. One of the major drawbacks of using plant-based proteins may be referred to as lacking one or two essential amino acids (Berghout et al., 2015). Combining different plant proteins with complementary amino acid profiles can improve protein quality (Wu, 2016).

Similar increasing trends as for plant-based market can be seen in the superfood market which has had a remarkable growth rate in recent years. These products are known to be mostly plant-based and high in nutrients such as antioxidants, fibers, vitamins and minerals, thus, they are receiving a lot of traction due to their health benefits. Market research analysts have predicted a compound annual growth rate (CAGR) of 17% for this market by the end of 2023 (Technavio, 2019).

ProViva is one of the most well-known companies in Sweden that produces probiotic fruitbased beverages and has now decided to add the ProViva high protein probiotic drink to its products. As a pioneer in producing functional beverages, ProViva is now exploring on combining three incredibly valuable food properties such as probiotic, vegan and high in protein and create a product that is framed as a '*super beverage*' on the supermarkets' shelves.

1.2. Danone and ProViva AB

Danone is a global food company focused on four different areas; dairy and plant-based products, early life nutrition, medical nutrition and water (Danone, 2019). Since 2010 Danone is the majority owner (51%) of the Swedish company ProViva AB. ProViva AB is located in Lunnarp, Sweden, and is a company that produces probiotic fruit drinks. The drinks are composed of two different parts, one fruit part, and one fermented oat part, which contains the probiotic bacteria *Lactobacillus plantarum* 299v. The fruit part generally contains around 35%-65% fruit, and the addition of vitamin C (ProViva, 2019).

1.3. Probiotics

There are different definitions of what probiotics are, the most common definition that are described by FAO/WHO (2006) is "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host". Another similar definition provided by ISAPP (2014) is "live microorganisms when administered in adequate amounts, confer a health benefit on the host". There are some aspects that are particularly important for a probiotic strain in order for it to be beneficial to humans. The bacteria must tolerate acid and bile. It has to be able to attach to the epithelial surface and mucosa. Another important aspect is safety, and therefore the bacteria must be non-pathogenic and should not have any ability to spread antibiotic resistance (Saarela et al., 2000).

1.3.1 Lactobacillus plantarum 299v

Lactobacillus plantarum 299v is a gram-positive, fermentative non-spore forming bacteria (de Vries et al., 2006) and is the probiotics used in the ProViva products. *L. plantarum* 299v can grow at pH between 4.0 and 8.0, but the bacteria can survive in pH between 2.0 and 9.0 (Hernandez-Sanchez et al., 2014). The consumption of *L. plantarum* 299v has shown health benefits to consumers in several different trials. Among others, L. *plantarum* 299v has shown to help in iron deficiency (Hoppe, Önning and Hulthén, 2017).

Like any other bacteria, *L. plantarum* 299v can grow in suitable and nutritious environments and survive in more undesirable conditions. pH values of the medium are crucial for bacteria survival and metabolism (Molin, 2015). According to a study done by Francois et al. (2010), the presence of weak organic acids was found to contribute to the reduction of bacterial metabolism in a fruit drink product. Probiotic bacteria can react differently to different food components. High composition of carbohydrates usually goes hand in hand with an effect on bacteria metabolism. *L. plantarum* consumes the carbohydrates and creates CO₂ and lactic acid (Filannino, et al., 2014).

1.4. Fruits

Fresh fruits and vegetables are a great source of vitamins, minerals and dietary fibers which are known for their health-promoting effects in the human diet. Among different fruits, berries are a high source of antioxidants plus dietary fiber which boost energy and decrease laxation (Block and Thomson, 1995). Fruits are often added to foods in order to improve the taste due to their appealing flavor (Papies, 2013).

One of the major factors in fruits are their low pH which helps the fruits to retain their flavor. Ascorbic acid or vitamin C is an antioxidant which contributes to this factor and it is found in many soft fruits (Ashurst, 1998).

Fruits are important sources of soluble sugars such as glucose, fructose and sucrose and the quality of fruits are usually determined by their sugar content which depends on factors such as type of fruits, environment and genetics of the fruit (Nookaraju et al., 2010). Therefore, using fruits with higher sugar content in the industry can eliminate or reduce the amount of added sugar to the products.

1.5. Protein

Proteins are one of the macronutrients in food and they are essential in the diet. Proteins are needed for cell maintenance and building of new cells in the body, but it is also required for hormones, enzymes and for some part of the immune system. They are the nitrogen holding compounds and contain amino acids. Proteins are built up of approximately 20 amino acids, of which nine are indispensable/essential for humans (Wu, 2016). The daily recommended value for proteins is 0.66 to 0.83 g/kg body weight (EFSA, 2018).

1.5.1. Essential amino acids

Essential amino acids (EAA) are amino acids that the human body cannot build itself. EAA, therefore, has to be ingested with food. In order for the body to function well, a certain amount of each EAA has to be consumed each day, see table 1. These values vary for children and adults. In table 1, the amount in mg of each essential amino acid that is required per g protein for adults and children can also be seen.

Essential amino acids	mg/kg per day children	mg/kg per day adults	mg/g protein children	mg/g protein adults
Histidine	15	10	18	15
Isoleucine	27	20	31	30
Leucine	54	39	63	59
Lysine	45	30	52	45
Methionine + cysteine	22	15	26	22
Phenylalanine + tyrosine	40	25	46	30
Threonine	23	15	27	23
Tryptophan	6.4	4.0	7.4	6.0
Valine	36	26	42	39

1.5.2. Amino acid score

The amino acid score (AAS) is an indication of how well balanced a food's amino acid profile is compared to the amino acid requirements for children, and it is evaluated for each essential amino acid. The AAS is calculated according to equation 1.

$$AAS = \frac{mg \, of \, amino \, acid \, in \, 1 \, g \, test \, protein}{mg \, of \, amino \, acid \, in \, requirement \, pattern} \tag{1}$$

The amino acid requirement pattern is based on children's requirement. An amino acid score above or equal to 1 is considered to be balanced, and that amino acid is not limited in the protein. The amino acid with the lowest AAS, below 1, is considered to be the limiting amino acid (WHO, FAO and UNU, 2007).

1.5.3. Protein digestibility

Digestibility is a measurement of how well the human body absorbs protein, and it is measured by comparing the nitrogen content in food with nitrogen content in feces after ingestion. In order to make the digestibility the most accurate, the fact that fecal nitrogen is endogenous needs to be taken into consideration. This is done by measuring nitrogen losses in a nitrogen-free diet. Digestibility is thereby calculated according to equation 2, where I is nitrogen intake, F is nitrogen loss in feces, and F_k is nitrogen loss in feces in a protein-free diet (WHO, FAO and UNU, 2007);

$$digestibility (\%) = \frac{I - (F - F_k) \cdot 100}{I}$$
(2)

1.5.4. PDCAAS

Protein digestibility corrected amino acid score (PDCAAS) is a method to evaluate the composition of essential amino acids in proteins/food, and how well the body can digest them. PDCAAS is calculated according to equation 3 (WHO, FAO and UNU, 2007);

$$PDCAAS = digestibility \cdot limiting amino acid score$$
(3)

If PDCAAS is above 1 it is truncated to 1. PDCAAS is currently the most common method to evaluate protein quality (Marinangeli and House, 2017).

1.5.5. DIAAS

The digestible indispensable amino acid score (DIAAS) is another method to calculate the protein quality in foods. The difference compared to PDCAAS, is that digestibility is measured for each essential amino acid. The digestibility for each EAA is measured in the same way as for total protein digestibility by examining the content in the food, and in the feces, except that each essential amino acid is measured, see equation 4 and 5;

$$AAS_{for \ each \ EAA} = \frac{EAA(mg) \cdot Ileal \ EAA \ digestibility \ coefficient \ perg \ total \ protein}{Reference \ pattern \ EAA \ (mg) \ perg \ protein} \cdot 100$$
(4)
$$DIAAS = EAA \ with \ lowest \ AAS$$
(5)

In contrast to PDCAAS, DIAAS values above 100 are not truncated. This method will, therefore, provide a more accurate evaluation of the protein quality (Marinangeli and House, 2017).

1.5.6. Protein Quality

Animal-based protein is considered to have a better quality than plant-based protein since animal protein usually contains essential amino acids in a balanced amount (de Gavelle et al., 2017). However, it is possible to receive a good amino acid profile with plant protein as well, but usually, a combination of different protein sources is needed, for example, a combination of pea and rice (Wu, 2016).

1.5.7. Antinutritive factors

Many vegetables rich in protein also contain a lot of antinutritive factors. These generally lower the digestibility and bioavailability of the food, and thus decreases the nutritional value. Antinutritive factors can also reduce the health gain that can be received by consuming a certain type of food (Nadathur, Wanasundara and Scanlin, 2016).

1.5.8. Protein Solubility

Solubility is a measurement of how much of a material can solubilize in a solution without precipitating (Golovanov et al., 2004). There are factors that contribute to the solubility of the protein which some can be manipulated, and some depend on the structure of the protein itself (Golovanov et al., 2004). Ambient conditions such as pH, ionic strength and temperature can to some extent affect the solubility of the protein. Salt can influence the solubility of the protein by affecting the ionic strength of the solution. Different salts can have different effects. By creating a double layer of electrons surrounding the proteins, based on DLVO (Derjaguin, Landau, Vervey, and Overbeek) theory, salt can increase the ionic strength and thus preventing the proteins from aggregating (Lam, *et al*, 2016).

The Solubility of the protein is highly dependent on its isoelectric point (pl). At the protein's pl, the protein net charge is zero thus, they tend to aggregate. As the pH values get far from the pl the proteins get more solubilized (Sumner, Nielsen, Youngs, 1981). By increasing the hydrogen ions in a protein solution with neutral pH, the NH₂ group absorbs the hydrogen and creates NH₃⁺. When all NH₂ groups have become protonated, the isoelectric point occurs, and the electrostatic repulsions between proteins vanish, due to one positive and one negative charge; thus, proteins precipitate. However, by adding more proton ions and exceeding the isoelectric point, the COO⁻⁻ group acts as a base and absorbs the hydrogens in the solution leading to an increase in electrostatic repulsion and thus the aggregation decreases (Encyclopedia Britannica, 2019).

Temperatures between 40 and 50°C generally provide the best solubility for proteins. However, when a higher temperature is used for a longer period, the protein will denature (Pelegrine and Gasparetto, 2005). The denaturation temperature for proteins are generally around 75°C (De Graaf, 2000), however, for pea protein, in general, the denaturation temperature is above 60°C (Lam et al., 2016) while for oat globulins the denaturation temperature is as high as 110°C (Webster and Wood, 2011). The denaturation occurs because of the secondary and tertiary structure of proteins, which is stabilized by noncovalent bonds and breaks due to high temperature. This makes hydrophobic groups, such as sulphur groups which are generally in the middle of the protein, to react with each other and this reduces the interaction with the water and thereby the solubility. Since the hydrophobic groups interact with each other they also cause aggregation, coagulation and sedimentation (Pelegrine and Gasparetto, 2005).

1.5.9. Protein sources

Oat, Pea and Soy proteins were the major sources that attracted the most attention and this report focuses mainly on these three proteins; even though other protein sources such as potato, pea+rice and hydrolyzed pea were also examined later in the trials. In a hydrolyzed protein, the protein has been broken down to its amino acids, partially or totally. This is usually done either with boiling it in strong alkaline or acidic condition, or its done by enzymes (Caballero, Trugo and Finglas, 2003).

1.5.9.1 Oat

As a classic cereal in the nordic diet, oat is now becoming more popular among public and manufactures due to its nutritious health effects. Such as wheat and rye, oat also belongs to grass family called Gramineae (Stewart & McDougall, 2014) Oat protein is a side stream of oat fiber extraction known as β -glucan, which provides several health benefits to human digestion system and has cholesterol-lowering impacts (Rasane et al., 2013). Compared to animal-derived protein, oat protein has significantly lower land and climate drawbacks and thus, it is a much more sustainable alternative for animal-based proteins (González, Frostell, & Carlsson-Kanyama, 2011).

1.5.9.1.1 Chemical composition

The quality of protein is mostly dependent on its essential amino acid composition. Among cereals, oats contain higher protein content (15-20%) with a relatively better amino acid profile compared to other cereals. The amino acid composition of oat is more distinct in comparison to other cereals due to having globulin as the major protein storage (unlike most cereals that have prolamine as their major protein storage) with higher amount of threonine which is among the limiting amino acids (Klose and Arendt, 2012). However, the lysine composition is still below the recommended amount by FAO which is 4.5% (FAO/WHO/UNU, 2007).

