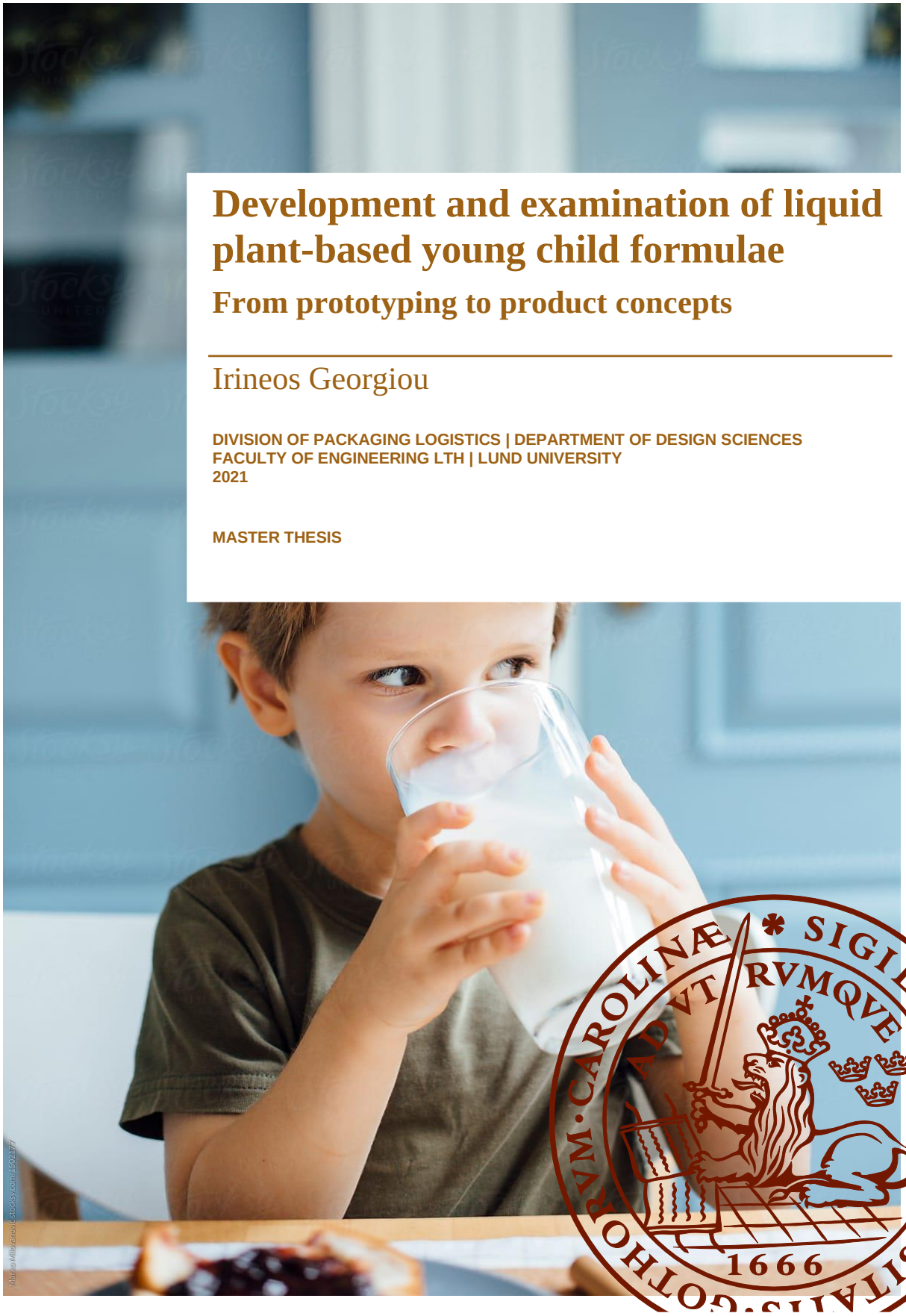

Development and examination of liquid plant-based young child formulae

From prototyping to product concepts

Irineos Georgiou

DIVISION OF PACKAGING LOGISTICS | DEPARTMENT OF DESIGN SCIENCES
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MASTER THESIS

This Master's thesis has been done within the Erasmus Mundus Joint Master Degree FIPDes, Food Innovation and Product Design.

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Abstract

This study evaluated the pilot-scale rapid-prototyping development of liquid plant-based young child formulae, by exploring different sources of proteins, carbohydrates, lipids, and flavours. The study was undertaken in collaboration with an industrial partner thus confidential information was blinded.

The raw materials examined consisted of protein blends; Legume 1, Cereal 1, and Cereal 2; Legume 1 and Legume 2; Legume 1, Seed 1, and Cereal 2; Legume 3, Legume 4, and Cereal 2; Legume 5, and Seed 2; Legume 2, and Pseudocereal 1; Legume 1, and Cereal 3; carbohydrates; Control carbohydrate, Whole cereal carbohydrate, Liquid cereal carbohydrate, Legume carbohydrate, Root carbohydrate, and lipids; Seed oil 1, Nut oil, Seed oil 2. The ingredients were examined in matrices with complete macronutrient and key minerals composition.

Functional examination on heat stability, and physicochemical examination on pH, calcium ion activity, viscosity, particle size distribution, and optical microstructure were performed on the samples after homogenization, after heat treatment, and upon storage for four weeks at 25, and 37 °C. Sensorial attributes, consumer perception, and nutritional properties as provided by the supplier; protein digestibility-corrected amino acid score, and dextrose equivalent were also evaluated.

Based on commercial availability, consumer insights, and the nutritional, physicochemical, and sensorial results, the ingredients with the most promising results for scale-up were selected; protein blends; Legume 1, Seed 1 and Cereal 2; Legume 1 and Cereal 3; carbohydrates; Liquid cereal carbohydrate, and Legume carbohydrate; and lipids; Seed oil 1, Nut oil, and Seed oil 2. Further, 31 flavours were examined at kitchen scale for their organoleptic properties when infused in the previously selected protein blends. Along with considerations on commercial availability, quality, and safety, the number of flavours was narrowed down to 11.

Together with a consumer perception research which took place simultaneously to this study, five product concepts were formulated, and were aligned with the selected raw materials. Packaging and labelling suggestions were created based on information obtained through workshops, and a benchmark analysis.

This study suggests further examination of the selected raw materials when combined together, and upon a complete nutritional composition. Moreover, scale-up considerations including adjustments on labelling and packaging should be applied for the product to be optimized and proceed to factory-scale development.

Keywords: plant-based; young child formula; development; physicochemical properties; proteins**;** carbohydrates; lipids; flavours

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Lund, June 2021

Irineos Georgiou

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1 Background

The first thousand days of our lives are considered as the most crucial for our growth and development (Gluckman & Hanson, 2004). Nutrition has been proven to have a major role in the appropriate growth and development of the body and brain (Godfrey et al., 2007; Vickers et al., 2000).

During the first six months of a new-born human, the exclusive source of energy should be constituted of maternal breast milk. Breast milk is the ideal food for an infant as it contains the essential nutrients for growth and development, and the antibodies necessary to protect an infant from infections and illnesses (EFSA, 2013). As the World Health Organization (WHO) suggests, additional foods should be introduced after the six months of age, while breastfeeding should be continued until two years of age or older (WHO, 2018).

Young children, of one to three years of age as defined by the European's Union (EU) Regulation, show rapid growth and development as their height and weight are increased by approximately 25 and 50 percent, respectively (European Comission, 2006; WHO, 2009). Due to early weaning, abandonment of young child formula (YCF), and the consumption of unbalanced diet, this transition period sets young children in risk of nutrient deficiencies (J.-P. Chouraqui et al., 2019).

YCF had been developed as an alternative to breast milk for ensuring adequate nutrient intake to young children during the transition period from breastfeeding to weaning, or when breastfeeding is not possible. Such formulae are commonly made of dairy ingredients, such as milk proteins, and lactose, fortified with essential nutrients, including vitamins, minerals, and fatty acids. It has been widely established that formulae intended for children of one to three years of age have a significant contribution to a sufficient nutrient intake (J. P. Chouraqui et al., 2020; Eussen et al., 2015; Lovell et al., 2018; Vandenplas et al., 2014; Verger et al., 2016; Zhang et al., 2020).

Today's nutrition trends are to a great extent shifting towards the consumption of plant-based (PB) foods. Vegan, vegetarian and flexitarian populations are showing a constant growth over the years (Wunsch, 2020). By default, parents following plant-sourced diets are interested in introducing these diets to their children. Despite that, PB products intended to young children are hardly available on the market. Products like PB milks intended to adults do not satisfy the nutritional criteria for a young child, and can set a child's health in risk (R. Mangels & Driggers, 2012).

2 Aim and objectives

The lack of availability and variety of PB formulae created the need for the development of PB YCF with not-widely examined raw materials. However, a challenge was also generated, as products that meet children's nutritional needs, and with appropriate sensorial, and physicochemical characteristics should be developed.

The aim of this study was the development of liquid PB YCF by following a rapid prototyping method, and the examination of nutritional, functional, physicochemical, and sensorial properties. In combination with information obtained from a consumer perception research which took place simultaneously to this study, product concepts were formulated, and packaging and labelling solutions were suggested.

For the completion of this research, certain objectives were set:

- Screen and source PB raw materials, including proteins, carbohydrates, lipids, and flavours, based on commercial availability, consumer perception, and nutritional compliance to the company's internal nutritional framework.
- Design formulations, and develop prototypes at kitchen and pilot scale for a liquid product, by focusing on the macronutrients' and key minerals' composition, and by being in compliance to the company's nutritional framework for the specific target group and product category.
- Evaluate the prototypes for their nutritional characteristics, organoleptic attributes, and physicochemical and functional properties; visual assessment, heat stability, pH, calcium ion activity, viscosity, particle size distribution, and optical microstructure.
- Select the most promising raw materials and align them with the product concepts identified through the parallel consumer perception study.
- Generate insights on packaging and labelling via workshops and a benchmark analysis, and suggest draft packaging and labelling suggestions for the product concepts.

3 Introduction

3.1 Categories of formulae

3.1.1 **Infant formula (0-6 months)**

Infant formula (IF) is usually made from cow's milk and is used as breast milk substitute, thus its composition aims to be close to human milk. In the EU, Regulation EC 2016/127 provides information on the minimum and maximum levels of the macro and micronutrients that can be present, as well as the appropriate ratio between the nutrients. Apart from cow's milk, the regulation allows the use of soy, usually for infants with galactosemia or congenital lactase deficiency, and the use of formulae made of hydrolysed proteins for infants who show allergenicity to proteins (The European Commission, 2016a).

3.1.2 **Follow-on formula (6-12 months)**

While infant formula is intended to be a replacement for human breast milk, follow on formula (FO) was created for substituting breast milk in addition to complementary feeding after six months (Codex Alimentarius, 2007). In Europe, the same regulation as for IF states the nutritional composition that should be followed. Compared to IF, FO mainly differs in the micronutrient composition, as FO contains higher amounts of iron (The European Commission, 2016a).

3.1.3 **Young child formula (12-36 months)**

YCF, also known as Growing up milk or Toddler's milk, is a milk-based drink intended to young children, and is designed to support the nutritional needs of young children as part of a balanced diet (The European Commission, 2016b).

In the European Union, YCF is currently not specifically regulated, and undergoes in the general food category. Nevertheless, nutritional requirements have been set by WHO (WHO, 2003). Based on WHO's framework, and from additional scientific studies conducted internally and externally, the company's nutritional framework was formed to satisfy the nutritional needs of products intended to young children.

YCF is commonly made of dairy proteins and less commonly of soy proteins, however, as YCF is not specifically regulated in many countries, its composition may vary. Nevertheless, YCF should contain less protein than IF and FO, as young children start consuming meals more frequently than infants. Protein intake usually exceeds requirements in Europe, though studies have shown that high protein consumption from an early age may cause obesity later in life. Therefore, it is important that amino acids requirements should be met with the lowest protein quantity. Additionally, dietary intakes of alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), iron, vitamin D, and iodine were found to be low in infants and young children in some European countries, thus fortification with the aforementioned micronutrients may be considered essential (EFSA, 2013).

3.2 Shifting towards plant-based diets

The current increase of the world's population which is estimated to be 9.7 billion by 2050, results in the need of greater food production (United Nations, 2019). In this manner, it has been projected that the demand for animal-based protein will be double by 2050 (Westhoek et al., 2011). However, the production of animal-based food raises sustainability concerns due to the production of higher greenhouse emissions compared to PB foods (Tilman & Clark, 2014). In addition to this, animal welfare, and exploration of healthier diets are some other reasons which initiated a shifting towards the consumption of foods from plant sources (Cherry, 2006). Veganism diet, which involves consumption of foods solely from PB sources, is showing a constant growth over the past years; in the UK, the number of vegans quadrupled from 2014 to 2019 (Wunsch, 2020).

Apart from vegans, diets including vegetarianism and semi-vegetarianism also hold a big proportion of plant-sourced food products' consumption. As a result, PB food production has shown a significant increase over the past years, with a global expected growth from 12.11 billion EUR in 2020 to 13.23 billion EUR in 2021 (The Business Research Company, 2021). As a result, the number of vegan and vegetarian children is also increasing, thus the demand for food products of the young age category is gaining a high demand.

Other than vegan and vegetarian children, PB food products intended to young children may benefit the general population of this age. As studies have shown, introduction of fruits, vegetables, and legumes in the first year of the children's lives can promote higher acceptance in later stages and can contribute to the prevention of allergenicity (Chan et al., 2018; Cooke et al., 2004; A. R. Mangels & Messina, 2001). Consequently, introduction of a variety of ingredients can bring greater diversity to a child's diet.

3.3 Soy formula

Soy formula is the most common dairy-alternative formula, holding a 12 to 25 percent of the Canadian and U.S. formula market (Bhatia & Greer, 2008; Canadian Paediatric Society, 2009; Rossen et al., 2016; Testa et al., 2018).

Soy's high prevalence lies due to its amino acid profile which is the closest to casein. Nevertheless, soy infant formula has been questioned in a number of studies in relation to its effect on human development, reproduction, and endocrine function, however, no conclusive evidence has been established (Badger et al., 2009; Bhatia & Greer, 2008; Messina et al., 2004; Strom et al., 2001; Testa et al., 2018). Despite that, soy formula is still not recommended by some national health services as, in addition to the aforementioned concerns, soy protein sensitization shows a prevalence of up to 35% in infants with cow's milk protein intolerance (NHS, 2018), and is considered as one of the eight most common allergens with a prevalence of 0.3% in the general population (Katz et al., 2014; NIH, 2010). Due to such concerns, consumer perception for soy varies (Jones et al., 2007; Schyver & Smith, 2005). Nevertheless, soy formula is the only dairy alternative IF and FO currently allowed in the EU (European Comission, 2006).

3.4 Moving forward from soy

Due to the allergenicity and controversial perception of soy, and as a greater variety of PB formulae is demanded, different plant proteins are currently being explored as identified in a benchmark analysis conducted in this study, and in previous studies (Alonso-Miravalles et al., 2021; Venlet et al., 2021). Despite the opportunity of new ingredients' exploration, challenges also arise. This is as PB ingredients other than soy do not exhibit a complete protein composition, meaning that not all nine essential amino acids; phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine, and histidine are present in adequate amounts (Marsh et al., 2012).

Pea protein is a current potential alternative to soy protein. This is due to the availability, and the nutritional and functional properties of pea which may be considered closest to soy, in addition to its non-allergenicity (Barać et al., 2015). Despite that, pea is not considered a complete protein, due to its low level of methionine (Gorissen et al., 2018).

Consequently, for a non-soy PB formula to meet the requirements of a complete protein, blending of different PB sources is necessary. Other than meeting the nutritional characteristics, requirements including processability, sensory attributes, and stability over storage add more challenges to the development of formulae using PB raw materials.

3.5 Challenges of plant proteins

3.5.1 **Physicochemical properties**

3.5.1.1 Heat stability

Thermal processing is essential in beverages like liquid formulae for safety and shelf-life stability to be ensured. Up to a certain degree, heating may be beneficial as proteins' emulsifying properties may be improved. Extensive heating, however, may affect such products as protein denaturation is induced which may lead to decreased solubility, increased flocculation and precipitation of proteins, and undesirable sensory attributes (Hui, 2005).

Important parameters during heating of proteins are temperature, heating time, pH, ionic strength, protein type, and protein concentration (Cui et al., 2014; Peng et al., 2016). Temperature, heating time, and protein concentration can indicate if interactions between unfolded protein molecules take place and if refolding is possible. The extent of denaturation may be depended on susceptibility of intra-molecular interactions, shear, pH, ionic strength, type of ions, and interactions with other compounds including carbohydrates, lipids, and additives (Hui, 2005).

3.5.1.2 Solubility

Solubility is essential for PB proteins to exhibit emulsifying properties and to be functional in beverages since phase separation can lead to shelf-life instability and undesired organoleptic attributes. The solubility of proteins depends on the pH, ionic strength, temperature, the presence of solvent additives, and the amino acids on the protein surface (Kramer et al., 2012).

Amino acid composition influences the solubility of proteins; proteins with polar amino acids enhance solubility in solvents such as water. On the contrary, proteins containing more hydrophobic amino acids, or amino acids with fewer charges on the surface have lower solubility (Hui, 2005).

Moreover, protein solubility depends on the pH of the solvent as pH will affect the surface charge of the proteins. Most proteins show similar pH-solubility profiles, with minimum solubility in the isoelectric region where electrostatic repulsion is maximal and net charge is zero (Nehete et al., 2013). At a pH far from the isoelectric point, protein charge and intermolecular repulsion increases which enhances solubility (Hui, 2005).

The addition of salts to a solution alters the ionic strength and can both increase and decrease the solubility of proteins. Generally, at low ionic strength, the solubility of proteins can increase, while at higher ionic strength, where water molecules are attracted by the salt ions, proteins approach each other because of the low

electrostatic repulsion, leading to aggregation and precipitation (Kramer et al., 2012).

Plant proteins are classified based on their solubility in different media. The different types include albumins, globulins, prolamins and glutelins. Albumins are generally characterized by a more hydrophilic surface which makes them have higher solubility in aqueous solvents. On the other hand, globulins have a relatively hydrophobic surface which reduces their solubility, however, their solubility is improved when the ionic strength is increased (Jarpa-Parra, 2018).

Other parameters which may alter protein properties and lead to increased solubility include sonication, the addition of charged amino acids or stabilizers, and hydrolysis.

3.5.1.3 Protein-mineral interactions

Salts and minerals are often added to beverages to fulfil nutritional needs. However, interaction between minerals, or between proteins and minerals may set physicochemical challenges. For instance, calcium and magnesium are divalent ions in solution, and can form salt bridges with proteins, which can result to coagulation and aggregation. The effect of minerals on protein functionality can differ depending on the protein type, the mineral form, and the product (Alonso-Miravalles et al., 2021).

3.5.2 **Bioavailability**

Digestibility and bioavailability of proteins may vary depending on the physiological state of the human, genetics, and the overall composition of the protein, since presence of antinutrients may reduce the bioavailability of the protein (Boye et al., 2012). The amino acid content of proteins is therefore not sufficient for the protein quality to be determined.

Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is a common method used for indicating the quality of a protein, and it has been adopted by the Food and Agriculture Organization of the United Nations (FAO), and WHO (Tome & Miller, 2000). PDCAAS is calculated based on the amino acid requirements of humans, and their ability to digest the protein (Marsh et al., 2012). The PDCAAS of a protein source is determined based on the protein content, amino acid profile and protein digestibility. The majority of animal-based proteins have a PDCAAS score which is either equal to or approximately 1. Digestibility of unprocessed plant proteins is lower than that of animal proteins, however, processing techniques including cooking, microwaves, and pressure may improve digestibility (Drulyte & Orlien, 2019; Navarrete, 2018). Soy protein has a PDCAAS score close to 1, whereas other PB protein sources generally have scores lower than 1 (Marsh et al., 2012). Consequently, blending of other plant proteins is essential for a PDCAAS value closer to 1 to be achieved.