1.5.9.1.2. Allergenicity

Prolamins can cause an autoimmune reaction in the small intestine due to mucosal inflammation which is known as celiac disease. Oat contains a low amount of prolamins, and studies have shown that celiac patients can tolerate oats especially in low quantities (Hoffenberg et al., 2000). However, due to cross-contamination occurrence while

transformation and storage conditions, it cannot be claimed as a completely safe product for celiac patients (Koerner et al., 2011).

1.5.9.1.3. Protein Solubility

Oat has an isoelectric point of 5 and it is relatively low solubility in water due to containing high amount of globulin. Globulin has a compact molecular structure, making it harder to solubilize in high protein solutions. It is typical for oat and other plant-based proteins to have an optimum pH of near neutral and slightly above, which makes it challenging for low pH products (Ma, 1984).

1.5.9.1.4. Digestibility

Studies have shown that oat protein has a digestibility higher than 90% which makes it a suitable option when it comes to plant-based proteins (Eggum, Hansen and Larsen, 1989).

1.5.9.1.5. Anti-nutritive Compounds

Although oats are known for their high nutritional value, nevertheless, they contain antinutrients which can be undesirable for human consumption. These antinutrients can be phytic acid and saponins. Saponins have shown to be affecting cell membranes and increase their permeability that leads to problems in transport mechanisms and the enzyme system in mammal intestines (Önning et al., 1996). Phytic acid inhibits the absorption of minerals (Urbano et al., 2000).

1.5.9.1.6. Taste

Oat has a characteristic taste, which can be described as sweet, cereal-like and a bit nutty. It also consists of a bitter and astringent after-taste, which is normally described as an off-taste if considered too intense (Günther-Jordanland et al., 2016).

1.5.9.2. Pea

Peas are seeds from the legume family which are known for their high protein content. The protein content of pea can vary from 18 to 30% depending on the cultivar and place of growth and 65-80% of this protein is coming from globulin storage. Peas comprise of 60-65% carbohydrates which are mainly composed of starch and relatively high ratios of dietary fiber compared to cereals which can go up to 17-27% (Chang et al., 2015).

1.5.9.2.1. Chemical composition

Even though pea proteins have a higher amount of lysine compared to other plant proteins, they are still lacking in methionine + cysteine composition. Proteins in pea are mainly in the form of storage proteins which can be seen as legumin (11S) and vicilin (7S) and convicilin that creates the globulin (Chang et al., 2015).

1.5.9.2.2. Protein Solubility

Pea protein has an isoelectric point at a pH of 4.5, and here the solubility is the lowest. However, the solubility increases rapidly both below and above this pl. The higher positive charge of the pea protein during more acidic conditions increases the solubility (Ladjal-Ettoumi et al., 2015).

1.5.9.2.3. Digestibility

The digestibility of pea protein has been estimated to be between 89.4 and 91.5% (Röös et al., 2018). However, the preparation of the pea can influence the digestibility. For example, hydration and heating increase the digestibility (Nadathur, Wanasundara and Scanlin, 2016).

1.5.9.2.4. Anti-nutritive compounds

In pea, there are a lot of anti-nutritive factors. Phytic acid is a component that stores phosphorus compounds in plant tissues. When ingested, it interacts with minerals in the stomach which reduces protein digestibility. Other compounds that reduce protein digestibility and bioavailability in the pea are small protein bodies, that inhibits trypsin and chymotrypsin. Pea also contains polyphenols and tannins which also reduces the digestibility and bioavailability by reacting with proteins and minerals. However, most of these compounds are found in the coat of the pea. The remaining antinutritive compounds in the pea are sensitive to hydration and thermal processing (Nadathur, Wanasundara and Scanlin, 2016).

1.5.9.2.5. Taste

Even though there is a big variation in taste among peas depending on growth conditions and cultivar, peas have a unique, earthy taste. Pea has a bitter after-taste due to saponin content. Pre-treating the pea with treatments such as cooking, roasting, hydrating or drying can reduce the off-taste (Nadathur, Wanasundara and Scanlin, 2016).

1.5.9.2.6. Allergenicity

Pea is not considered to be an allergen; however, it is possible and quite common to receive an allergic reaction when consuming it (Livsmedelsverket, 2018).

1.5.9.3. Soy

Yellow soybean has a high protein content, usually between 37 to 42%. The carbohydrate content is between 8 and 13%, fat content between 18 and 22% and a fiber content between 13 and 17%. Green soybean has a similar protein and fiber content, but a fat content around 1% and a carbohydrate composition around 39% (Redondo-Cuenca et al., 2007).

1.5.9.3.1. Chemical composition

In soy-derived foods, it is common to use protein isolate in order to have a high protein content. Usually, these soy protein isolates contain around 90% protein (Kalman, 2014). Soy protein isolates are a high-quality protein, containing all the essential amino acids (MacDonald, Pryzbyszewski and Hsieh, 2009), with a total amount of essential amino acids that is above the requirements for adults. Some amino acids occur in a limited amount, especially methionine and isoleucine (Gorissen et al., 2018). The protein in soy is mainly storage proteins, 7S- and 11S-globulins which are called beta-conglycinin and glycinin (Chang et al., 2015).

1.5.9.3.2. Protein Solubility

The solubility of soy protein isolate changes when the pH is changed (Chang et al., 2015). The lowest solubility is reached at the isoelectric point which is between 4.5 and 5.0 for soy (Yuan et al., 2002) and is at this pH almost 0. The best solubility is reached at acidic pH around 3.0, where the solubility is around 70% due to the charges of the protein at this pH (Chang et al., 2015).

1.5.9.3.3. Digestibility

The true ileal digestibility for soy protein isolate is considered to be 95% (Röös et al., 2018).

1.5.9.3.4. Antinutritive compounds

Soy contains lectins and saponins that are considered to be antinutritive compounds (Sunilkumar, 2016).

1.5.9.3.4. Taste

Soy has a slightly beany taste (Shin, Kim and Kim, 2013), and due to the antinutritive compounds lectins and saponins also a slightly bitter taste (Sunilkumar, 2016).

1.5.9.3.5. Allergenicity

90% of all food allergies in the world are believed to come from eight foods. Soy is one of them and are considered to be an allergen. In the entire world-population, between 0.3-0.4% are allergic to soy. Several different protein components in the soybean have been linked to allergies, and the most common one is lecithin (L'Hocine and Boye, 2007).

1.5.9.4. Whey

Currently, the most popular protein source in protein-rich beverages is whey, which is a milkderived product. During cheese production whey is separated from the cheese and is therefore a by-product from the cheese production (Hoffman and Falvo, 2005); whereas, in plant-based proteins the extraction is more challenging and is highly influenced by the presence of interfering compounds such as carbohydrates, terpenes, phenolics, lipids and etc (Niu et al., 2018). Compared to plant-based proteins in general, whey protein has a better essential amino acid profile (Hoffman and Falvo, 2005), see table 2. According to this table, whey protein is not limited in any essential amino acid (Arla Foods Ingredients, 2018). However, the amino acid composition can vary depending on the brand. The digestibility of whey protein is estimated to 95% (Röös et al., 2018), and this gives a PDCAAS of 0.95, and histidine becomes the limited amino acid. Whey protein is astringent, just as many plant-based proteins, however, it has less off-taste and are perceived as less sandy (Bull et al., 2017). Whey protein also has a better solubility compared to plant-based alternatives. However, just as with plant-based protein the solubility is the least at the isoelectric point at pH 4.5, at pH around 3.5, and 5.65 the solubility is between 85 and 95% (Pelegrine and Gasparetto, 2005).

Whey		l	1	1	1				1	
		Met+	Phe+							Total
Essential amino acids	Trp	Cys	Tyr	His	Lys	Val	lle	Thr	Leu	EAA
Amount (g AA / 100g protein)	1.8	5.0	6.3	1.8	10.3	6.3	7.1	7.7	11.5	57.8
Requirements children (g AA/100g protein)	0.74	2.6	4.6	1.8	5.2	4.2	3.1	2.7	6.3	31.0

Table 2. Amino acid profile of whey protein isolate (g AA / 100g protein).

1.6. Texture

Food texture is a broad sensational parameter for describing food features that involve visual, audio and tactile organs. The texture is one of the most crucial parameters for individuals to perceive and experience food and gives them the opportunity to describe and elaborate on different features of the food that they are consuming. The components of the food and food texture are parallel in this concept. Solid foods, beverages, emulsions etc. all have their own specific characteristic (Luckett and Seo, 2015). In beverages, rheological and sensory perception of the product plays a key role in defining and creating a new beverage. In the process of developing ProViva high protein beverage, factors such as viscosity and fluidity were crucial as the aim was to create a product that has a viscosity between a normal ProViva drink and ProViva smoothie. The major concern with this product were parameters such as sandiness, viscosity and astringency.

1.6.1. Viscosity

Viscosity is defined as the resistance of fluid while flowing when shear stress is applied and it is feasible when motion is applied within the liquid (Murphy and Morrison, n.d.). One of the main characteristics of a drink is its flowing properties and that it is easy to consume. This characteristic is influenced by the viscosity of the drink and the particle size of the blended components. The quantity of the liquid base and the selection of ingredients helps to control the blend viscosity.

The plant fibers coming from oat protein, oat fiber and pea protein, have a major impact on functional properties of the product. These functional properties can be their swelling properties, water holding capacity and gel formation. Thus, a change in the viscosity is observed because of the matrix structure, which is formed by polysaccharide chains after absorbing water since the product is being refrigerated and plant fibers have enough time to swell (Figuerola et al., 2005).

Dietary fiber has a great impact on viscosity when solubilizing in aqueous solutions. Soluble fibers absorb water when encountering an aqueous solution and create a gel which is an ability to enhance viscosity and volume of the product (Murphy and Morrison, n.d.).

1.6.2. Sandiness

One defect in texture is called sandiness or grittiness and is when fine particles can be sensed in different types of food (Engelen et al., 2005). In a study, done by Imai et al. (1999) where

sandiness was evaluated it was concluded that increased particle size and concentration of particles strongly affected the perceived sandiness. However, the shape of particles, spherical or oval, did not affect the sandiness. In the same study, they also examined if viscosity affected the perceived sandiness, which it did. The more viscous, the higher threshold value. The conclusion from the study was that particle concentration affected the perceived sandiness the most, followed by viscosity and particle size (Imail, Hatea and Shimada, 1995). The perceived sandiness is strongly linked to the type of food, and therefore it is not possible to predict a threshold value for particle size in a given food. However, by comparing the range of detectable particle sizes, an estimation can be made. For example, the threshold value for perceived sandiness in ice cream is 55 μ m, in condensed milk it is 10 μ m and in chocolate it is 25 μ m (IMAI et al., 1999).

1.6.3. Astringency

Astringency can be described as different sensations such as dryness, tightness of muscles in the mouth and in the oral mucosa, but also roughness (Laguna, Bartolomé and Moreno-Arribas, 2017). Astringency is not considered to be a taste; however, it affects the overall taste of a food a lot, and is a tactile sensation. Astringency is thought to appear when astringent compounds react with proline-rich proteins in the saliva, which creates increased friction and therefore is perceived as astringent (Beecher et al., 2008). Polyphenolic compounds are generally considered to be astringent, and these compounds are present in many plant sources as secondary metabolites. Sucralose, sucrose, polydextrose and milk have all shown an effect on reducing astringency (Ares et al., 2009).

1.7. Food Sustainability

One common definition of food sustainability from FAO/Biodiversity International (2010) is: "Sustainable diets are those diets with low environmental impacts which contribute to food and nutrition security and to a healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources."

Plant-based proteins are considered to be more sustainable than animal-based proteins (Gorissen et al., 2018). When comparing greenhouse gases (GhGs) milk products are considered to be medium with an emission of 1-4 kg CO₂ eq/kg edible weight, while oat and pea are considered low GhGs with 1 kg CO2 eq/kg edible weight (Meyer and Reguant-Closa, 2017). When comparing the carbon footprint for protein concentrates, oat protein concentrate is considered to have a carbon footprint of 10 kg CO₂-eq/kg concentrate, pea is considered to be 2.5 and whey protein concentrate around 20 kg CO₂-eq/kg concentrate. This suggests that plant-based protein concentrates have a lower carbon footprint compared to animal-based protein concentrates have a lower carbon footprint compared to animal-based protein concentrates have a lower carbon footprint compared to animal-based protein concentrates (Pulkkinen, Hietala and Katajajuuri, 2018).

1.8. Processing steps

1.8.1. Pasteurization

Pasteurization is partial sterilization and is used to limit the number of pathogens in a food, usually a beverage, and increases its shelf-life. Generally, a temperature below 100°C is used for between 10 seconds and 30 minutes, depending on the type of food that is being pasteurized (Dündar, Akyıldız and Ağçam, 2018).