3.6 Role of packaging and labelling

Primary packaging and labelling have four major functions in food products; containment, protection, convenience and communication (Robertson, 2016).

In terms of communication, packaging and labelling serve as the first contact with the consumer, which is what defines the first moment of decision, and what will lead to a product's purchase. Labelling is responsible for the product's marketing, since the different visuals, wordings, and claims are communicated through it. Following today's trends, such claims include information on the packaging's recyclability, the product's carbon footprint, the locality of the product, as well as health and nutritional claims. Additionally, labelling provides information on among others, the product's nutritional composition, list of ingredients, shelf-life, storage conditions, way of using the product, and traceability details. Labelling is specifically regulated for misleading towards the consumer to be avoided (European Commission, 2011).

Packaging is responsible for the product's containment, protection, and preservation. Additionally, in relation to convenience packaging provides the product's usability, which is one of the aspects the consumer will judge the repurchase of the product (Robertson, 2016).

Due to the annual increase of packaging production, and as the majority of them are being heaped in the landfills which has an impact on the environment, awareness for sustainability has been raised by governments and consumers (European Bioplastics, 2018; Eurostat, 2020b; Geyer et al., 2017). Additionally, the increase of animal-derived food production added to the concerns on sustainability (Eurostat, 2020a; Ritchie & Roser, 2019). As a result, the usage of more sustainable food and packaging sources and production methods are nowadays demanded, and can influence the overall product's image (Boz et al., 2020; Wandosell et al., 2021). For this reason, researchers are currently exploring different techniques for more sustainable food and packaging production, and ways for communicating such improvements through labelling (Bos, 2019; Boz et al., 2020).

4 Materials and methods

In this chapter, the materials and methods of each trial's production and analyses are discussed. Moreover, an explanation on the parallel collaborative consumer

study is briefly explained, as well as the methodology used for the raw materials' initial selection. Furthermore, the steps followed for the product concepts' development are briefly revealed. Last, the methodologies used in the different packaging and labelling activities are demonstrated.

4.1 Consumer perception research

For the initial evaluation of the different raw materials, and the creation of labelling and product concepts, a primary consumer perception study was conducted in parallel with the current study, throughout the current study's duration. The study included a sample of consumers who were selected based on the following criteria:

- Are mothers of one to three years old children
- Their children have no food allergies
- Are using YCF or FO

The consumers were involved in a series of polls and workshops in relation to perception or brainstorming on different raw materials, product attributes, and packaging and labelling concepts.

4.2 Selection of raw materials

Each category of raw materials; proteins, carbohydrates, lipids, and flavours were initially selected. The initial selection was done based on the ingredients' commercial availability, safety and quality compliance by following the company's criteria for ingredients of the specific age group, techno-functional suitability for YCF, and nutritional quality, by obtaining information from the supplier and previous internal studies. Further, the raw materials were examined by the consumers on their perception.

Initially, a number of 15 protein sources, including different cereals, pseudocereals, and legumes was examined by the consumers. Based on the consumers' evaluation, 11 proteins were selected and sourced; Cereal 1, Cereal 2, Cereal 3, Legume 1, Legume 2, Legume 3, Legume 4, Legume 5, Seed 1, Seed 2, and Pseudocereal 1.

Sequentially, seven carbohydrate sources were evaluated by the consumers, including carbohydrates from cereals, roots, pseudocereals, and legumes. Based on the availability, five carbohydrate-rich ingredients were selected and sourced; Control carbohydrate, Whole cereal carbohydrate, Liquid cereal carbohydrate, Legume carbohydrate, and Root carbohydrate.

Further, seven lipid sources were evaluated by the consumers, including seeds, nuts, fruits, and algae oils. From these, Seed oil 1, Nut oil, and Seed oil 2 were sourced.

Lastly, flavours were selected based on previous internal studies which evaluated the different flavours' perception by consumers. Following, a number of 31 flavours was screened for their perception by the consumers. Based on consumer perception from the current and previous internal studies, and from an internal workshop in which different flavours were matched with the identified product concepts, 19 flavours were selected for further evaluation.

4.3 Recipe design and development

Recipes were designed using an internal software, for examining their nutritional and regulatory compliance, quality, and safety for the needs of the target consumer group. The recipes were designed for a liquid form.

For the development of the products, a rapid prototyping method was used. The method was created by the company but follows a similar philosophy as other prototyping methods (Elverum et al., 2016). For the specific study, prototyping included the exploration and evaluation of a big range of raw materials and their behaviour in a prototype matrix, and payed less attention to the in-depth understanding of each raw material's physicochemical properties. Rapid prototyping generally does not focus on a specific supply chain or country of launch, but on the selection of raw materials based on pre-defined criteria, which in this study included nutritional, sensorial, and basic physicochemical and stability properties.

The recipes contained all necessary macronutrients; proteins, carbohydrates, lipids, and a complex of minerals which may have an influence on processability. Complete compliance with the nutritional framework is to be ensured in later development stages. Details in the recipes' composition are not presented due to confidentiality reasons.

Four pilot-scale trials, and one kitchen-scale trial were performed. In all pilot-scale trials, the ingredients were incorporated according to the standard dissolving sequence used at the company. The samples were homogenized and pasteurized at a pressure, temperature, and time set by the internal guidelines. Adjustment of pH to approximately 7 was done after emulsification, for ensuring protein stability during the process. The samples undergone sterilization at a temperature and time set by the internal guidelines.

4.3.1 **Trial 1**

Trial 1 had as scope the examination of different protein blends and the selection of the most desired ones. Seven samples were prepared with different protein blends, as demonstrated in Table 1. All samples contained a standard blend of Control carbohydrate, standard oil blend, and a complex of minerals.

Table 1 Samples' number and protein composition

Sample	***Protein composition***
1.0	Legume 1, Cereal 1, Cereal 2
1.2	Legume 1, Legume 2
1.4	Legume 1, Seed 1, Cereal 2
1.5	Legume 3, Legume 4, Cereal 2
1.6	Legume 5, Seed 2
1.7	Legume 2, Pseudocereal 1
2.0	Legume 1, Cereal 3

4.3.2 **Trial 2**

The purpose of Trial 2 was the examination of different carbohydrates, aiming to the identification of raw materials with improved nutritional and sensorial properties, and with higher consumer acceptance compared to the current raw material used. Five samples were prepared with different carbohydrate sources, as demonstrated in Table 2. Sample 2.0 served as a control. All of the samples contained a standard protein blend, standard oil blend, and a complex of minerals.

Table 2 Samples' number and carbohydrate composition

Sample	***Carbohydrate composition***
2.0	Control carbohydrate
2.1	Liquid cereal carbohydrate
2.2	Whole cereal carbohydrate
2.3	Root carbohydrate
2.4	Legume carbohydrate

4.3.3 **Trial 3**

Trial 3 was carried out to evaluate different lipids, aiming to the identification of raw materials with improved nutritional and sensorial properties, and with higher consumer acceptance compared to the current raw material used. Three samples with

different lipid sources were prepared as seen in Table 3. All samples contained a standard protein blend, Control carbohydrate, standard oil blend, and a complex of minerals.

Table 3 Samples' number and lipid composition

Sample	*Carbohydrate composition*
2.0	Seed oil 1
2.5	Nut oil
3.0	Seed oil 2

4.3.4 **Trial 4**

Trial 4 had as purpose the initial evaluation of flavours' sensorial attributes when infused in the samples of Trial 1. Trial 4 was performed in a form of a workshop at the company's Design Kitchen. Initially, a number of 19 flavours was infused in different protein blends (w/v), and the samples were evaluated by four team members. Subsequently, based on the sensorial attributes of the flavours, their number was narrowed down to 12. The flavours were distributed in eight samples, as noted in Table 4.

Table 4 Samples' number, their protein composition, and flavour composition

Sample	*Protein composition*	*Flavour composition*
4.1	Legume 1, Seed 1, Cereal 2	Flavour 1
4.2	Legume 1, Cereal 1, Cereal 2	Flavour 2
4.3	Legume 5, Seed 2	Flavour 3 & Flavour 4
4.4	Legume 1, Legume 2	Flavour 5, Flavour 6 & Flavour 7
4.5	Legume 1, Legume 2	Flavour 8
4.6	Legume 1, Cereal 1	Flavour 9
4.7	Legume 1, Cereal 1	Flavour 10
4.8	Legume 3, Legume 4, Cereal 2	Flavour 11 & Flavour 12

4.4 Analyses

Unless otherwise stated, all samples were examined after homogenization, after heat treatment, and after four weeks of storage at 25, and 37°C, as accelerated shelf-life. Analyses on the samples after homogenization and after heat treatment were conducted for gaining understanding of the samples' processability and

physicochemical properties, thus assist the selection of raw materials for the final formulations. Analyses on the samples after four weeks of storage helped in assessing the products' stability over shelf-life. All samples were shaken prior to the analyses. Due to the fast-moving nature of the project, not all samples were analysed in replicates. Trial 1 was also analysed after 12 weeks of storage, due to time allowance. Trial 4 was not analysed as the samples were prepared at a kitchen scale.

4.4.1 **Visual assessment**

For obtaining an indication on the appearance and shelf-life stability, such as creaming, sedimentation, flocculation, inhomogeneity, insolubility of particles, colour, and thickness, the samples were visually assessed after homogenization, after heat treatment, and after four weeks of storage at 25, and 37°C. Possible phase separation was first measured with a ruler in millimetres. Shaking of the samples then took place, by manually shaking the samples five times within five seconds for observing possible improvement in the samples' phase separation, such as sediment removal or re-homogenization after creaming.

4.4.2 **Heat stability**

Heat stability analysis was conducted after homogenization for an indication of samples' behaviour on aggregation and gelling to be obtained. The samples were observed for their heat stability using an oil bath at 140 °C for ten minutes. An amount of 10 ml was placed in 40 ml DURAN tubes, and the tubes were closed with a lid. The samples were placed in the oil bath and were rotated every 30 seconds. The aggregation and gelling points were noted.

4.4.3 **pH**

The pH was measured for gaining an indication on the products' microbiological stability and potential protein aggregation if pH values reach closer to the proteins' isoelectric point. The pH of the samples was measured using an X pH electrode.

4.4.4 **Calcium ion activity**

Calcium ion activity was measured in the samples for determining potential proteins' aggregation due to binding with free soluble calcium. Calcium ion activity was determined by an X Calcium ion selective electrode.

4.4.5 **Viscosity**

Viscosity of the samples was examined for determining possible processability constrains of the products, and to obtain the rheological behaviour of the samples in the mouth or under more harsh conditions. Viscosity of the samples was performed at 100-s and 1000-s using a Rheometer X and a X probe in the settings for finished liquids, at 20°C. The first speed was applied to mimic the conditions in the mouth, while the second was applied for observing the products' behaviour upon extreme conditions.

4.4.6 **Particle size distribution**

Particle size distribution was measured for gaining an understanding on potential aggregation and sedimentation which may occur in the samples during the different treatments. Particle size distribution was characterized by static light scattering, using an X particle size analyser in a range of 0,01 μm and 3500 μm at 25°C. The internal standard operating procedure for liquid milk products was used with demineralized water as dispersant. The resulting graphs were observed for determining changes of particle size distribution upon the different treatments. Additionally, D [0.9] was noted, which describes that 90% of the particles were below the specified size.

4.4.7 **Optical microstructure**

Microstructure of the samples was visualized using an X microscope, for the samples' aggregate size to be determined. An amount of 1 ml of the sample was diluted in 9 ml of water. Then, an amount of 6.5 μm was placed on microscope glass slide, covered with a coverslip, and observed at 100x magnitude and 25°C. Scale of 10 μm was added in the obtained pictures.

4.4.8 **Organoleptic properties**

Descriptive sensory analyses took place after three weeks of storage for understanding each samples' organoleptic attributes. The analyses were performed by non-trained panellists, all members of the project. The samples' attributes were assessed individually, followed by a group discussion on the overall acceptance on the samples. The evaluated attributes included appearance, smell, flavour and taste, mouthfeel and afterfeel, and general comments or overall acceptance.

4.4.9 **Digestibility-Corrected Amino Acid Score (PDCAAS)**

The theoretical protein digestibility value of the proteins was examined in Trials 1 and 2, for determining the nutritional suitability of the protein blends for the specific age category. PDCAAS was calculated by dividing the amino acid with the lowest score in the specific recipe, by the quantity of the same amino acid in the recipe, multiplied to the protein digestibility. Protein digestibility of each raw material was obtained through in vivo or in vitro studies (Boye et al., 2012). PDCAAS values above 1 were truncated to 1.

4.4.10 **Dextrose equivalent (DE)**

The dextrose equivalent (DE) of the carbohydrates at Trial 2 was evaluated based on the information obtained from the suppliers, for determining the nutritional suitability of the carbohydrates in comparison to the already used Control carbohydrate.

4.5 Product concepts

Based on the results obtained through the initial steps of the consumer study, different product concepts were formulated. Subsequently, through an internal brainstorming workshop, the concepts were aligned with various ingredients, sensory cues and related product attributes. Further, based on insights obtained by the involved teams, the concepts were being reshaped throughout the project's duration. By combining the results collected during the project's span the final concepts were formed.

4.6 Packaging and labelling

Simultaneous activities were involved in the project for obtaining information, and for providing suggestions on potential packaging and labelling for the product. Through a combination of the activities conducted, and based on scaling-up considerations, suggestions of packaging and labelling solutions were formulated.

4.6.1 **Ingredients' perception and labelling ideation workshop**

A workshop on ingredients exploration and labelling ideation was conducted as part of the consumer study. The workshop aimed in obtaining information on ingredients

that are most preferred by consumers, nutritional and health claims that consumers are looking for in toddlers' products, as well as packaging and labelling ideas.

The participants were asked to link certain product attributes with foods and drinks they could think of. Further they were presented with certain raw materials for which they were asked for their perception. Lastly, the participants were asked to draw a packaging and label which would include any of the previously presented raw materials, or additional ones which they wish to have in YCF.

4.6.2 **Concepts and labelling perception study**

A labelling perception study took place during the consumer study for a perception on the current study's product labels to be evaluated. Each product's overview was given together with the approximate ingredient list and in some cases with a nutritional information table. Each ingredient list and/or nutritional information table was shown with different variations. Specifically, the concepts' labels differed by having more or less ingredients listed, more or less complete nutritional tables, and more or less complex labelling of the ingredients. The purpose of the variations was to obtain information on consumer perception on the ingredients when written in different forms.

4.6.3 **Benchmark analysis**

A benchmark analysis on ten competitors' products of the same age category was performed. The analysis took place for understanding competitors' trends, their way of communication through labelling with the consumers, the raw materials they are using, and the products' nutritional composition. Information of the analysis included the brand, country of origin or launch, age category of the product, ingredients list, serving size, calories per serving, packaging material, nutritional information in comparison to the company's internal nutritional framework, front label communication, and additional information on the ingredients contained in the product or from the product's website. Ultimately, the information obtained was used for establishing suggestions on potential packaging and labelling concepts for the resulted products of this study.

4.6.4 **Packaging and labelling design workshop**

A workshop on packaging and labelling design was conducted with a number of participants, for facilitating the development of concepts that can be used in the products of the current study. The participants were separated in six groups, and were aligned with one concept. Each concept included information on the ingredients, sensorial and product properties, and a consumer persona. The

participants were then given time to brainstorm, ideate, design, and present a packaging and label idea.

5 Results and discussion

In this chapter, the results of each trial are discussed, along with the considerations that lead to each ingredient's selection. Furthermore, the insights obtained from the different activities on packaging and labelling are presented. By the combination of the trials' results, and the insights obtained from the packaging and labelling activities, the product concepts are demonstrated, together with suggestions on potential packaging and labelling solutions.

5.1 Trial 1

Trial 1 was developed twice due to microbial spoilage occurred from temperature drop in the processing line during the first production. The two batches were performed in different processing lines due to equipment availability. Due to the duplicate production, the samples of the first batch were stored for longer time which allowed to examine the samples' physicochemical properties after 12 weeks of storage at 25, and 37 °C. Analyses on homogenized samples was done the same day as the production while analyses on the heat treated samples was done three days after the production. Sample 2.0 was not examined after 12 weeks of storage as it was produced in a later batch, thus its storage had not reached 12 weeks.

5.1.1 **Visual assessment**

The samples after homogenization and after heat treatment exhibited similar attributes, showing no phase separation. Some insolubility of particles was observed in the samples after homogenization, which was improved after heat treatment, due to the additional homogenization step that occurs during heat treatment.

After four weeks of storage at 25 and 37 °C, Samples 1.0, 1.2, 1.5, and 1.7 showed suspended particles, possibly due to a thin layer of creaming. Such phenomenon was also observed in Samples 1.5 and 1.7 after twelve weeks of storage at 25, and 37°C. The particles in suspension were however not observed in all bottles of the same

sample, indicating physicochemical variations between the products, possibly due to processing fluctuations.

After four weeks of storage at both storage temperatures, Samples 1.0 and 1.2 showed sedimentation of around 1 mm, which was persistent upon gentle shaking. Sample 2.0 showed sedimentation of around 3 mm, which could be removed upon gentle shaking. As it may be observed in Figure 1, Samples 1.4, 1.5, and 1.6 showed sedimentation of approximately 10 mm, which was however easily removable upon gentle shaking. Sedimentation was not increased further after 12 weeks of storage.

Figure 1 Visual assessment of the samples. Samples from left to the right; 1.0, 1.2, 1.4, 1.5, 1.6, 1.7, and 2.0.

Particles' insolubility was observed by a study which examined partially-substituted dairy infant formulae with pea and fava bean proteins, compared to dairy formula (Le Roux et al., 2020). The study proved that different processing parameters may improve the solubility of plant proteins.

5.1.2 **Organoleptic properties**

A descriptive organoleptic analysis was conducted on the samples with 11 non-trained panellists, all members of the project's team. The most common attributes of each sample noted by at least two panellists are displayed in Table 5. Sample 2.0 was sensorially evaluated only by three panellists at the same day of production, as later the product showed microbiological spoilage thus could not be tasted by other panellists.

Table 5 Main organoleptic attributes of the samples, noted by at least two panellists.

Sample	*Appearance*	*Smell*	*Taste / Flavour*	*Mouthfeel, aftertaste and afterfeel*
1.0	Dark Grey Brown Pink	Neutral Grassy	Cereal Mushroom Cardboard Bitter Milky Nutty	Watery Dry Thick
1.2	White/whitest	Neutral	Sweet Watery	Watery Dry Creamy
1.4	Grey/greyest	Neutral Beany Nutty Lentils/pea	Sweet Beany Oat milk Milky/creamy	Nutty Astringent
1.5	-	Neutral	Beany Milky Nutty	Fatty Creamy Powdery Watery/fluid Dry
1.6	-	Neutral Cardboard	Cereal Beany Grassy Nutty Playdough Cardboard Milky	Thick Creamy Astringent Watery/thin Nutty/walnut
1.7	Floating particles	Neutral Grassy	Fresh Bitter Milky Nutty	Oxidized Dry
2.0	White	Beany Pea Oat	Watery Oily Creamy Neutral	Astringent

In terms of acceptance, Sample 1.4 was shown to have the highest acceptance, by having received five most-liked votes. Sample 1.5 gained a positive impression by at least two panellists while Sample 1.2 was described as neutral. On the other hand,

1.0, 1.6, and 1.7 had received negative feedback by at least two panellists. Sample 2.0 was perceived positively by the three panellists who evaluated it.

Common attributes were noted between the different samples. Such attributes included:

- Smell: Neutral, beany
- Flavour: Cereal, cardboard, bitter, milky, nutty, sweet, beany
- Mouthfeel and aftertaste: Watery, dry/astringent, nutty, creamy

Overall, it may be expected that addition of fibres, different lipids, and flavours in later stages may contribute to increased viscosity and creaminess, contributing therefore to the improvement of the watery attributes. Moreover, the addition of flavours is expected to influence positively the overall flavour of the samples, which may therefore mask certain undesirable attribute of all samples.