1.8.2. Protein Hydration

During protein hydration water is added to dry protein up to the level that the protein hydration occurs and by adding more water there will not be any more hydration and instead, the solution gets more diluted. The hydration capacity of a protein can differ in different proteins and can be up to 10 to 20 percent of the protein weight. In proteins, there are hydrophobic and hydrophilic sides which are dependent on the protein amino acid structure. Due to their dipolar structure, water can bond with both sides of the protein which carry hydroxyl group and also with the side which holds the amide group. The 2D hydrogen-bonded network between the protein and the water hydrogen molecules has a major role in protein hydration (Encyclopedia Britannica, 2019).

Protein hydration has a significant impact on its solubility, whereas protein aggregation has an opposite effect. By increasing the concentration of proteins in an aqueous solution, the unfolded proteins and folded proteins, which are in equilibrium with each other, can combine together and result in aggregation of the protein particles. This is due to the less free accessible area for the proteins' surfaces to attach to the water molecules and also with an increase in water entropy, the water between hydrated or coated protein shells would run into the bulk and thus the hydration coating will get disturbed (Vajda and Perczel, 2014). In the production of protein isolates, the isolates are usually dried. In order for the protein isolates to aggregate as little as possible in the water, a rehydration step can help separate the aggregated protein particles (Oladele et al., 2018). When heat is included in the rehydration step, it denatures the protein more, and when partial denaturation occurs it has been shown to be beneficial for the proteins water hydration ability due to the fact that unfolded proteins form a matrix where water is easily trapped and thereby the hydration increases (Rao et al., 2002).

1.8.3. Homogenization

Plant-based proteins, used in an acidic beverage, may contain a significant amount of fibers, protein aggregates or other abrasive substances derived from grains that can be invisible to naked eye. Due to the varying density of different particles in the product, they can get settled. In this case, homogenization is performed in order to create a homogenous product with well distributed particles (Dairy Processing Handbook, 2018). There are two common types of homogenizers, single-stage and two-stage homogenizers. The first step reduces the particle size, and the second step reduces the particle size further and keeps the particles from aggregating (Dairy Processing Handbook, 2018).

For the protein drink, it is important that the protein particles are evenly distributed in the drink to avoid graininess and to create a smooth texture. ProViva drinks are clean products and do

not contain any emulsifier nor stabilizers, thus, homogenizing the product can inhibit the option of having additives.

1.9. Quality parameters

Several parameters are important from a food quality perspective which food safety is among them. Food safety includes avoidance of contamination of microorganisms such as yeast, moulds and enterobacter among others, but also chemical and physical material that can harm or be a hazard for the consumer (FAO, 2019). Allergenicity is also an important quality parameter in order to avoid cross contamination (FAO, 2014). For probiotic products, the amount of the bacteria that is stated on the package also needs to be fulfilled (de Simone, 2019), and this is done by viable count of the probiotic strain. All these parameters are of great importance when developing a product, however, due to lack of time the main focus has been on the following parameters that is discussed in this section.

1.9.1. Brix

Brix is the mass fraction of sucrose in the solution. By adding 1 gram of sucrose in 100 grams of solution which is solely water, the Brix would increase 1 unit, therefore, it measures the sweetness of the sample. In theory, Brix is a calibration of the refractive index of a solution against dissolved sucrose (Ashurst, 1998). A refractometer is used to measure the Brix of the sample and it is simply done by putting a few drops of the sample prism and the percentage value will be shown (Sella, 2008).

1.9.2. pH

pH is a logarithmic scale that shows how acidic and basic a solution is. It is negative log10 of the hydrogen ion concentration that is shown as mol/L (Impey and Child, 2012). In developing a beverage, pH is one of the important parameters for describing its characteristic and improving its shelf-life. Fruit derived beverages are considered as low pH drinks. Low pH beverages help with microbial spoilage, flavor and appearance enhancement (Ashurst, 1998). Therefore, in the case of ProViva an acidic condition is preferred in order to accomplish the ideal requirements.

1.9.3. Color

Color is primarily one of the main factors that make foods and beverages appealing. It shows the freshness, healthiness and identity of what we consume. The color of the product is unquestionably dependent on its composition. The color characteristic of the strawberry beverage is mainly due to the presence of anthocyanins which are water-soluble phenolic compounds and their color can range from blue and purple to red in different plants (Khoo et al., 2017). Pelargonidin-3-glucoside is a type of anthocyanins which can be found in large amounts in strawberries (Giampieri et al., 2012).

pH is a crucial factor for anthocyanin in order to demonstrate its desired color. Due to its ionic molecular nature, this pigment is more stable in low pH conditions and appear to be more

reddish. The red color of anthocyanins is in the form of flavylium cations which increases the water solubility of the anthocyanins in low pH (Khoo et al., 2017).

1.9.4. Shelf-life

In order to create a product with acceptable quality and also making it possible to launch in a large scale as a reliable profitable product, it is important to consider its shelf-life when it is going to be put on the shelves for a specific time. One of the methods for measuring shelf life of a product is to perform a stress-test by accelerating the spoilage time by changing the storage conditions parallel to having it stored for its exact storage period by increasing the ambient temperature. This gives a good insight to decide whether to continue with shelf life studies or to give it up (Han and Ng, 2013).

A lot of factors influence the shelf-life in a probiotic beverage. pH is one important aspect and since acidic pH usually inhibits the growth of pathogens, a low pH is useful. At the same time, the probiotics need to survive, but preferably not grow. Therefore, a pH below 4 is recommended (Hernandez-Sanchez et al., 2014). As mentioned previously, weak organic acid can inhibit probiotic growth (Świątecka, et al., 2010).

1.10. Product Characterization

1.10.1. Protein content

The Dumas method is a method for measuring protein content. The method is based on combustion at temperatures between 800 and 1000°C. The combustion makes the nitrogen in the sample form nitrogen oxide, and finally to nitrogen gas (N_2) by reduction. The nitrogen gas is then measured by a thermal conductivity detector. The Dumas method is time efficient and is a relatively safe measurement compared to alternatives. However, when comparing the Dumas method with the Kjeldahl method the protein content is slightly higher in the Dumas method (Jung et al., 2003).

In order to convert the nitrogen content to protein content, a nitrogen-to-protein conversion factor is used. This value is based on the amount of nitrogen in different protein and is usually considered to be 6.25. However, not all protein contains the same amount of nitrogen. Therefore, it is recommended to use different nitrogen-to-protein conversion factor depending on the food that is being analyzed (Mariotti, Tomé and Mirand, 2008). For example, the nitrogen-to-protein conversion factor for pea is 5.40 (Sosulski and Imafidon, 1990), and the conversion factor for oat is 5.83 (FAO, 2002).

1.10.2. Particle Size

Two common numbers for particle sizes are D[3,2], surface area moment mean, and D[4,3], volume moment mean. These means are correlated to moments of inertia, which means that they are the center of gravity for the surface area distribution and for the volume distribution. The benefit with these numbers is that the amount of particles is not taken into consideration (Rawle, 1993).

1.10.3. Sensory evaluation

Sensory analysis is important information when developing a new product which can be collected either from a trained panel or from potential consumers (MacFie, 2007). The data involves human's response to some certain food. Information such as perceived sense of taste, smell, sight and hearing are into consideration in this test. The gathered information helps the producers to create a product based on what the consumers need and help the producers to refine and develop a product based on their needs to create a more successful product. It is important that no extrinsic factor such as noise or smell would bother the participants, however, even in the case of a trained panel the individuals may get affected by different factors such as hunger or food habits (Sharif et al., 2017). The data from the sensory analysis is quantitative and it can vary considerably between different participants. This heterogeneous data can be assessed using statistical analysis to declare whether there is a real relationship between the participants and product's characteristics (Lawless et al., 1998).

In the early stages of developing a new product, a descriptive analysis is suitable. It is an analytical evaluation and a trained panel is usually used for this purpose (Lawless, 2016). In this type of test, the different samples are provided, and the panel elaborates their ideas about the different samples, by describing the products with words, and also the intensity of the attributes. Therefore, it is possible to gain a lot of important information about how the product are perceived and to see development. It is useful with a trained panel for this purpose, and during this training, the attributes that will be examined should be stated (MacFie, 2007). The question that a descriptive test answers is how the sensory characteristics differs between different samples (Lawless, 2016).

Another important part during product development is measuring liking with a sensory panel, and this is often referred to as an effective test (Lawless, 2016). There are different ways to do this, one is to provide two different samples and ask which one that is preferred. Another is to use a hedonic scale, where the participant grades the sample on a 1-9 scale, where 9 is considered, like extremely, and 1 is dislike extremely (MacFie, 2007). The question that is answered with this method is either which sample that is preferred, for the ranking, or how much the panel likes the products, with the 9-grade scale. However, these analyses are often performed at a later stage of the product development (MacFie, 2007).

1.11. Aim

The aim of this project was to develop a new ProViva product with a protein content between 4 and 8%. The protein should be of plant-based origin and have an AAS similar to whey, but without the drawback of the allergenicity and animal origin from milk derived products. There should be two tastes of the product, made with 2-3 different fruits/berries per taste, and the fruit content should be between 10-40%. In normal ProViva drinks, the Brix is approximately within 10-14, thus, it was decided to aim for a Brix within the same range, preferably with no added sugar. In order for the shelf-life to be as good as possible, a pH below 4.0 was aimed for the product. The viscosity of the product was aimed to be between the current ProViva fruit drinks and their Smoathies, approximately between 640 and 1640 cP. The aim was also to detect necessary processing steps in order to achieve a smooth texture of the beverage.

2. Material and Method

The material and method is divided into six sections including ingredients for product development, screening of suitable protein sources, recipe development, processing steps, product characterization and statistical analysis.

2.1. Ingredients for Product Development

2.1.1. Different proteins tested

For the nutritional composition of the protein concentrates and isolates in this section, see table 3, and for amino acid composition, see table 4.

The first protein that has been tested was an oat protein concentrate, PrOATein, from Tate & Lyle, with a protein content of 51%. PrOatein is limited in lysine and has an amino acid score of 0.58. The true ileal digestibility of PrOATein is 91% and this gives a PDCAAS of 0.46.

A yellow pea protein isolate, S85 plus D-EXP, form Roquette with a protein content of 80%, has also been used. S85 plus D-EXP has an AAS of 0.81 and is limited in methionine + cysteine. The true ileal digestibility for this pea is 90%, which gives a PDCAAS of 0.73.

A soy protein isolate, PRO-FAM 974 IP, from Kiranto Foods AS, with a protein content of 90%, has also been tested. This soy protein has an AAS of 1.0, and digestibility of 0.95, giving a PDCAAS of 0.95.

Potato protein isolate, Solanic 100F, from Avebe with a protein content of 80% has been tested. The potato protein isolate has an AAS of 1.0. The digestibility of potato isolate is not known.

A combination of pea and rice, ProDiem 7415, from Kerry, with a protein content of 80%, has been tested. This rice and pea isolate has an AAS of 0.8 and is limited in methionine and cysteine. The digestibility of the combination of ProDiem is not known.

A hydrolyzed pea protein isolate, ProDiem refresh 7304, with a protein content of 80% was also tested. The hydrolyzed pea isolate was limited in methionine and cysteine and had an AAS of 0.49. The digestibility of the hydrolyzed pea protein is not known.

	-	Carbo	-		Satura		Sodiu	Enormy	Enorg	
Protein Sources	Protein	hydrate	Sugar	Fat	ted fat	Fiber	m	(kcal)	y (kJ)	Ash
Oat, Tate & Lyle	51.0	16.9	0.4	16.0	2.8	1.9	0.0	445	1865	0.0
Pea, Roquette	80.0	0.0	0.0	9.0	2.0	1.0	1.4	400	1635	5.0
Soy, Kiranto Foods AS	90.0	0.5	0.0	4.0	1.0	0.0	1.2	380	1590	2.3
Potato, Avebe	80.0	1.0	0.5	4.1	1.5	4.5	0.1	370	1548	1.9
Pea + Rice, Kerry	80.0	4.0	0.1	9.6	1.9	2.8	0.7	424	1787	2.8
Hydrolyzed Pea, Kerry	80.0	2.5	1.5	0.4	0.1	0.0	0	350	1470	6.0

Table 3. Nutritional composition of the	different proteins,	expressed a	as g/100g
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Table 4. Reference amino acid for children, and amino acid profile and score for the different proteins used, expressed as g amino acid per 100 g protein.

		Met+	Phe+							Total	
Essential amino acid	Trp	Cys	Tyr	His	Lys	Val	lle	Thr	Leu	EAA	AAS
Reference	0.7	2.6	4.6	1.8	5.2	4.2	3.1	2.7	6.3	31.2	-
Oat, Tate & Lyle	1.3	3.6	8.8	2.0	3.0	4.8	3.7	2.7	7.0	36.9	0.58
Pea, Roquette	1.0	2.1	9.3	2.5	7.1	5.0	4.7	3.8	8.2	43.7	0.81
Soy, Kiranto Foods AS	1.1	2.6	9.6	2.7	6.4	4.7	4.7	3.9	8.3	44.0	1.00
Potato, Avebe	1.2	3.7	11.6	2.2	7.3	6.4	5.4	5.4	9.8	53.0	1.00
Pea + Rice, Kerry	0.7	2.1	8.5	2.1	6.1	4.7	4.0	3.2	7.5	38.8	0.80
Hydrolyzed Pea, Kerry	0.4	1.3	6.2	2.0	6.5	3.6	3.2	2.9	5.8	31.8	0.49

2.1.2. Other Ingredients

Soluble oat fiber (Tate & Lyle, Kismstad, Sweden).

Due to confidentiality agreements; the suppliers, all the ingredients used and the nutritional information regarding the ingredients are not mentioned.

2.2. Screening of Suitable Protein Source

In order to get an overview of the different protein sources, all available proteins were tested in a strawberry, banana and blackcurrant drink. The parameters that were evaluated for each protein source were taste, after taste, aroma, sandiness, sedimentation, amino acid score and if it was considered an allergen or not. Each protein source was then ranked as good (green) acceptable (yellow) or bad (red), for each parameter. The taste, aftertaste, aroma and sandiness were at this stage tasted and evaluated by the three people working at Research & Development at ProViva, together with us. This was done by a tasting session, where all different proteins were tasted, based on descriptive sensory analysis. Everyone then told their opinion regarding the different proteins, based on taste, aftertaste, aroma and sandiness, and stated if they considered the product as good, acceptable or bad, for more information see section 2.3.2.1.

2.2.1 Sedimentation

The sedimentation was measured by pouring approximately 300ml of product in a 350ml bottle and after 24 hours refrigeration the sedimentation was measured and calculated according to equation 6. Sedimentation between 0 and 0.1 was considered as good, 0.1-0.2 as acceptable and higher than 0.2 as bad. These criteria were based on observing different protein sources' sedimentation and their comparison with each other and this grading was an approximation based on discussions within the group.

$$Sedimentation = \frac{height of sedimented material}{height of beverage}$$
(6)

2.2.2. AAS

An AAS of 1 was considered as good, 0.8 and higher as acceptable and below 0.8 as bad.

2.2.3. Allergen

The allergen parameter was based on literature studies, where allergens were marked as red, common to receive allergic reaction or likely for cross-contamination as yellow, and neither allergen nor common for allergic reaction as green.

2.3. Recipe development

In order to decide on two different flavors for the protein beverages, a tasting session with all current ProViva products was performed to decide on which main flavor that the protein beverage should be developed from. The first trials were therefore based on a strawberry and on a blackcurrant recipe with the addition of protein. However, when the blackcurrant was dismissed a new banana-based recipe was invented, based on the same components as other ProViva beverages (see section 3.2.1.).

For an overview of the different parameters changed during the development along with the evaluation method and the aim, see table 5.

Evaluated parameters	Strawberry drink	Banana drink	Evaluation method	Aim
	Passionfruit	Passionfruit		
	Lime	Tropical compound	Descriptive sensory	
Flavor combinations	Pomegranate	Raspberry	analysis	2-3 fruits/taste
	Pea 25%, Oat 75%	Pea 25%, Oat 75%	Descriptive sensory	Loss candinass
	Pea 30%, Oat 70%	Pea 30%, Oat 70%	analysis	less off-taste
Protein combinations	Pea 37%, Oat 63%	Pea 37%, Oat 63%	AAS from suppliers	Achieve better AAS profile
	Citric acid	Citric acid	pH measurements	pH below 4
Acid	Lactic acid	Lactic acid	Shelf-life test, see section 2.3.6.	Good shelf-life
		Increasing banana		
	Increasing white grape juice conc.	Increasing tropical compound	Descriptive sensory analysis	
Sugar	Decreasing sucrose	Decreasing sucrose	Brix measurement	No added sugar
	Pectin	Pectin	Descriptive sensory analysis	Viscosity between
Viscosity	Soluble oat fiber	Soluble oat fiber	viscosity measurement	640 and 1640 cP, and reduced sandiness

Table 5. Overview of the recipe development.

2.3.1. Evaluation Parameters

2.3.1.1. Flavor

Since the project outline stated that the beverage should combine two or three fruits/berries, different combinations were tested. Strawberry was mixed with pomegranate, passionfruit and lime in three different batches. Banana was tested with passionfruit, raspberry and a mixture of different tropical fruits, referred to as tropical compound. The tropical compound was a mixture of orange concentrate, white grape concentrate, pineapple concentrate, banana concentrate, mango puree and passionfruit concentrate. Coconut cream was also added to the banana flavored product. The flavors were analyzed by descriptive sensory analysis according to section 2.3.2.1.

2.3.1.2. Protein

Initially 4, 6 and 8% of different protein sources were tested in two current ProViva drinks, ProViva strawberry and ProViva blackcurrant and later on, 4% of the different proteins in the newly invented recipe based on banana. For information regarding the different proteins, see section 2.1. Combination of pea and oat proteins were tested according to table 5 in order to achieve a good amino acid profile and reduce the sandiness. The protein composition was

evaluated according to section 2.3.2.1. Using descriptive sensory analysis with extra focus on perceived sandiness and off-taste.

Pea protein (%)	Oat protein (%)
25	75
30	70
37	63

Table 5. The different ratio between pea and oat protein that was tested.

2.3.1.3. Acids

Two different acids were available for pH reduction, citric acid and lactic acid, and these were compared in different trials. The pH was optimized with different concentrations of the two different acids. Later in the trials, it was decided to replace citric acid with lactic acid. The pH and shelf-life tests were used for evaluation of the acids and was measured according to section 2.3.2. The aimed pH was below 4.0, and the CO_2 content after shelf-life should be below 16%. The products should not be perceived as too sour either, and this was evaluated according to section 2.3.2.1.

2.3.1.4. Sugar

At the beginning of the trials, the sugar amount, sucrose, was kept constant at the same level as in the original ProViva-Strawberry recipe for strawberry, and in a similar amount in the banana. Later on, trials were made in order to reduce the amount of added sugar, to achieve the aim of no added sugar. In the banana beverage, the amount of banana (sugar content 13.9%) and tropical compound (sugar content 38%) varied at the same time as the added sugar were reduced. In the strawberry, the white grape juice (sugar content 64%) was varied to increase the natural sweetness. Trials were made without any added sugar at all. The sugar content was measured with Brix according to section 2.3.2.2., and with sensory analysis according to section 2.3.2.1.

2.3.1.5. Viscosity

In order to increase the viscosity, two different thickeners were available, pectin and soluble oat fiber (which contains 35% β -glucan), and these two were evaluated based on sandiness and taste by a sensory analysis along with viscosity measurement, according to section 2.3.2.

2.3.2. Evaluation Method

2.3.2.1. Sensory evaluation

During the product development descriptive sensory analysis have been performed after each trial to evaluate the result. The panel have been consisted of the R&I staff together with us. In all test, approximately 50 ml of each product have been poured up in plastic cups. Then all participants declared their opinion about the sample mainly describing; taste, off-taste, sandiness, viscosity, acidity and overall liking. The samples were not anonymous.

2.3.2.2. Stress-test, pH, Brix and viscosity

In order to measure the shelf life of the product, a stress test was made. 250g of the product was poured in a 350ml bottle. The bottle was then put in 25°C for 7 days. After 7 days the CO_2 and O_2 in the bottle were measured with a CheckPoint Handheld Gas Analyser (PBI Dansensor, Ringsted, Denmark). After the products had been stored for 25°C in 7 days, they were also tasted. This measurement was performed on the banana pea and oat and banana oat, and also for strawberry pea and oat and strawberry oat products. In order to improve the shelf-life, citric acid was changed to lactic acid, and the white grape juice content in the strawberry beverage was reduced.

pH was measured with a pH 340i (WTW, Weilheim, Germany) and Brix was measured with a refractometer BS200 (TODAY'S instruments co., Ltd, New Taipei City, Taiwan). The pH electrode was left in the sample until the pH was stable. These measurements were conducted on all trials to make sure that the product had a suitable pH and also enough sweetness. pH and Brix were measured both before and after the stress-test.

The viscosity was measured with a Brookfield DV-II+ Programmable Viscometer (Brookfield Engineering Laboratories Inc, Middleboro, USA). Approximately 140 ml of the product was poured into a 250 ml beaker with a diameter of 6.5 cm. Spider 2 was used with a speed of 10 rpm. The measurement was conducted for 30 seconds. The viscosity was measured both before and after the stress-test.

2.4. Processing steps

The beverages were made according to figure 1, with hydration, or without hydration, homogenization, mixing, pasteurizing, cooling, mixing with fermented oat and finally packaging.



Figure 1. Flow-chart over processing steps, with different initial steps, either hydration or without hydration.

2.4.1. Hydration

Based on recommendations from suppliers, a hydration step with the protein was made for one hour at 60°C. All water in the recipe was used in the hydration step. Before the other ingredients were added, the hydrated protein and water were weighted, and more water was added to compensate for the water that had evaporated during the heating. For ratio between water and protein, see section 3.2.2. Half of the samples were made with hydration and the other half without hydration, referred to as not hydrated.

2.4.2. Homogenization

Two different homogenizers were used, due to an equipment failure. A two-step homogenization was performed on the protein and water mixture, by a lab scale homogenizer called Panda PLUS 2000 (GEA Niro Soavi S.p.A., Parma, Italia). The first pressure was set to 200 bar and the second step to 50 bar. However, for the initial homogenization trials, as well as for the homogenization of protein used for particle size measurement of only protein and water mixture a two-step homogenizer called RANNIE 5-12.38 (APV, Copenhagen, Denmark) was used. The same pressure was used as with the previous homogenizer.

2.4.3. Pasteurization, Cooling, Mixing, Bottling and Storage

After the homogenization all other ingredients (except fermented oat) were added to the protein and water and mixed together. The samples were then pasteurized in a steam-water bath at 90°C for 15 seconds. Directly after, they were put in a cold-water bath for cooling until a temperature of maximum 8°C were reached. The fermented oat was then added and mixed in with the other ingredients and the beverage could be poured in bottles. The bottles should be stored in maximum 8°C but preferably in 4°C.

2.5. Product Characterization

As mentioned in section 3.2.1.2., two different protein sources (oat and pea+oat) were decided for the product and 2 different processes, hydration and homogenization, were considered for the proteins in order to improve their texture in the final product. The goal was to have a hydration step for both protein sources to compare it with a not hydrated protein mixture, however, due to facing problems with the homogenizer, the not hydrated oat protein mixture was eliminated from the rest of the steps. After finalizing the products formulation, four different analysis were performed on the three final varieties of the two different tastes; oat protein, pea and oat protein and pea and oat protein not hydrated, including protein content, protein solubility, particle size and sensory analysis. Figure 2 shows the steps from choosing the protein sources to processing and analyzing the final product.



Figure 2. The implemented processes and analysis on the product

2.5.1. Protein content

The nitrogen content was measured with a Flashea 1112 Series (Thermo Scientific, Waltham, USA) on the liquid beverage, for three different varieties of both strawberry and banana, same as mentioned above. Since the analyzer only accepted dry matter, the water was absorbed with Chromosorb W/AW (SÄNTIS analytical). Approximately 30mg of the absorber was added, half in the bottom, and half on the top of the beverage. Approximately 50mg of the beverage was used. Duplicates of each product were made, and one week later another measurement of each product was made. The nitrogen-protein conversion factor used was 6.25. The protein content was also calculated for the nitrogen-to-protein conversion factor for oat (see section 1.10.1.) for the oat samples. For a combination of the nitrogen-protein conversion factor of pea and oat for the pea and oat sample (for conversion factor of pea see section 1.10.1.). This conversion factor was calculated according to equation 7 and was 5.70.

conversion $factor_{oat+pea} = 0.7 \cdot conversion factor_{oat} + 0.3 \cdot conversion factor_{pea}$ (7)

2.5.2. Protein solubility

The solubility was measured by centrifuging in an Allegra X-15R Centrifuge (Beckman Coulter, Indianapolis, USA) 50 ml of each beverage in a centrifuge tube at 4000 rpm for 10 minutes. Then the protein content, conducted as previous for protein content, was measured on the supernatant. The sample was collected in the upper third of the centrifuge tube. In order to evaluate the protein solubility, the protein content in the supernatant was then compared with the result from the protein content measurement in the beverage, according to equation 8.

$$Solubility = \frac{Protein \ content \ in \ centrifuged \ sample}{Protein \ content \ in \ beverage}$$
(8)

The solubility was measured at two different dates, at 2019-04-25, duplicates of all samples were measured, and at 2019-05-06 one measurement of all samples was made. Between the two measurements the samples were stored at a temperature of 3°C.