5.1.3 **Heat stability**

As noted in Table 6, no sample showed gelling after ten minutes of heating. Aggregation of the samples 1.4 and 1.6 was observed after half and one minute, respectively. Phase separation was displayed in Sample 1.4 immediately after its immersion in oil, which however disappeared upon rotation. Sample 1.5 showed late aggregation, at approximately eight minutes, while Samples 1.0 and 1.7 aggregated after three minutes. Samples 2.0 and 1.2 showed aggregation after four and five minutes, respectively.

Table 6 Aggregation and gelling points (minutes) of the samples during heat stability test.

Sample	*Aggregation point*	*Gelling point*
1.0	3	> 10
1.2	5	> 10
1.4	0.5	> 10
1.5	8	> 10
1.6	1	> 10
1.7	3	> 10
2.0	4	> 10

The products' relatively low protein content of 0,9 – 2%, and low total dry matter of around 10-15% may explain this no gelation pattern. It may be indicated that the samples can be sufficiently processed at a factory scale, however, early aggregation

may create processing issues such as fouling, or lead to inhomogeneity of the product. As stated by Bogahawaththa *et al.*, (2019), heat stability of plant proteins may depend on pH, isoelectric point, protein concentration, and heat treatment.

5.1.4 **pH**

As seen in Table 7, pH changes of maximum ±0.1 occurred between the samples after homogenization and after heat treatment, indicating no significant changes. Sample 1.4 showed a slight decrease in pH after heat treatment, whereas the rest of the samples showed a slight increase (±0.1). All samples maintained a pH range between 7.3 and 7.4, which was alike the samples' initial pH adjustment. Over four weeks of storage at 25 °C, all samples showed a pH reduction of up 0.2. The pH was reduced further in the samples stored at 37 °C, with the lowest pH to had reached approximately 7.

Table 7 pH of the samples after homogenization, after heat treatment, after four weeks of storage at 25 °C and 37 °C, and after 12 weeks of storage at 25, and 37 °C.

	Sample						
Treatment	***1.0***	***1.2***	***1.4***	***1.5***	***1.6***	***1.7***	***2.0***
After homogenization	7.42	7.37	7.42	7.36	7.38	7.35	7.41
After heat treatment	7.45	7.47	7.34	7.37	7.44	7.44	7.37
After four weeks at 25 °C	7.37	7.36	7.23	7.22	7.34	7.23	7.28
After four weeks at 37 °C	7.25	7.17	7.17	7.14	7.08	7.20	7.01
After 12 weeks at 25 °C*	7.36	7.40	7.55	7,27	7.31	7.24	-
After 12 weeks at 37 °C*	7.23	7.23	7.11	7.10	7.17	7.04	-

**Different batch was analysed. Average value of two repetitions.*

The pH was measured in a different batch after storing the samples for 12 weeks at 25 and 37 °C. The results had shown that the samples maintained pH equal to or above 7, indicating no spoilage or risk of proteins' aggregation due to reduced pH. Similar values of 6.9-7.4 were observed in a previous study conducted within the company on a similar product throughout a 12-week stability study at the same temperatures.

As a 12-week storage at 37 °C gives an indication for one-year shelf-life, the products may be expected to maintain an acceptable pH value throughout their shelf-life (WFP, 2020). As aforementioned, Sample 2.0 was not examined over a 12-weeks storage due to time constrains. As the sample had shown a pH value of 7 after four weeks at 37 °C, it is suggested to be examined over a 12-weeks storage, for an indication of the product's shelf-life stability to be determined.

5.1.5 **Calcium ion activity**

Calcium ion activity values were noted in Table 8. Due to possible equipment error occurred during the measurements after homogenization and after heat treatment, the absolute numbers were considered inaccurate. Despite that, the values' trends were considered logical, as under all treatments Sample 1.2 showed the highest calcium activity values, followed by Samples 1.7 or 1.0. Considering the values taken after 4 and 12 weeks of storage at the two temperatures, Sample 1.2 exhibited a maximum calcium ion activity of around 43 ppm. Deviations of up to ±12 ppm were observed between the same samples stored for 4 and 12 weeks at 25 and 37 °C. Such deviations were not considered impactful.

Table 8 Calcium ion activity of the samples after homogenization, after heat treatment, after four weeks of storage at 25 °C and 37 °C, and after 12 weeks of storage at 25 °C and 37 °C. Values in ppm.

	Sample						
Treatment	***1.0***	***1.2***	***1.4***	***1.5***	***1.6***	***1.7***	***2.0***
After homogenization*	46.11	63.37	52.04	42.98	46.44	69.59	41.27
After heat treatment*	130.52	156.20	65.44	100.61	84.68	140.87	33.55
After four weeks at 25 °C	31.76	33.60	18.14	25.43	24.80	33.49	25.26
After four weeks at 37 °C	28.27	36.55	13.37	16.60	18.90	26.77	19.30
After 12 weeks at 25 °C**	24.50	42.82	26.84	24.82	23.43	37.83	-
After 12 weeks at 37 °C**	32.32	37.94	23.61	19.37	19.70	32.78	-

**Possible error occurred during measurements.*

***Different batch was analysed. Average value of two repetitions*

In a previous internal study on a similar product, calcium ion activity exhibited values between 33 and 36 ppm after heat treatment. In the current study, all samples exhibited values between approximately 20 and 40 ppm throughout a maximum of 12 weeks storage at 25 and 37 °C. It may be concluded that calcium ion activity can be maintained in acceptable values throughout a one-year shelf-life. However, it is important to be noticed that the form, thus the solubility, of calcium of the current study differed from the compared study. Differences in calcium content arising from the different protein sources, as well as the different calcium forms of the supplemented minerals, may resulted in differences in the calcium ion activity of the samples.

5.1.6 **Viscosity**

As it can be seen in Tables 9 and 10, no great changes were observed between the two rotation speeds. The viscosity was decreased in all samples after heat treatment. The viscosity of the samples after homogenization ranged from around 3 to 5 mPa-s, while after heat treatment the viscosity in all samples was reduced to values around 2 (±0.2) mPa-s. Sample 1.4 exhibited the highest viscosity (5 mPa-s at 100s-1) after homogenization, however, its viscosity reached similar values to the rest of the samples after heat treatment, with values at around 2.5 mPa-s. It is important to be noticed that heat treatment includes an additional homogenization step, which may explain the reduction of viscosity in the samples after heat treatment.

Table 9 Average viscosity of the samples after homogenization, after heat treatment, after four weeks of storage at 25 °C and 37 °C, and after 12 weeks of storage at 25 °C and 37 °C, under 100s-1 rotation speed. Values in mPa-s.

	Sample						
Treatment	***1.0***	***1.2***	***1.4***	***1.5***	***1.6***	***1.7***	***2.0***
After homogenization	3.50	3.23	5.12	3.36	3.70	3.30	3,82
After heat treatment	1.91	1.98	2.02	2.47	2.18	2.20	2.12
After four weeks at 25 °C	2.13	2.10	2.57	2.94	2.22	2.35	2.20
After four weeks at 37 °C	2.26	2.53	2.34	3.04	2.01	2.36	2.22
After 12 weeks at 25 °C*	2.12	2.12	2.40	2.93	2.45	2.38	-
After 12 weeks at 37 °C*	2.15	2.40	2.29	2.89	2.25	2.42	-

**Different batch was analysed. Average value of two repetitions.*

Table 10 Average viscosity of the samples after homogenization, after heat treatment, after four weeks of storage at 25 °C and 37 °C, and after 12 weeks of storage at 25 °C and 37 °C, under 1000s-1 rotation speed. Values in mPa-s.

	Sample						
Treatment	***1.0***	***1.2***	***1.4***	***1.5***	***1.6***	***1.7***	***2.0***
After homogenization	3.15	2.84	4.21	2.92	3.06	2.84	3,30
After heat treatment	1.90	1.95	1.98	2.25	1.94	2.09	2,11
After four weeks at 25 °C	2,08	2,06	2,36	2,57	2,02	2,22	2,17
After four weeks at 37 °C	2,18	2,36	2,23	2,65	1,95	2,24	2,18
After 12 weeks at 25 °C*	2,07	2,09	2,21	2,52	2,12	2,24	-
After 12 weeks at 37 °C*	2,10	2,27	2,17	2,55	2,05	2,25	-

**Different batch was analysed. Average value of two repetitions.*

Over time and at the different temperatures, the viscosity between the same samples showed a deviation of ±0.4, which was considered insignificant. Sample 1.5 exhibited the highest viscosity among all samples, showing a value of around 3 mPa-s.

Alonso-Miravalles et al. (2021) examined and compared three reconstituted spray-dried formulae made of lentil, soy, and rice proteins of 1.9% content, compared to 0.9-2% content of the present study's samples, and with corn maltodextrin and sunflower oil. The study found viscosity values of 18 to 21 mPa-s, which were considered to be significantly higher to this study's values. Despite this, the differences in viscosity may be explained as the aforementioned study's products consisted of approximately 30% total solids, compared to the current study's samples which consisted of around half the amount of total solids.

In a previous internal study on a similar product, the viscosity of the sample post heat treatment was 2.6 mPa-s, while over time the viscosity showed a minor rise of 0.2 mPa-s. Despite that the values of the study were alike to the current study's, it should be noted that the former contained fibres which may have influenced its viscosity. Conclusively, based on the results the viscosity of the samples is not expected to significantly rise throughout their shelf-life.

5.1.7 **Particle size distribution**

As it may be observed in Figure 3, particle size was decreased in all samples after heat treatment. Such decrease in particle size may be explained as an additional homogenization step takes place during heat treatment. Sample 1.4 showed the highest particle size after heat treatment, with 90% of the particles being below approximately 36 μm, followed by Samples 2.0 and 1.5 which exhibited values of around 7 and 4 μm, respectively. The rest of the samples' particle size ranged in 0.6-0.8 μm under the same treatment. The fibre containment in the protein isolates used in Sample 1.4, of 4.6% for Legume 1 and Seed 1 blend, and 1% for Cereal 2, may have contributed to the higher particle size, due to the fibres' insoluble nature.