2.5.3. Particle size

The particle size of the products was measured using Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, United Kingdom). The samples were collected from the middle part of the container in which the sample was added. The stirrer speed was set on 2000 rpm in the Mastersizer container. The refractive index used was 1.52 (Ye et al., 2016) which is the refractive index of whey and pea protein which was considered to be accurate for the pea and oat proteins. When the laser obscuration reached approximately 75%, the Samples (strawberry and banana drink) were added to the container in separate steps. Triplicates of all samples were measured.

The particle size of homogenized protein and water (not mixed with the other ingredients) was also measured, to analyze the hydration step as well as the protein source. Triplicates of all samples were measured.

The mean oat protein particle size in the oat protein concentrate was 123 μ m with D[3,2] of 61.2 μ m. The particle size of the pea protein isolate was unknown.
2.5.4. Sensory evaluation

A sensory evaluation was performed with 13 people who all worked at ProViva and whom all were used to sensory evaluations. The products tested were the same as previous which included hydrated oat, hydrated pea and oat, and not hydrated pea and oat in both strawberry and banana flavor. Approximately 50 ml sample were put in small plastic cups in a different order on a tray and was marked with a randomized three-digit number, created with the program used for the questions, Eyequestion (EyeQuestion, Amsterdam, Netherlands). The products had been stored in a fridge with a temperature of 4°C for one week before the evaluation. The products were poured up 15 minutes before the start of the sensory evaluation.

The sensory evaluation was divided into two different parts for the two different tastes. The first part was to grade each product on a 1-9 scale where 9 was very good and 1 was very bad. The parameters graded where; viscosity, sandiness, taste, off-taste, astringency and total liking. For each question, it was also possible to leave a comment. It was mandatory to leave a comment regarding the taste. The second part was to rank the three different products in relation to each other, with the most and least preferred ones, for questions see appendix A1.

2.5.4.1. PCA

A principal component analysis (PCA) was performed on the result from the first sensory part in order to find correlations between the different parameters and the different products.

2.6. Statistical Analysis

The average and standard deviation was calculated for all the different analysis, including brix, pH and viscosity. Grubbs outlier test was calculated for solubility and for particle size to detect outliers. $g_{critical}$ was 1.1543 for $\alpha_{0.05}$, when triplicates were used, and when the calculated g-value was higher than $g_{critical}$ that sample was considered an outlier and was excluded.

A two-tailed, heteroscedastic t-test was made, comparing all different samples to detect if there were a significant difference between the different samples, in solubility, particle size and in sensory analysis, respectively. The significant cut-off was set to 0.05.

3. Result

3.1. Screening of Suitable Protein Source

The result from the screening showed that oat and soy were the most promising protein sources while potato was the worst option, see table 6 (green is ranked as good, yellow as acceptable and red as bad). The hydrolyzed pea protein was considered to have a milder taste but a too weak amino acid profile, compared with the other pea, which was more of a concern for the product. The protein source decided for the further development was oat. The main drawback for soy was the allergenicity. For better understanding the criteria regarding the rankings, see section 2.2.

Protein	Oat	Soy	Potato	Реа	Pea+Rice	Hydrolized Pea
Taste				•		•
After taste						0
Aroma						
Sandiness		0				
Sedimentation						
AAS				0	0	
Allergens	•			\bigcirc		

Table 6. The different parameters for comparing the plant proteins.

3.2. Recipe Development

3.2.1. Evaluation Parameters

3.2.1.1. Flavor

From the tasting session with all different ProViva products, strawberry and blackcurrant were decided to be the most favored ones, and therefore the first trials were based on these recipes. However, very soon it was concluded that the blackcurrant flavor did not go so well with the taste of the plant-based protein. Strawberry flavor, on the other hand, was a very good complement to plant-based protein. Since strawberry is mild in its taste and went well together with plant-based protein, other mild fruits and berries were considered to find a good combination. Since banana is both mild in its flavor and also is a common flavor for protein-rich beverages (same for strawberry) it was decided to make trials with banana flavor instead of blackcurrant.

Tabel 7. Summary of the different fruits/berries that the strawberry and banana were mixed with and if the respective fruit/berry were perceived as good (green), acceptable (yellow) or bad (red).

Fruit/Berry	Lime	Passionfruit	Pomegranate	Raspberry	Tropical compound
Strawberry					
Banana					

The combination of strawberry and lime was refreshing. The lime provided the strawberry beverage with an appealing acidity; however, the lime taste could not really be detected and therefore the combination with lime and strawberry was only considered as acceptable, see table 7. Passionfruit also provided the strawberry beverage with refreshing acidity, but it also enhanced the strawberry taste and provided the beverage with a deeper taste and not just the plain strawberry. The mixture of pomegranate and strawberry was considered as less successful. The pomegranate did not provide the beverage with acidity nor with enhanced/improved flavor. Even when 4% of pomegranate juice was added it was impossible to detect. Therefore, the combination of strawberry and passionfruit was selected for the following development of the recipe. The final fruit content in the strawberry beverage was 25.9%. The amount of passionfruit was based on enhancing the strawberry flavor without taking over the taste.

Passionfruit was an appealing combination to banana as well, with the refreshing acidity and good combination together with the banana. However, since passionfruit had been selected for strawberry, and the combination of strawberry and passionfruit was considered to be slightly better, the banana-passionfruit was not continued with. The banana beverage was also mixed with raspberry, which however, was a very dominant flavor compared to the banana, and made the banana taste disappear. The raspberry also removed the mild taste from the beverage and did not go so well with the plant-based protein. The combination between banana and the tropical compound, on the other hand, was a successful mixture with appealing acidity and the tropical compound enhanced the banana flavor in a preferred way. The total fruit content in the banana beverage was 24.2%. This was a result from descriptive sensory analysis during the product development, where the ratio between banana and the tropical compound at this fruit level was considered appealing at the same time as the amount decreased the taste of the proteins.

3.2.1.2. Protein

The three different concentrations (4, 6 and 8%) of protein were all considered to be extremely sandy, and the sandiness increased with the protein concentration. Based on this result and after discussions with the different suppliers, who considered 2% of plant-based protein to be high, it was decided to have 4% as our new aim. Still, the 4% oat protein (which was the most preferable protein based on the screening) sample was still very sandy. In order to reduce the sandiness and improve the amino acid profile of the beverage, a combination of pea and oat protein were tested. A summary of the perceived sandiness, the taste and the amino acid score for the three different ratios of pea and oat proteins are seen in table 8, where the three different combinations have been compared with each other and ranked.

Table 8. Summary of the result from sandiness, taste and amino acid score, where the best is marked with green, acceptable with yellow and the worst with red.

	Sandiness	Taste	AAS
25% Pea 75% Oat			
30% Pea 70% Oat	0	0	0
37% Pea 63% Oat			

The sandiness was reduced when the pea protein was included, and the amino acid profile was also improved. Both the amino acid profile and the sandiness were improved with 37% pea and 63% oat protein. However, the aftertaste from the pea was quite dominant at this ratio. Since the taste is one of the most important parameters for a beverage to be successful on the market, and the after taste was much more acceptable in the 30% pea, 70% oat protein sample, this ratio was determined to be used in the product, even though the amino acid profile and the sandiness was slightly better with a different ratio. Even though the combination of pea and oat was promising, oat as an only protein source was still included in the following development trials due to its appealing taste, since further processing steps could reduce the perceived sandiness. The amino acid profile for the different protein combinations are seen in table 9.

Table 9. The amino acid profile for three different ratios between oat (Tate & Lyle) and pea (Roquette) protein.

		Met+C	Phe+t							Total	
Protein	Trp	ys	yr	His	Lys	Val	lle	Thr	Leu	EAA	AAS
Reference	0.7	2.6	4.6	1.8	5.2	4.2	3.1	2.7	6.3	31.2	-
25%Pea 75%Oat	1.2	3.2	8.9	2.1	4.0	4.9	4.0	3.0	7.3	38.6	0.77
30%Pea 70%Oat	1.2	3.2	9.0	2.2	4.2	4.9	4.0	3.0	7.4	38.9	0.81
37%Pea 63% Oat	1.2	3.0	9.0	2.2	4.5	4.9	4.1	3.1	7.4	39.4	0.87

3.2.1.3. Acidity

The acidity in the beverage when the plant-based protein was added was around 4.2 (compared to 3.35 in the original strawberry product), indicating that the protein had a slightly buffering effect. Therefore, the added acid concentration had to be increased in order for the product to keep its refreshing taste and appealing color which in the case of strawberry the redness becomes more intense. A pH of 3.7 for strawberry and 3.9 for banana was considered to provide the beverages with the most refreshing acidity, along with an appealing color and taste. For the final pH of the product, see table 12.

3.2.1.4. Sugar

Initially, the strawberry beverage was considered to be quite sweet, and the added sugar was reduced to 3.5%, which was considered to be a suitable amount. The added sugar was removed completely, and in order to compensate for that, the amount of strawberry, passionfruit and white grape juice was increased. However, the taste was considered as

slightly more astringent, as too weak and not sweet enough, and therefore some added sugar was included in the recipe again. In order to reduce the amount of added sugar, the grape juice was increased, however, due to shelf-life problem, see section 3.3.1., the amount of white grape juice was decreased again, and the final added sugar content was 3.3%.

In the banana, the sugar content was 4% in the beginning. Just as in the strawberry beverage, trials were made to remove the added sugar and instead, banana and tropical compound were increased. However, a similar result as with the strawberry beverage was noticed, the beverage was more astringent, tasteless and not sweet enough, and therefore 2.9% added sugar was included in the recipe again. For the final Brix, see table 12.

3.2.1.5. Viscosity

The viscosity in the two beverages were slightly different since the banana provided much more viscosity to the beverage than the strawberry. Pectin was included in both recipes from the beginning and provided some viscosity in them, however, more pectin was added to the strawberry recipe than to the banana recipe. The soluble oat fiber provided a lot of viscosity. The result from pectin and soluble oat fiber were really similar but since oat fiber was believed to sound more appealing to the consumer in the ingredient list, the oat fiber was included in the recipe and the pectin removed. For the final viscosity measurement, see table 12.

3.2.2. Final recipe

The final composition of the ingredients for banana and strawberry drinks with different protein sources are demonstrated in table 10. Due to confidentiality, the materials except from protein, oat fiber, water and sugar are marked with X.

Ingredients Banana	weight % Oat	weight % Pea+Oat	Ingredients Strawberry	weight % Oat	weight % Pea+Oat
Fruits	24.2	24.2	Fruits	25.9	25.9
Coconut cream	1.90	1.90	Flavor	Х	Х
Oat fiber	0.48	0.57	Oat fiber	0.76	1.05
Sugar	2.85	2.85	Sugar	3.33	3.33
Acidifier	Х	Х	Acidifier	Х	Х
Water	64.03	64.77	Water	65.69	66.22
Oat protein	7.45	5.22	Oat protein	7.45	5.22
Pea protein	0	1.43	Pea protein	0	1.43
Fermented oat	X	X	Fermented oat	Х	Х
Total	100	100	Total	100	100

Table 10. The final recipe of the banana and strawberry drinks, where the amount of some ingredients are marked with X due to confidentiality.

3.2.3. Nutritional Composition

The nutritional composition of the final products is seen in table 11 and is based on theoretical values from the suppliers. Banana oat had the highest energy content with 68.2 kcal / 100 g, while strawberry pea+oat had the lowest with 60.7 kcal /100 g. The percentage of energy that was provided from protein is the lowest in banana oat with 23.5% and the highest in strawberry pea+oat with 27.7%.

Amount /100g	Banana Oat	Banana Pea+Oat	Strawberry Oat	Strawberry Pea+Oat
Energy (kcal)	68.2	63.9	64.4	60.7
Energy (kJ)	286.1	270.5	271.0	256.8
Protein	4.0	4.0	4.3	4.2
Carbohydrates	8.7	8.3	8.4	8.2
of which sugars	6.2	6.1	5.3	5.2
Fat	1.8	1.5	1.4	1.1
of which saturated	0.2	0.2	0.2	0.2
Fiber	0.6	0.6	0.5	0.6
Salt	0.0	0.0	0.0	0.0
%energy from protein	23.5	25.0	26.7	27.7

Table 11. The nutritional composition for the four final recipes per 100g of product.

3.3. Measurements

3.3.1. Stress-test, pH, brix and Viscosity

In the first measurement for the stress-test (see table 12), where the measurements from when the products were made are marked as before stress-test, the pH decreased a lot in both samples. There were also a harsh sour and slightly bitter taste in the products, and it was considered to be a bit slimy. The first stress-test was performed at the end of the product development, when the recipes were close to the final recipe.