The values were compared to a research which examined three reconstituted and heated spray-dried formulae made of lentil, soy, and rice proteins (Alonso-Miravalles et al., 2021). Lentil protein formula's D [0.9] values 9 (≈ 35 μm) agreed with the values found in Sample 1.4. Rice formula exhibited values alike Sample 2.0, with 90% of the particles being below 8.6 μm, while soy formula had values of 14 μm.

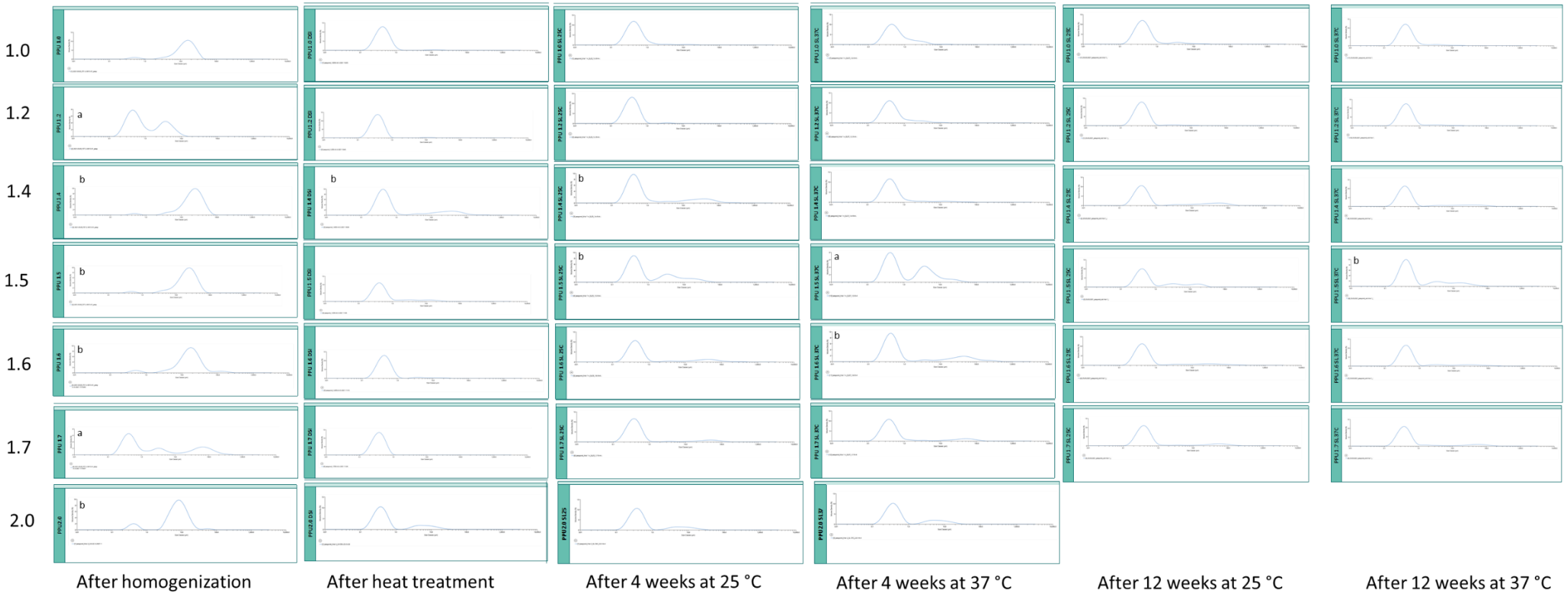

Figure 2 Particle size distribution of the samples after homogenization, after heat treatment, after four weeks of storage at 25 and 37 °C, and after 12 weeks of storage at 25 and 37 °C. X axis represents size classes (µm); 0.01, 0.1, 1, 10, 100, 1000, 10000. Y axis represents volume density (%); 0, 5, 10, 15.

[a] volume density (%); 0, 2, 4, 6, 8

[b] volume density (%); 0, 2, 4, 6, 8, 10

Different batch was analysed for the samples after 12 weeks at 25°C and after 12 weeks at 37°C.

Over storage for four weeks at 25 °C, Sample 1.6 showed the highest particle size with 90% of the particles being below 33 μm. The fibre content in the two protein sources used in the sample, of 13% in total, may explain the increased particle size. Sample 1.4 followed by maintaining a value of 32 μm. Increase in particle size was observed by the samples 1.5 and 1.6, as 90% of their particle size distribution was at values below 10 and 8 μm, respectively. Sample 2.0 showed similar values (6 μm) compared to day 0. Samples 1.0 and 1.2 maintained low particle sizes, with values 0.9 and 0.6 μm, respectively. At the same storage period and at a temperature of 37 °C, the particle size of Sample 1.6 showed a further increase to 53 μm at D [0.9]. Following, Sample 1.5 maintained similar size (7.7 μm) as in the previous storage temperature. Sample 1.4 showed a decreased value, with 1.4 μm. Minor increase in particle size was observed in Samples 1.0 and 1.2, with values of 2 and 1.5 μm, respectively. Sample's 1.4 decreased particle size may be explained as the sample showed increased sedimentation, which may have led to inhomogeneity of the sample thus measurement of the supernatant.

Under a 12-weeks storage at the two temperatures, the samples showed decreased particle size values, with all the samples being between a range of 0.4 and 1 μm at D [0.9]. Such decrease is expected to have been shown due to increased sedimentation which resulted to the measurement of the samples' supernatant. It is however important to be noted that a different batch was analysed, which was produced in a different processing line and with a different batch of raw materials, thus may have contributed to the significantly different results in some samples.

At a previous internal study on a similar product, the particle size of the sample after heat treatment was at values 3-4 μm, while throughout a 12-week storage the sample's particle size was increased to 5-10 μm. Such values are considered to be similar to the particle size of some of the current study's samples. Conclusively, it may be expected that the samples may show somewhat increased particle size which may lead to sedimentation, however, such results are not considered to limit the products' stability over a one-year shelf-life.

5.1.8 **Optical microstructure**

As observed in Figure 4, increased protein aggregation was observed prior to heat treatment in Samples 1.0, 1.4, 1.5 and 1.6. Overall, heat treatment contributed to the reduction of aggregates' size in all samples. Sample 1.4 maintained particles with size above 20 μm. Samples 1.0, 1.2 and 1.7 showed the highest homogeneity with decreased particle sizes after heat treatment, while 1.5, 1.6, and 2.0 sustained some particles between 5 and 10 μm. It should be noted that heat treatment includes an additional homogenization step, which may explain the improved results of the samples after heat treatment.

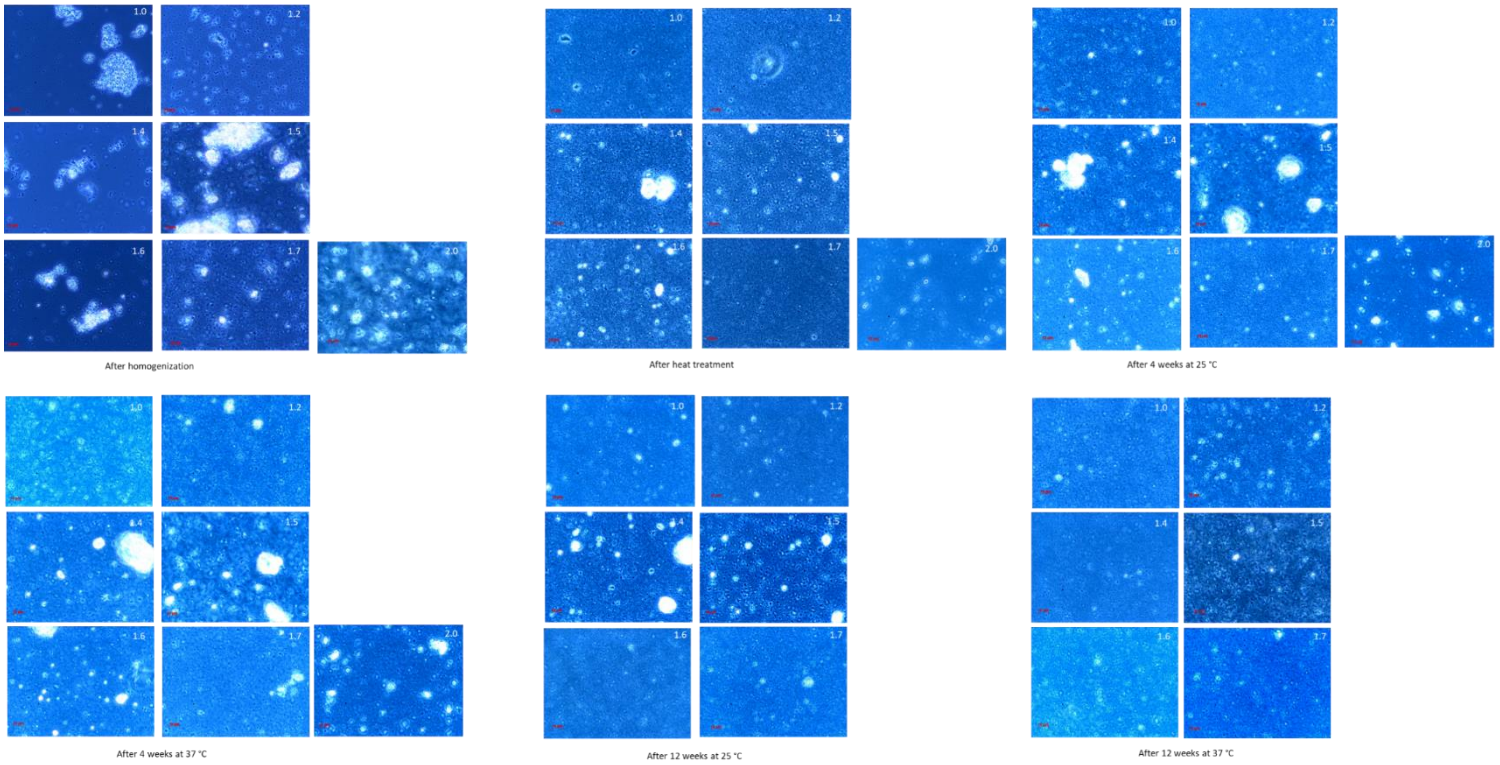

Figure 3 Optical microstructure of samples after homogenization, after heat treatment, after 4 weeks at 25°C, after 4 weeks at 37°C °C, after 12 weeks at 25°C, and after 12 weeks at 37°C. Different batch was analysed for the samples after 12 weeks at 25°C and 12 weeks at 37°C. Scale indicates 10 µm.