To increase the shelf-life, the acidifier and the amount of fruits were changed. The change of these two items led to less decrease in the pH after the stress test. The Brix and the viscosity of the products was also decreased. The viscosity of the strawberry beverages was decreased more than the banana. The taste was still sour, but not as much as in the first stress-test. The sliminess had disappeared. However, the exact causes of this milder decrease in pH, brix and viscosity were not closely studied; nevertheless, the group decided to keep the latest changes

in the recipe as they showed relatively better results compared to the results from the initial stress-tests by having less decrease in pH and less increase in CO₂ content.

When the strawberry oat sample from the first test was analyzed, it was noted that the lid was not properly closed, which resulted in the odd ratio between O_2 and CO_2 .

Samples	O ₂	CO ₂	рН	pH (before stress- test)	Brix	Brix (before stress- test)	Viscosity (cP)	Viscosity(cP) (before stress- test)
Strawberry Pea+Oat first								
test	15.1	15.2	3.19	3.76	-	-	-	-
Banana Pea+OAt first test	14.9	15.8	3.36	4.10	-	-	-	-
Strawberry Pea+Oat not hydrated	19.9	5.9	3.36	3.75	10.5	11.0	369	680
Strawberry Pea+Oat	16.3	9.6	3.36	3.76	10.7	11.2	388	616
Banana Pea+Oat not hydrated	15.4	13.2	3.43	3.97	10.7	11.2	518	662
Banana Pea+Oat	15.6	12.6	3.43	3.96	10.1	10.8	560	604
Strawberry Oat	20.0	0.3	3.37	3.65	11.0	11.7	638	1000
Banana Oat	16.0	9.0	3.48	3.84	9.8	10.6	635	1000

Table 12. Result from stress-test, for the first stress-test and for the last recipe. The measurement from when the samples were made are marked with (before stress-test).

3.4. Product Characterization

3.4.1. Protein content

The result from the protein measurement is seen in figure 3, and the average protein content is slightly above 4% for all samples. However, when the protein content was calculated based on the actual nitrogen-to-protein conversion factor, seen in figure 3, the average protein content was only above 4% for the banana pea+oat sample. For the rest of the samples, the protein content was just below 4%.

Figure 3. The average protein content (%) and standard deviation for the different samples based on the nitrogen-to-protein conversion factor of 6.25 (dark colors), but also for the actual nitrogen-to-protein conversion factor for pea+oat (5.70) and for oat (5.83) (light colors).



3.4.2. Protein solubility

Four outliers were detected, one in the hydrated pea and oat sample with banana taste (g-value=1.262), one in the not hydrated pea and oat sample with banana taste (g-value=1.407), one in the oat sample with strawberry taste (g-value=1.378) and one in the not hydrated pea and oat with strawberry taste (g-value=1.299). Therefore, these samples were eliminated from the results. After the elimination, the average and standard deviation were demonstrated in figure 4. The result from the t-test showed that there was a significant difference between oat protein and the combination of pea and oat protein. No significant difference was possible to detect between the banana and the strawberry tastes, nor between the hydrated and not hydrated samples.



Figure 4. The Solubility (%) of the different products. The significant difference is shown with different letters.

3.4.3. Particle size

The particle size distribution was obtained for both banana and strawberry drinks after protein solutions were homogenized and mixed into beverages. Figure 5 demonstrates a polydisperse particle size distributions which show that the samples have a broad size distribution with two distinct peaks that appear in the graphs. Figure 5 (a) on the left shows the particle size distribution of banana drink and figure 5 (b) shows the particle size distribution for strawberry. The same range of particle size was obtained for both samples, but the volume differed based on the product medium and type of protein used. The modes for the banana are 12μ m for not hydrated pea+oat, 16μ m for pea+oat and 28μ m for oat. For the strawberry beverage the mode for the not hydrated pea and oat are 11μ m, 15μ m for pea+oat and 25μ m for oat.



Figure 5. Particle size distribution for banana (a) and strawberry (b) drinks with 3 different protein sources.

The results from D[3,2] (surface area moment mean) and D[4,2] (volume moment mean) have been collected from all three samples. Based on the data obtained from the t-test, see figure 6, banana pea+oat not hydrated has the smallest particle size, while banana oat, strawberry oat and strawberry pea+oat have the largest particle size based on surface area moment



mean. The banana samples were observed to have the smallest and strawberry samples had the largest particle size based on the volume moment mean.

Figure 6. The particle size distribution for the 3 different protein sources and tastes, with D[3,2] surface area moment mean (a) and D[4,3] volume moment mean (b).

In order to have a more precise look at particle distribution of the protein sources individually and how they can affect the end product, another measurement was performed on the proteins based on their particle size distribution which had been homogenized in advance. Figure 7 shows the particle size distribution of the three different protein solutions. The size range of protein particles in figure 7 decreases significantly compared to figure 5, while the volume increased slightly in pea and oat. Not hydrated pea and oat and oat solutions have bigger particles size distributions based on the figure. The modes for the different samples are 10μ m for pea and oat, 15μ m for oat and 16μ m for pea and oat not hydrated.



Figure 7. Particle size distribution of the three different protein solutions without the fruit base.

The t-test results on D[3,2] shows that the surface weighted mean values demonstrated significant difference among all different samples, see figure 8 (a). However, the volume moment mean values show that there is no significant difference between oat protein and not hydrated pea+oat protein based on volume weighted mean value, see figure 8 (b).



Figure 8. The D[3,2] surface area moment mean (a) and D[4,3] volume moment mean (b) of the three different homogenized samples.

3.4.4. Sensory evaluation

The sensory analysis did not show any significant difference regarding viscosity, see appendix A2. For the sandiness, on the other hand, there was a significant difference between banana pea+oat and the strawberry oat, see figure 9. For the rest of the samples, there was no significant difference. For the taste, there was a significant difference between banana pea+oat and the strawberry pea+oat not hydrated, see figure 10, but there was no significant difference between the other samples. For off-taste there was a significant difference between banana oat and all the strawberry pea+oat, see figure 11, however, there were no significant differences among the rest of the samples. In the results from the question regarding the astringency, there was no significant difference, see appendix A3. For the total liking, there was a significant difference between banana pea+oat and the strawberry oat and the strawberry oat and the strawberry oat the samples. There was no significant difference between banana pea+oat not hydrated, see figure 12. There was no significant difference between the rest of the samples.



Figure 9. Sandiness, where significant difference is marked with different letters.

Figure 10. Taste, where significant difference is marked with different letters.



Figure 11. Off-taste, where significant difference is marked with different letters.



Figure 12. Total liking, where significant difference is marked with different letters.

The result from the ranking showed no significant difference between the different samples, see appendix A4. The results from the comments are seen in the appendix A5. The taste in both products was commonly described as too weak fruit taste and as a chemical taste.

3.4.4.1. PCA

A principal component analysis was performed in order to find correlations between the different samples and parameters. A biplot was made in order to detect correlations, see figure 13. The degree of explanation for the first two principal components is very good with a total of 94.8%. There is a positive correlation between banana pea+oat and banana pea+oat not hydrated with sandiness, astringency, total liking and taste. For the banana oat sample, it is hard to see any correlation. The strawberry oat and strawberry pea+oat not hydrated are negatively correlated with the sandiness, astringency, total liking and taste. The strawberry pea+oat is positively correlated with viscosity and negatively correlated with off-taste, indicating that it had a lot of off-taste. The correlation regarding the total liking confirms the result from figure 12.



Figure 13. Biplot for the sensory analysis, with a degree of explanation of 71.91% for PC_1 and 22.9% for PC_2 . The banana samples are marked with yellow, the strawberry with red and the parameters with blue.

3.4.5. Overview

Table 13 demonstrates a summary of all the analysis that were performed on the products including all the parameters from sensory analysis. The colours are based on the comparison between the product where the best products are marked with green and the worst with red and yellow is acceptable. In this table, in the rows in which all three colors are presented, there is only a significant difference between the green and the red icons. Based on the number of green icons that each product has received, it can be seen that banana pea and oat is considered the most preferable product both from sensory analysis and protein evaluation perspective. However, there are no significant differences between the combination of pea and oat protein and only oat protein.

Table 13. A summary over all analysis performed on the three different varieties of the two different flavors. The best results are marked with green, the middle result as yellow, and the worst result with red.

	Barano cost	Barata Paircat	sprang period	Stoutentot	strantern portoat	stantern parton nated
Protein content						\bigcirc
Protein solubility		0	0		0	0
Particle size						•
AAS	0			0		
Sandiness						•
Off-taste		•	0			
Taste						
Ranking	0	0	0	0	0	0
Viscosiy						•
Asstringency	0	•	0	0	0	0
Total liking						

4. Discussion

4.1. Protein

As mentioned in the aim of the project, one of the main goals was to create a plant-based high protein product that can compete with similar products in the market. The texture was one of the major concerns in developing the new recipe; thus, proteins played an essential role as they were causing the major problem in this case. The final pH of the product was between 3.7 to 3.9, which is considered to be an acidic pH. The isoelectric point for pea and oat are 4.5 and 5, respectively. Proteins tend to aggregate more as the pH gets around or near their isoelectric point due to the loss of electrostatic repulsion between them. This phenomenon can create a chalky and gritty mouthfeel in the beverage (Golovanov et al., 2004). In addition, proteins have a buffering capacity, meaning that pH does not decrease in proportion to the hydrogen ions that are being added to the medium, thus, after adding acid to a certain extent (usually to the pH around 3 to 4), the pH would not decrease significantly. This phenomenon is due to the fact that the proteins become protonated thus, leaving the solution with fewer hydrogen ions and this would show a higher pH compared to the actual amount of acid that has been added (Encyclopedia Britannica, 2019). This can be problematic while adjusting the pH of the product by adding more acid, the product would become more sour, but the pH wouldn't change. This was experienced and is the main reason why the pH of the beverages is closer to four than to three, even though a lower pH probably would increase the solubility and the particle size. According to Beecher et al. (2008) the reason why the drink is considered more sour even though the H⁺ has bound to the proteins is because the pH changes in the drink when it comes into contact with the saliva (at pH 7). The pH comes closer to the pl and the H⁺ is therefore released and can be detected by the receptors in the mouth, which creates the sour sensation. This also contributes to the astringency of the beverage and likely even to the perceived sandiness. As the proteins combine with the salivary proteins and reach pl, they start to aggregate and create a scenario similar to what we see in polyphenols. Beecher et al. (2008) also mentions that, astringency in the pH of 2.6 and 6.8 were perceived the least. Therefore, it can be assumed that if the pH decreases to lower amounts, the astringency of the product can be perceived less.

Moreover, since the protein used in the beverage have been extracted, high temperature, strong alkaline or acids have probably been used, in order to extract the proteins, and this can lead to loss of noncovalent bonds and causes the denaturation in the proteins (Pelegrine and Gasparetto, 2005). Thus, the hydrophobic groups within the proteins will react with each other and this will cause less water binding capacity and more aggregation.

In pea, there are a lot of anti-nutritive factors. Phytic acid is a component that stores phosphorus compounds in plant tissues. The remaining antinutritive compounds in the pea are sensitive to hydration and thermal processing (Nadathur, Wanasundara and Scanlin, 2016), the presence of anti-nutrients can be considered as a drawback in using plant-based proteins. However, the protein sources used in this product were concentrate and isolate proteins which are heat treated due to the tremendous amount of water used for their extraction, and most of the anti-nutrients are possible degraded (Nadathur, Wanasundara and Scanlin, 2016).

4.2. Recipe Development

4.2.1. Protein Source

The decision to use oat as the primary protein source was based on the screening, where oat was the most preferred protein in terms of taste and aftertaste. Potato showed bad results (bad taste, off-taste, high sedimentation and high perceived sandiness) and was not considered to be an option. The different pea combinations did not have an appealing after taste and this was the primary reason why they were excluded as a major protein source. Soy was considered to be a good option based on taste, but since one aim with the project was to find a protein source comparable to whey, but without the drawback of allergenicity, soy was not considered a good option, based on the fact that it is an allergen (L'Hocine and Boye, 2007). Even though oat generally can't be consumed by people with celiac disease (Hoffenberg et al., 2000), this was not considered to be a significant concern since the current ProViva products already contain fermented oat, and the addition of more oat does thereby not make the drink less available for people with allergies. The remaining drawbacks of oat were the very strong sandiness and the low AAS. Since the most prominent benefit with the pea proteins were that they were not perceived as sandy, and had a relatively good amino acid profile, which also was a suitable complement to the oat, due to oats lack of lysine, and peas high content of lysine, it was decided to try a combination. The hydrolyzed pea had a slightly more appealing after taste, but the AAS was low and this combined with that the word "hydrolyzed" would need to be included in the ingredient list. The regular pea protein also had almost the same after taste in combination with oat, and therefore it was decided to move forward with the blend of regular pea and oat. The difference between the pea and the pea+rice was that the amount of lysine was higher in the pea, and this was needed to compensate for the oat proteins lack of lysine.

4.2.2. Sugar

One aim was to have a no-added-sugar product. However, as described in the result, the taste when the sugar was removed was not that appealing. Sucrose have shown an effect on reducing astringency (Ares et al., 2009), and this was noted, since more astringency was perceived when the sucrose amount was removed. It was discussed to add some steviol glycoside for the sweetness, however, since off-taste in the aftertaste was already present due to the plant protein, adding another kind of off-taste in the form of sweetener did not seem like a good idea. Therefore, some sugar was included in the final recipe; however, in a lower concentration compared to other ProViva products.

4.2.3. Viscosity

The viscosity and sandiness have an opposite impact on one another. It is more likely that by increasing the viscosity, the perceived sandiness reduces (Imail, Hatea and Shimada, 1995). The sandiness was perceived higher in strawberry compared to banana, and this was likely due to higher viscosity provided by the banana. Therefore, a higher amount of soluble oat fiber needed to be included in the strawberry recipe. However, the sandiness was not completely removed due to large protein aggregates that were present in the drink. Therefore, a homogenization step was introduced to reduce particle sizes. When comparing the oat with the pea and oat proteins, the oat proteins provides a lot more viscosity than the combination of pea and oat. This is due to the lower protein content in the oat protein of 51%, compared to 80% in the pea protein, which leads to that a bigger percentage of the oat protein powder needs to be added compared to the total percentage of pea+oat protein isolate and concentrates. This affects the viscosity a lot since more protein concentrates and less water were added to the oat protein product.

4.2.4. Nutritional Composition

In terms of nutritional composition, all four products are similar regarding the macronutrients and energy content. A product is allowed to be labeled as high/rich in protein if the energy content of protein in the food is equal to or higher than 20% (European Commission, 2012). This means that all recipes can be claimed as high in protein since all of them had more than 20% of their energy provided from proteins. This aim was therefore, accomplished.

The AAS for the combination of oat and pea was as previously mentioned 0.81. Comparing this with the AAS for whey, which is 1.0, we did not reach the goal with the same AAS. Since the beverage is considered to be an in-between meal and therefore, it can't be considered as a meal; thus, other food sources are to be consumed to accomplish the daily need.

4.3. Processing steps

4.3.1. Homogenization

Since the homogenizer at ProViva broke down, all analyses were delayed. This led to the fact that hydrated protein couldn't be homogenized right away and was kept in the fridge for almost a week. Another homogenizer had to be used, and this homogenizer was sensitive to both particles and viscosity. Since the mixture of proteins and water included a lot of relatively large

particles along with a quite high viscosity, the homogenizer broke down several times as a result. It was therefore not possible to homogenize as much material as was planned.

4.4. Measurements

4.4.1. Stress-test, pH, Brix and Viscosity

Based on the results from stress test, viscosity, pH and Brix of the product changed. The metabolism of the bacteria is mostly based on the amount of carbohydrate it consumes (Filannino, et al., 2014). Considering sugar and fiber as the major sources of carbohydrates, the viscosity would decrease when the bacteria consume these carbohydrates and produces lactic acid and CO₂. The changes in Brix can also indicate that the bacteria consumes the sugar and this can leave the product with a lower Brix compared to the original product.

4.5. Product Characterization

4.5.1. Protein Content

When the protein contents were calculated based on the nitrogen-to-protein conversion factor of 6.25, all samples reached the aimed 4 % protein content. This was also the conversion factor that the suppliers said they had used when measuring the protein content in their products. However, since this nitrogen-to-protein conversion factor for oat is 5.83 (FAO, 2002), and for pea is 5.40 (Sosulski and Imafidon, 1990), it seems to be more accurate to use these numbers. This is also what Mariotti, Tomé and Mirand (2008) suggests. Since the needed amount of respective protein was based on the information from the suppliers, the actual amount of protein in the beverages is therefore slightly below 4%, see section 3.4.1. Another aspect to take into consideration is that the Dumas method, which was used, generally provides higher values than the Kjeldahl method (Jung et al., 2003), see section 1.10.1. Based on the Kjeldahl method, the protein content would therefore, probably be even lower. To ensure that the protein content is above 4%, in the end-product, it would be better to increase the amount of added protein.

4.5.2. Protein Solubility

The result from the solubility showed that there was a significant difference between oat protein and the combination of pea and oat protein, but not between anything else. This indicates that the pea protein has a better solubility than the oat protein. This correlates with results from Ladjal-Ettoumi et al. (2015) where pea protein had a solubility around 20% at a pH between 3.5-4.0. The results from the oat protein solubility correlates well with results from Loponen et al. (2006), that oat protein solubility in the pH range around 4.0 is approximately 10%. As discussed previously, pH does affect protein solubility (Sumner, Nielsen, Youngs, 1981), however, it is not possible to detect from this result. Since the pH of the strawberry beverage was 3.7 and the banana beverage was 3.9, it was likely to believe that the strawberry beverage should have had higher protein solubility since that pH was further away from the proteins' pl. However, no such thing was possible to detect based on these results.

Based on the results from protein solubility regarding hydrated and not hydrated oat+pea, no significant differences were observed. Proteins have different hydration capacities. By adding more water to protein solution, there will not be any more hydration and instead, the solution gets more diluted (Encyclopedia Britannica, 2019). Since the concentration of the proteins in all hydrated and not hydrated samples were similar, therefore, the hydration capacity which collaborates with its solubility had been almost constant as the hydration capacity depends on the protein type and also its concentration in the solution.

Even though the solubility is really low, sedimentation does not occur in the beverages. This is probably due to the relative high viscosity, and also to the high particle concentration in the beverages, leading to hindered sedimentation, and thereby a really low sedimentation velocity (Beckett, 1996).

Four outliers were detected, which seemed to be a lot. However, two out of four of the outliers were from the last measurement, which was made almost one week after the other. Worth to mention was that all measurements from the last measurement were higher than the measurements from the first measurements. Since the samples had been stored for some time before they were measured, the samples were heavily shaken before centrifugation. This might have been the reason for the outliers. Another possible reason is that the later samples were taken from new containers, however, since the protein content in the centrifuged sample were compared with the protein content in an uncentrifuged sample, and both samples came from the same container and were measured the same day, this should not have affected the solubility.

4.5.3. Particle Size

From the graphs describing the particle size distribution of the beverages it can be seen that the size distribution is quite broad, with particles from the range of $1-1000\mu$ m. Since the threshold value for some different foods are in the range between $10-55\mu$ m (IMAI et al., 1999), it can be concluded that the particles can be perceived as sandy. See section 1.6.2. When the mode for the various products are compared, it can be seen that the mode is larger for the oat protein compared to the pea and oat proteins. This might be because the oat particles are bigger than the pea protein particles, even after the homogenization. When the banana and strawberry beverages are compared, strawberries have a bigger second peak. This is most likely not due to bigger protein aggregations in strawberry compared to banana, since the pH in strawberry is further away from the pI compared to the pH in banana, and it would therefore be more likely that the banana should have bigger aggregates. One difference in the two recipes is that strawberries contain two times more fiber than the banana. Moreover, this might be the reason why the second peak is bigger in the strawberry.

Depending on whether the particle size is based on surface area moment mean or volume moment mean the results are different. For the surface area moment mean there are similar results between the same proteins/processes in both banana and strawberry recipe, but for the volume moment mean, the particle size can vary depending on the recipe whether it is the strawberry or banana. Since the aim of the particle size measurement was to analyze the particle size of the oat compared to pea+oat and hydration compared to no hydration, the fact

that the volume in strawberry is bigger than in banana seems to be irrelevant. These differences are probably linked to the fact that the strawberry recipe contains more soluble fiber and also presence of strawberry kernels to some extent. Thus, for comparing the results from particle size measurements, the volume moment mean is selected, since it correlates to the results from sandiness in sensory analysis which demonstrates higher perceived sandiness in strawberry drink.

It is easy to see that the other ingredients in the beverage interfere with the particle size measurement when comparing the particle size distribution of the protein and water with the one for the beverages. In the result of the protein water solution, the particle size is $1-100\mu m$, see figure 7. However, the mode is between 10 and 15μ m. This leads to the conclusion that the biggest particles come from the other ingredients, but there is a possibility that the other ingredients in the beverage also increases the protein aggregation, for example, pH close to the isoelectric point increases protein aggregation (Sumner, Nielsen, Youngs, 1981), as mentioned in section 1.5.8. However, even in the protein-water mixture, a lot of the protein particles are still likely to contribute to the perceived sandiness. Worth to mention is that another homogenizer was used for this measurement, and even if the same pressure was used for both steps, it is not unlikely that the two different homogenizers might have produced different particle sizes of the proteins. Another important aspect when comparing the results is also that the particle size of the beverage was measured one week after mixing the ingredients, while the water-protein mix was measured the day after the homogenization. Therefore, the results from the beverages and the protein-water mixture should not be considered to be completely comparable. However, comparing the oat protein with pea+oat protein, and hydrated with not hydrated protein should not be a concern.

The results from the particle size measurement of only protein and water show more similar result between surface area moment mean and volume moment mean. For the D[3,2] there is a significant difference between all three of them, indicating that the hydrated pea+oat has the smallest particle size. This is also confirmed based on D[4,3] where the hydrated pea+oat also has the smallest particle size. However here there is no significant difference between the hydrated oat and the not hydrated pea+oat. From the beginning, it was believed that the hydrated proteins would have a smaller particle size due to less aggregation of the proteins and these results correlate well with that. When comparing the D[3,2] rolue of 61.2μ m for the original oat protein particles (see section 2.5.3.) with the D[3,2] for the oat protein mixed with water of 9μ m, it is clear that the homogenization has beneficially decreased the particle size. One thing that should be mentioned is that the pH in the water and protein mixture is different from the pH in the beverage. The pH was not measured, but it is likely to assume almost neutral pH due to the water. Since the aggregation occurs the closest to the pI, more aggregation is likely in the beverages compared to in the water and protein mixtures (Sumner, Nielsen, Youngs, 1981).

From the particle size distribution results on hydrated and not hydrated protein solutions, before being mixed with other ingredients, it can be seen that the hydrated pea+oat and not hydrated pea+oat had significant difference and the pea and oat not hydrated had a bigger particle size compared to the hydrated sample. According to section 1.8.2. hydrated proteins, even if they are aggregated, bind with water better and water molecules can penetrate the aggregated proteins (Rao et al., 2002).

4.5.4. Sensory Analysis

The expected results were to see a difference between the protein sources and the hydration step. As mentioned earlier, section 1.8.2, a hydration step can increase the solubility (Vajda and Perczel, 2014). However, no such things were possible to detect. The banana samples seemed to be preferred over the strawberry samples.

The sensory analysis did not reveal so many results since most of the questions did not show a significant difference between the samples. Perhaps the questions should have been asked in another way in order to gain more information from them, for example, ranking the samples in relation to each other for each question, as preferred most and preferred least, instead of grading them separately. Worth to mention is also that the samples were stored for one week before the evaluation, and that this could have affected the result. This might have been the reason why some samples were described as "tasting a bit old".

According to section 1.5.9.2.5. hydration can also affect the taste of pea (Nadathur, Wanasundara and Scanlin, 2016). However, this could not be concluded based on the sensory evaluation. This might in this case be that mixing the protein in water before adding the other ingredients is enough to improve the taste, and that the heating step during the hydration is not necessary in order to achieve an improved taste.

When comparing the results from sensory analysis regarding the sandiness with particle size measurements, it is possible to see similar tendencies. In oat samples the average score from sensory in sandiness is relatively lower compared to other samples which correlate with the results from the particle size of the same product, that shows larger particle size based on surface area moment mean values. Therefore, as the particle size increased, the perceived sandiness is also higher. This correlates well with the result from Imai et als. Study (1999), see section 1.6.2. Also, since the oat protein contained lower protein fraction compared to pea (51% in oat and 80% in pea), the amount of protein used in the only oat protein drink was higher in order to reach 4%. Thus, more oat protein powder is added to these drinks which provides a higher particle concentration than in pea and oat and this contributes to their higher perceived sandiness. Similar result was described by Imai, Hatea and Shimada (1995) where particle concentration was what affected the perceived sandiness the most, see section 1.6.2.

One thing to take into consideration is that the standard deviation is relatively high for most samples. For example, in the ranking, see appendix A4, there seems to be a relatively clear difference in the average between the hydrated and not hydrated pea and oat. However due to the high standard deviation, a significant difference could not be detected. The reason for this was that people perceived the products really different. One theory can be that this test was performed at 13 o'clock which was after people have had lunch and this could have been one of the major factors that influenced people's grading.