After four weeks of storage, all samples showed increased protein aggregation, with Samples 1.4 and 1.5 showing sizes above 20 μm. The rest of the samples showed aggregates of mainly smaller or equal to 10 μm. Slight increase of particle sizes were observed at 37 °C compared to 25 °C.

The samples stored for 12 weeks showed smaller aggregates compared to the samples stored for four weeks. However, the former was analysed from a different batch which may explain the differences. Differences in homogenization may have been caused from the different processing line used upon production of the two batches. Additionally, it is possible that the samples' supernatant was measured, which may did not allow the measurement of the sedimented solids.

As in the samples stored for four weeks, Samples 1.4 and 1.5 stored for 12 weeks at 25 °C exhibited the largest size of aggregates compared to the rest of the samples. In contrast, samples 1.4 and 1.5 stored for 12 weeks at 37 °C showed smaller aggregates. Such differences may arise due to variations between the samples or from an error during the measurements, even though the two treatments were analysed the same day.

5.1.9 PDCAAS

As seen in Table 11, the PDCAAS of the samples 1.6 and 1.7 was not within the set limit of 0.9. Sample 2.0 exhibited the highest PDCAAS (1), followed by Sample 1.4 (0.95) and Sample 1.2 (0.94). Samples 1.0 and 1.5 showed lower values, of 0.91, and 0.9, respectively.

Table 11 PDCAAS value of the samples.

Sample	*PDCAAS value*
1.0	0.91
1.2	0.94
1.4	0.95
1.5	0.90
1.6	0.89
1.7	0.75
2.0	1.00

5.1.10 Conclusions

Overall, samples 1.0, 1.2, and 1.7 showed the best results in terms of homogeneity and low particle size. Despite that, all samples showed acceptable physicochemical results, showing no gelation, stability over shelf-life, and similar viscosity, pH, and

calcium ion activity values. Therefore, the selection of the samples was done based on the PDCAAS value, the consumer perception, the sensory analysis, and based on the samples' suitability for each of the identified product concept.

Sample 1.0 had received negative feedback by two panellists. Due to Cereal's 1 controversial perception, it was decided to not proceed further with the sample.

Sample 1.2 did not receive negative feedback, and it was characterized as neutral, which could be suitable as a base product. The sample exhibited a high PDCAAS value (0.94). However, the sample contains Legume 2 which has a contradictory perception due to health concerns and sustainability reasons as it is not considered a local ingredient where the consumer study took place. As a result, the sample was not selected for the final examination.

Sample 1.4 which showed the highest particle size and bigger aggregates was perceived as dry, creamy or rich, and powdery, by five, two and one panellist, respectively. The increased particle size may explain the dryness observed by the panellists. However, it may be considered contradictory that the sample was described as powdery by only one panellist. Despite this, the sample showed the most acceptable sensorial characteristics, as well as a high PDCAAS value (0.95). The sample was thus selected for further examination. It should however be noted that the sample showed phase separation upon heat stability analysis which may be important to be taken into consideration when scaling up. Additionally, consumers' controversial perception of Cereal 2 due to its contaminant content should be taken into consideration. Lastly, considerations on the presence of natural flavouring in Legume 1 and Seed 1 blend should be taken into account for the product's labelling.

On the other hand, Sample 1.5 which also showed higher particle and aggregates' size compared to the rest of the samples was perceived as powdery by four panellists, and milky, creamy, fatty and dry by two panellists. In terms of sensorial acceptance, the sample obtained positive impression by at least two panellists. On the other hand, as the sample was at the limit of the desirable PDCAAS value, it was decided to not proceed further.

Sample 1.6 received a negative sensorial impression by two panellists. In addition to that, the sample showed a PDCAAS of 0.89, which was considered to be lower than the acceptable limit. As a result, the sample did not proceed for further examination.

Sample 1.7 was considered unsuitable for scaling up due to its low PDCAAS value of 0.75. In addition, the sample showed low organoleptic acceptance. The containment of Legume 2 in the sample was considered as another limiting factor for the sample due to the contradictory perception of the legume.

Sample 2.0 had shown acceptable physicochemical characteristics as well as the highest PDCAAS value (1). The sample's ingredients familiarity and perception added an advantage to the sample, and therefore resulted to its further examination. It should however be noted that the sample was sensorially examined by only three

panellists, thus a more objective opinion should be obtained for the sample's organoleptic attributes.

5.2 Trial 2

Analyses on samples after homogenization were done the day of trial and the day after. Analyses on the samples after heat treatment was done a day after the trial. Due to temperature drop in the processing line, microbiological spoilage occurred in Samples 2.2 and 2.3, which did not allow for particle size and calcium ion activity to be measured after four weeks of storage.

5.2.1 **Visual assessment**

After homogenization and after heat treatment, all samples other than 2.3 exhibited persistent sedimentation which was not easily removed upon shaking of the samples. This may be due to the use of Cereal 3, as Legume 1 proved to have high solubility when used in Trial 1. As it can be observed in Figure 5, Sample 2.2 containing Whole cereal carbohydrate showed increased sedimentation of approximately 5 mm after heat treatment. The decreased solubility of Whole cereal carbohydrate was observed from the dissolving stage during the mixing of the ingredients.

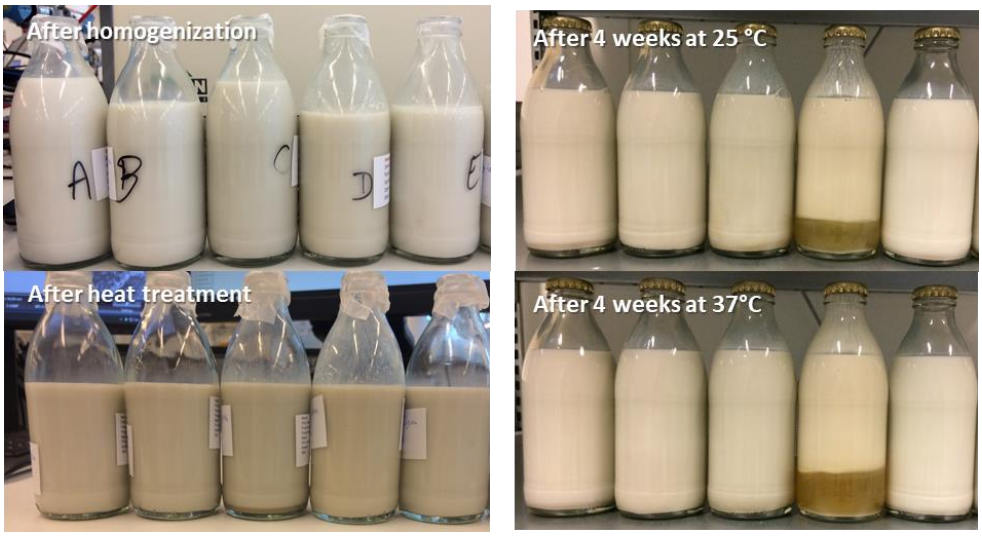

Figure 4 Visual assessment of the samples. Samples from left to the right; 2.0, 2.1, 2.2, 2.3, and 2.4.

Phase separation was observed visually in Sample 2.3 after four weeks of storage; however, the sample became homogeneous for at least four hours after shaking.

Destabilization in the emulsion may occurred due to ionic strength, poor heat stability of the carbohydrate, or interaction of the dispersed phase with the continuous phase (Maphosa & Jideani, 2018).

After four weeks of storage at both 25 and 37 °C, Samples 2.0 and 2.1 showed sedimentation of around 3 mm which was removable upon shaking. Sample's 2.0 decreased sedimentation was expected since the sample served as the control, while Sample 2.1 may have shown low sedimentation due to the carbohydrate's liquid nature, in contrast to the rest of the carbohydrates which were in a powder form. Sample 2.2 exhibited the greatest sedimentation of around 5 mm, as observed in Figure 5, which was easily removable upon gentle shaking. Insoluble particles were observed at the same sample, as it was previously noticed at the sample after heat treatment. Visually, Sample 2.2 showed the highest viscosity among all samples.

The sedimentation observed in all samples is expected to have arisen from Cereal 3 due to its high content of carbohydrates and fibres, of 21 and 4.6%, respectively, and since Legume 1 was evaluated in Trial 1 and did not exhibit such insolubility. In addition, the hypothesis may be confirmed as Samples 2.0 and 2.1 containing the Control carbohydrate and Liquid cereal carbohydrate, respectively, would not be expected to sediment. This is as the reference carbohydrate Control carbohydrate has shown increased solubility in Trial 1, and the liquid nature of Liquid cereal carbohydrate makes the ingredient less likely to sediment.

5.2.2 **Organoleptic properties**

A sensorial description of the samples was performed on the samples' production day by three non-trained panellists. Further examination was not done due to the samples' spoilage over time. The samples' organoleptic attributes were noted in Table 12.

Sample 2.2 displayed more intense smell compared to the rest of the samples, which however was perceived as positive. Increased sedimentation was observed in the sample, which appeared also in the sample's texture as it was described powdery.

Sample 2.3 was observed to have spicy and peppery notes which were considered undesirable, especially as the product is intended to children.

Some common attributes were described for the rest of the samples, including nutty, beany, creamy, neutral, watery, sedimented, and with off notes. Despite this, in most of the samples such attributes were present in low intensity, indicating that most of the carbohydrates did not greatly influence the organoleptic properties of the samples. It should be noted that one of the oils was added at an earlier processing step than normal, which may explain the identified off notes at samples 2.0 and 2.4.