Another factor influencing the result is that these products were unique and different compared to ProViva's other products and they are targeting a specific group, that are consuming high protein products. The sensory panel was not provided with a reference sample (since there are no similar types of products in the market) which could perhaps lead them to compare this product with the current ProViva products, and thus, the result could deviate. Also, based on the comments that some participants had made, most of them were not a regular consumer

of the high-protein product as such, and since this product was mainly targeting a significant group of society, these participants couldn't represent these consumers. However, if the participants have had more information about the product's features, their opinion could be influenced by them.

4.5.4.1. PCA

In the principal component analysis, see figure 13, the PC_1 is believed to be total liking. This is based on the fact that the total liking is close to the x-axis, but also because banana pea and oat had the highest mean for total liking, see figure 12, and this correlates well to the biplot. Based on this, the pea and oat seem to be the most preferred and the oat the least preferred by looking at the biplot. This also correlates with the ranking test, see appendix A4, where the mean for pea and oat, in both tastes, was the highest. However since there was not a significant difference for this in the total liking nor the ranking, this correlation should not be considered to be accurate, however, it is an indication of whether oat alone or a combination of oat and pea protein is considered to be the best, along with if hydration is necessary or not.

4.5.5. Overview

Based on the overview banana pea and oat is supposed to be the best option. However, as mentioned in the result, a different color does not necessarily indicate a significant difference, but it can be seen as an indication. Based on these results, the combination of pea and oat protein are preferred before only oat protein. There is also indication that a hydration step is preferred. However, there is not a significant difference that supports this except from in the particle size measurement based on only protein and water. Another factor that is beneficial for a hydration step, is that it can remove some off-taste from pea, decrease anti-nutritive compounds and improve the digestibility in the pea. However, since the pea isolate likely has been processed a lot in order to extract the isolated proteins, it is questionable if further hydration can affect the taste, anti-nutrients and digestibility. The sensory evaluation did not show a difference for the taste, and the other factors such as its effect on anti-nutritive and digestibility have not been analyzed. Since the hydration step also is time and energy consuming due to the hydration of one hour at 60°C, and no significant results supports the hydration, it is therefore not recommended. Overall, the banana recipe was preferred over the strawberry.

4.6. Conclusion

The aim was to develop a new ProViva product with a protein content of 4-8%. The final protein content was 4%. A final AAS of 0.81 was achieved with a combination of pea and oat protein with a ratio of 70% oat protein concentrate and 30% pea protein isolate, and the product is plant-based without allergens. There are two different tastes of the product, one strawberry-passionfruit with a total fruit content of 25.9% and one banana-tropical with a total fruit content of 24.2%. The final Brix was 10.7 in both drinks, and the pH was 3.7 in strawberry and 3.9 in banana. The viscosity was around 650 cP for both beverages. One necessary processing step that was detected for these protein-rich beverages was homogenization. Hydration is not recommended since it is a time as well as energy-consuming step, and the evidence that it improves the product in a preferable way is questionable.

4.7. Future work

In order for these products to be launched, the biggest challenge is the sandiness and taste. For the taste, probably the amount of fruits/berries along with flavors should be elaborated to an even greater extent than was done in this thesis.

In order to reduce the sandiness, more focus should be put on homogenization, trying to find the optimum pressure in order to reduce the sandiness as much as possible. It can also be tried to homogenize the product several times to see if the result improves. Also, considering the fact that the homogenized protein solution has a neutral pH, by mixing the ingredients in the following steps and thereby adding acid to the solution, the protein mixture starts reaching its isoelectric point (at pH around 4-5) and exceeding this pH and reaching 3.7 to 3.9, makes the proteins aggregate again. Thus, in future, it can be more relevant to have a homogenization step after adding acid to the protein solution.

In a study based on protein solubility done by Golovanov et al. (2004), the addition of the charged L-amino acids Arg and Glu (Arg+Glu) to the dilute protein solution helped reducing aggregation in the solution. This method also could suppress aggregation at pH close or at isoelectric point. This can also be an alternative in such kinds of protein applications with low pH mediums.

Since the particle size distribution was different for the beverage compared to just the mixture of protein and water, it would be interesting to measure particle size of the beverage without added protein. This would detect if there is a specific ingredient that contributes to the big particle size distribution, or if it is the aggregated particles due to the pH.

Another idea would be to measure the particle size during different pH to find a minimum particle size. Based on this, the recipe could be developed. Probably more acidic fruits would need to be included in the recipe in order to achieve a lower pH without perceiving the product as too sour. However, as mentioned before this can be a problem due to the buffering capacity of the proteins.

As the main challenge in this study was the protein source from different suppliers, utilizing other protein sources such as lentils or hemp, depending on their quality and compatibility on applications as such can also be examined.

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Appendixes

A.1 Sensory Evaluation Questions

- Q1. Hur upplever du **viskositeten?** How do you perceive the **viscosity?**
- Q2. Kommentarer/Comments
- Q3. Hur upplever du **sandigheten?** How do you perceive the **sandiness?**
- Q4. Kommentarer/Comments
- Q5. Hur upplever du **smaken?** How do you perceive the **taste?**
- Q6. Vad **gillade/gillade du inte** med smaken? (Denna fråga är obligatorisk) What **did you like/didn't like** with the taste? (This question is mandatory)
- Q7. Kan du känna en **bismak**? Can you sense a **off-taste**?
- Q8. Kommentarer/Comments
- Q9. Upplever du **torrhet** i munnen? Do you perceive **astringency** in the sample?
- Q10. Kommentarer/Comments
- Q11. **Totalt gillande** av denna produkt? *Total liking* of the product?
- Q12. Kommentarer/Comments

A.2 Viscosity





A.3 Astringency

A.4 Ranking



A5. Results from comments

Banana Oat

Viscosity	Sandyness	Comment Taste	Off-taste	Astringency	Total liking
NA	NA	God banan smak	NA	NA	NA
NA	NA	Saknar fruktsmak	NA	NA	NA
NA	NA	Den var för sandig	NA	NA	NA
För tjock	NA	Syrlig, besk	NA	NA	NA
NA	NA	Bra	NA	NA	NA
really good texture	not really sandy	I like the strong oat taste	really weak off notes	NA	slightly sandy but good taste

Ngt tjock	Speciellt sandig efteråt	Ngt sur	NA	Pga sandighet	NA
NA	Väldigt sandig	Mycket sandig, ingen direkt tropisk smak.	Banan	Torr och sandig	NA
NA	NA	För syrlig banan	NA	NA	
NA	NA	Inte lika fruktig som de andra	NA	NA	NA
NA	NA	Smakar bränd	NA	NA	NA
Lite väl tjock	NA	Bra balans av smakerna	NA	NA	NA
NA	Alldeles för mkt sandig känsla på tungan	För syrlig och för låg intensitet av fruktsmak	NA	NA	NA

Banana Pea+Oat

Viscosity	Sandyness	Taste	Off-taste	Astringency	Total liking
NA	NA	Fin smak av banan	NA	NA	NA
NA	NA	Smakar kemiskt. Plus för ok banansmak	NA	NA	Ok banansmak
NA	NA	För lite smak av banan och väldigt sandig	NA	NA	NA
NA	NA	Ngt söt, nötaktig	NA	NA	NA
NA	NA	Bra smak, balans mellan frukt och protein	NA	NA	NA

really good viscosity	slightly sandy but acceptabl e	good banana taste - not too acidic	NA	NA	Good product with a good texture - taste is well balanced
NA	NA	Tyckte den var ganska god	NA	NA	NA
NA	NA	Mycket banan, nästan växte i munnen	Banan	NA	Väldigt mycket banan
Bästa hittils	Ok	Fifa lite för syrlig unken havre	Unken havre	NA	NA
NA	NA	Fräsh	NA	NA	NA
NA	NA	Saknar smaken av banan endast torrhet i munnen	NA	NA	NA
Eventuel It lite rinnig	NA	Lite bitter	NA	NA	NA
NA	NA	God banan smak	NA	NA	NA

Banana Pea+Oat Not Hydrated

Viscosity	Sandyness	Taste	Off-taste	Astringency	Total liking
NA	NA	Bismak	NA	NA	NA
Ngt tjock för att vara ProViva	Ngt mindre sandig känsla i munnen	Saknar fruktsmak	NA	NA	Luktar kemiskt, smakar inte tydligt av frukt
NA	NA	Den är för sandig men annars god	NA	NA	NA
NA	NA	För lite banan	NA	NA	NA
---------------------------------------	---------------------------------	--	--	----	---
NA	NA	Bra	NA	NA	NA
good viscosity	quite sandy	like the banana taste in this product	can't feel any strong off notes	NA	really good taste just slighlty too sandy
NA	NA	Ngt besk	Något besk	NA	NA
NA	NA	Hade velat ha lite mer att tropisksmaken trädde fram mer	NA	NA	God
NA	NA	Ngt unken banan	NA	NA	NA
NA	NA	Bra sötma	NA	NA	NA
Ser tjock ut men ok i munnen	Kanske mer torr än sandig	Saknar banan smak	NA	NA	Skulle inte köpa den men köper inte heller andra protein drycker
NA	NA	Bra balans	NA	NA	NA
NA	NA	Önskar mer fruktsmak	NA	NA	NA

Strawberry Oat

Viscosity	Sandyness	Taste	Off-taste	Astringency	Total liking
NA	NA	Den är för sandig, men än annars god smak	NA	NA	NA
Tjock för att vara proviva	NA	Alldeles för sandig	NA	NA	NA

NA	NA	Smaklös	Besk	NA	NA
Tjock, fyllig	NA	Aningen smaklös	NA	NA	NA
NA	NA	Dålig fruktsmak	NA	NA	NA
Tjock i mugg, vattnig i munnen	NA	Konstgjord smak, som sitter som en hinna på tungan	NA	NA	NA
NA	NA	Otydlig jordgubbe smak, dock bättre än 964	NA	NA	NA
Good texture - really full-filling	sandy in mouth	i like the oat taste in this sample	NA	NA	Good oat taste and fruit taste in this sample
NA	NA	Smakar som lera	Banan mm	NA	NA
Ngt tjock	NA	Ngt besk	Ja beskhet	Ganska sandig	NA
NA	NA	Eftersmaken är inte så god	Lite fodersma k	NA	NA
NA	NA	God	NA	NA	Lite för sandig
NA	NA	För lite jordgubb	NA	NA	NA
NA	NA	Vill ha en friskare duktigare smak	NA	NA	NA

Strawberry Pea+Oat

NA	NA	Viskositeten var bra, men den var sur i smaken	NA	NA	NA
Tjock för att vara ProViva	NA	Sandig, saknar tydlig fruktsmak	NA	NA	NA
NA	NA	Besk eftersmak	NA	NA	NA
NA	Aningen smaklös, typ pulverkäns la	Aningen smaklös, lite pulverkänsla.	NA	NA	Bäst av de tre
NA	NA	För syrlig, ingen egentlig smak	NA	NA	NA
Utseende i koppen motsvara r munkänsl an	NA	Lite bränd?	Bränd	NA	NA
NA	NA	För lite jordgubbe smak, mycket bismak	NA	NA	NA
Like the thickness of the product -	slightly sandy but acceptable	good fruit taste - good balance	NA	NA	Good product - little bit too acidic
NA	NA	Smakade lera,konstgjord	Konstgjor t	Väldigt sträv	Känns inte som om det är gjort på rena råvaror utan endast på konstgjord väg som massa aromer mm.

NA	NA	Också lite besk i eftersmaken	Ngt besk	NA	NA
NA	NA	Smaklös luktar frukt men smakar ingen	Gammal havre	NA	NA
NA	NA	Slightly too sour, but othervise good	NA	NA	NA
NA	Torr	Smakar papper, för lite jordgubbb	Pappsma k	Ngt torr	NA
NA	NA	Vill ha mer fruktsmak	NA	NA	NA

Strawberry Pea+Oat Not Hydrated

Viscosity	Sandyness	Taste	Off-taste	Astringency	Total liking
NA	NA	Bra smak och inte för sandig	NA	NA	NA
Tjock för att vara Proviva	NA	Syrlig och sandig, inte tydlig fruktsmak	NA	NA	NA
NA	NA	God jordgubbe	NA	NA	NA
NA	NA	Kändes just sandig	NA	NA	NA
NA	NA	Konstgjord jordgubbssmak	NA	NA	NA
NA	NA	Smakar inte jordgubbe eller annan frukt	NA	NA	NA
NA	NA	Ngt lite jordgubbe smak	NA	NA	NA
really good texture	quite sandy	good strawberry taste	acidic off taste	NA	little bit too acidic for me

NA	NA	Smakar som lera	Banan	NA	Smakar för mycket aromer mm
Ngt tjock	NA	Besk	Ja beslutet och sur	NA	NA
NA	NA	Äcklig smak och konsisten	Gammal havre	NA	NA
NA	NA	Bitter	NA	NA	NA
Ngt för tjock	NA	För syrlig	Pappsma k	NA	NA
NA	NA	Vill ha mer frukt smak	NA	NA	NA