Table 12 Organoleptic attributes of the samples.

Sample	*Appearance*	*Smell*	*Flavour*	*Mouthfeel, aftertaste and afterfeel*
2.0	Whitest	Beany Pea Oat	Watery Oily Creamy Neutral	Astringent
2.1	-	Neutral	Oat meal Cereal Sweet Nutty	Smooth Creamy
2.2	Sedimented Viscous Dispersed particles	Cereal Powdery	Powdery Nutty Strong taste	Creamy Viscous
2.3	Slightly sedimented Viscous Dispersed particles Beige	Neutral	Milky Nutty Earthy Peppery/spicy Hay	Smooth Mouthcoating Creamy
2.4	-	Neutral	Beany Nutty Cereal Off-notes	Least viscous Creamy

5.2.3 **Heat stability**

As noted in Table 13, all samples showed no gelling after ten minutes of heating. Early aggregation, at 0.5 minutes, was observed in Sample 2.3. Samples 2.1 and 2.4 aggregated after one minute, followed by Sample 2.2 which showed aggregation after 1.5 minutes. Sample 2.0 showed the latest aggregation, after four minutes. Appearance of discoloration was observed in Sample 2.2, which however disappeared after rotating the sample. Phase separation was displayed in Sample 2.3 immediately after its immersion in oil, which then disappeared upon rotation. Such separation was in line with the sample's behaviour over storage, which may have occurred due to the carbohydrate's poor heat stability (Maphosa & Jideani, 2018).

Table 13 Aggregation and gelling points (in minutes) of the samples during heat stability test.

Sample	*Aggregation point*	*Gelling point*
2.0	4	> 10
2.1	1	> 10
2.2	1.5	> 10
2.3	0.5	> 10
2.4	1	> 10

As expected from the samples' low protein and total solid content, no gelation occurred, indicating that the samples may be sufficiently processed in a factory-scale. Early aggregation may reduce samples' processability and lead to impaired functionality, however, such phenomenon is common in dairy formulae, meaning that no additional challenges may expected to arise upon heat treatment.

5.2.4 **pH**

As observed in Table 14, pH reduction of maximum 0.3 were observed between all samples after homogenization and after heat treatment, indicating no significant changes. After heat treatment, all samples maintained an acceptable pH range between 7.1 and 7.3, a value close to the initial pH adjustment on target 7.4.

Table 14 pH of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C.

	Sample				
Treatment	***2.0***	***2.1***	***2.2***	***2.3***	***2.4***
After homogenization	7,41	7,40	7,42	7,41	7,39
After heat treatment	7,35	7,37	7,35	7,15	7,32
After four weeks at 25 °C	7,28	7,30	7,11	6,86	7,14
After four weeks at 37 °C	7,01	7,07	6,98	6,70	7,02

After four weeks at 25 °C, the pH of Samples 2.0, 2.1, and 2.4 was reduced by maximum 0.2, reaching to values between 7.1 and 7.3. Samples 2.2 and 2.3 that showed spoilage exhibited a reduction of maximum 0.3, showing a pH value of 7.1 and 6.8, respectively. The pH of all samples was further reduced when the samples were stored at 37 °C, by maximum 0.3. At 37 °C, Sample 2.3 showed a pH of 6.7. Samples 2.0, 2.1, 2.2, and 2.4 had a pH 7.

In an internal study conducted on a similar product, the pH of the product displayed values between a similar pH range (7.1 and 7.3) after a 4-weeks storage, while the samples' pH was reduced to 6.9-7.2 after a 12 weeks storage. Most of the current study's samples showed values above the minimum acceptable value (close to7), indicating no spoilage or risk of increased protein aggregation. As a 12-week storage at 37 °C indicates a year of shelf-life, it is recommended that the samples are examined after 12 weeks, for an indication of the products' stability throughout their shelf-life to be obtained.

5.2.5 **Calcium ion activity**

Table 15 demonstrates the calcium activity values of the samples. Calcium ion activity was decreased in all samples after heat treatment, and further reduced over time. Sample 2.3 showed the highest calcium ion activity compared to the rest of the samples, with values at around 74 and 39 ppm after homogenization and after heat treatment, respectively. Apart from Sample 2.3, the rest of the samples showed values between 33 and 41 ppm after homogenization, and 23 to 33 after heat treatment. The deviations of 10 ppm were not considered to impact the products properties at this low level. After four weeks of storage, Samples 2.0, 2.1, and 2.4, exhibited values between 15 and 25 ppm.

Table 15 Calcium ion activity of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C. Values in ppm.

	Sample				
Treatment	***2.0***	***2.1***	***2.2***	***2.3***	***2.4***
After homogenization	41,27	32,74	38,47	74,31	34,42
After heat treatment	33,56	28,28	29,39	39,10	22,90
After four weeks at 25 °C	25,26	19,96	*	*	16,83
After four weeks at 37 °C	19,3	19,09	*	*	15,23

**Values were not taken due to spoilage observed in the samples.*

In a previous study conducted in the company on a similar product, calcium ion activity exhibited values between 24 and 100 ppm after homogenization and heat treatment. In the current study, at the same treatments all samples displayed values between 22 and 74 ppm, indicating similar results to the aforementioned study. After four weeks of storage, the samples of the current study showed values between 15 and 25 ppm, which are comparable to the values displayed at the previous internal study (22 ppm). As previously mentioned, it is important to be noticed that the form of calcium of the current study differed from the compared study.

Overall, the calcium activity values shown in the samples indicated no risk of increased aggregation due to calcium's binding with proteins. Nevertheless, for an

indication of the calcium ion activity throughout a one-year shelf-life, it is suggested that the samples are examined after 12 weeks of storage at 37 °C.

5.2.6 **Viscosity**

As it can be seen in Tables 16 and 17, viscosity of the samples was decreased in all samples after heat treatment, as a result of the additional homogenization step included during heat treatment. Sample 2.2 displayed the greatest viscosity compared to the rest of the samples, with values of approximately 8 to 11 mPa-s. Sample 2.3 also shower higher values between around 5 and 6 mPa-s. The rest of the samples maintained average viscosity values of approximately 2 mPa-s.

Table 16 Average viscosity of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C, under 100s-1 rotation speed. Values in mPa-s.

	Sample				
Treatment	***2.0***	***2.1***	***2.2***	***2.3***	***2.4***
After homogenization	3,82	3,85	11,36	5,35	4,72
After heat treatment	2,13	2,56	9,95	6,01	2,32
After four weeks at 25 °C	2,20	2,91	11,54	7,51	2,55
After four weeks at 37 °C	2,22	3,02	11,57	7,44	2,50

Table 17 Average viscosity of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C, under 1000s-1 rotation speed. Values in mPa-s.

	Sample				
Treatment	***2.0***	***2.1***	***2.2***	***2.3***	***2.4***
After homogenization	3,30	3,34	11,31	4,78	3,96
After heat treatment	2,11	2,43	7,98	4,97	2,29
After four weeks at 25 °C	2,17	2,66	8,32	5,77	2,46
After four weeks at 37 °C	2,18	2,72	8,52	5,80	2,41

A recent study by Alonso-Miravalles et al. (2021) who examined and compared three reconstituted spray-dried formulae made of lentil, soy, and rice proteins, along with corn maltodextrin and sunflower oil, found viscosity values of 18 to 21 mPa-s. Such values were considered significantly higher to the values of this study. However, the current study's samples lower total solid content of approximately 10-15% compared to 30% of the aforementioned study may explain the reason of the significant differences.

Over storage, the viscosity of all samples was increased. Samples 2.2 and 2.3 showed an increase of around 1.5 and 1 mPa-s, respectively. The rest of the samples showed an increase of maximum 0.5 mPa-s. Between the two storage temperatures, all samples showed a maximum increase of 0.2 mPa-s, a change which was considered insignificant. The highest viscosity over a four-weeks storage was observed at Sample 2.2 (11.5 mPa-s), followed by Sample 2.3 (7.5 mPa-s). The rest of the samples showed values equal to or below 3 mPa-s. Sample's 2.2 high values may be explained due to the carbohydrate's fibre content of 13%. The solubility of the fibre included in the carbohydrate source may have influenced the viscosity values, as soluble fibres contribute to increased viscosity. On the contrary, Sample's 2.3 carbohydrate source had a higher fibre containment of 33%, which however may been consisted of insoluble fibres thus contributing in a lower degree to viscosity's rise (Fahey, 2014).

The higher viscosity values observed in the two samples may lead to processing issues such as temperature or pressure instability, and fouling. Compared to a previous internal study in which similar product was observed over a four-week storage, the viscosity values ranged at 2.5-2.6 mPa-s, which are considered comparable to some of the samples of this study. Despite this, it should be noted that the aforementioned study's sample contained additional ingredients which may have influenced its viscosity. It is therefore recommended that the viscosity of the current's study samples is re-evaluated upon the final product's composition. Additionally, a 12-week analyses at 37 °C would be suggested for an indication of a one-year shelf life to be obtained.

5.2.7 **Particle size distribution**

As seen in Figure 6, all samples except 2.2 showed decrease in particle size after heat treatment. Such decrease in particle size may be explained as an additional homogenization step takes place prior the heat treatment. Sample 2.2 showed significantly higher volume of bigger particle size after heat treatment, compared to after homogenization, showing an increase of 80 μm. The sample had also noticeably higher particle size compared to the rest of the samples at the same treatment, with 90% of the distribution being below 116 μm. Samples 2.0, 2.4, 2.1, and 2.3 showed similar values after heat treatment, with 90% of the samples being at 7, 7, 8, and 9 μm, respectively. Similar to viscosity, Sample's 2.2 high particle size may be explained due to the carbohydrate's fibre content of 13% fibre, which may be consisted of insoluble fibres due to the wholegrain nature of the carbohydrate. Sample's 2.3 decreased particle size as compared to Sample 2.2 may be due to differences in soluble fibres containment. Further information on the soluble and insoluble fibres' containment of the two samples may confirm the results

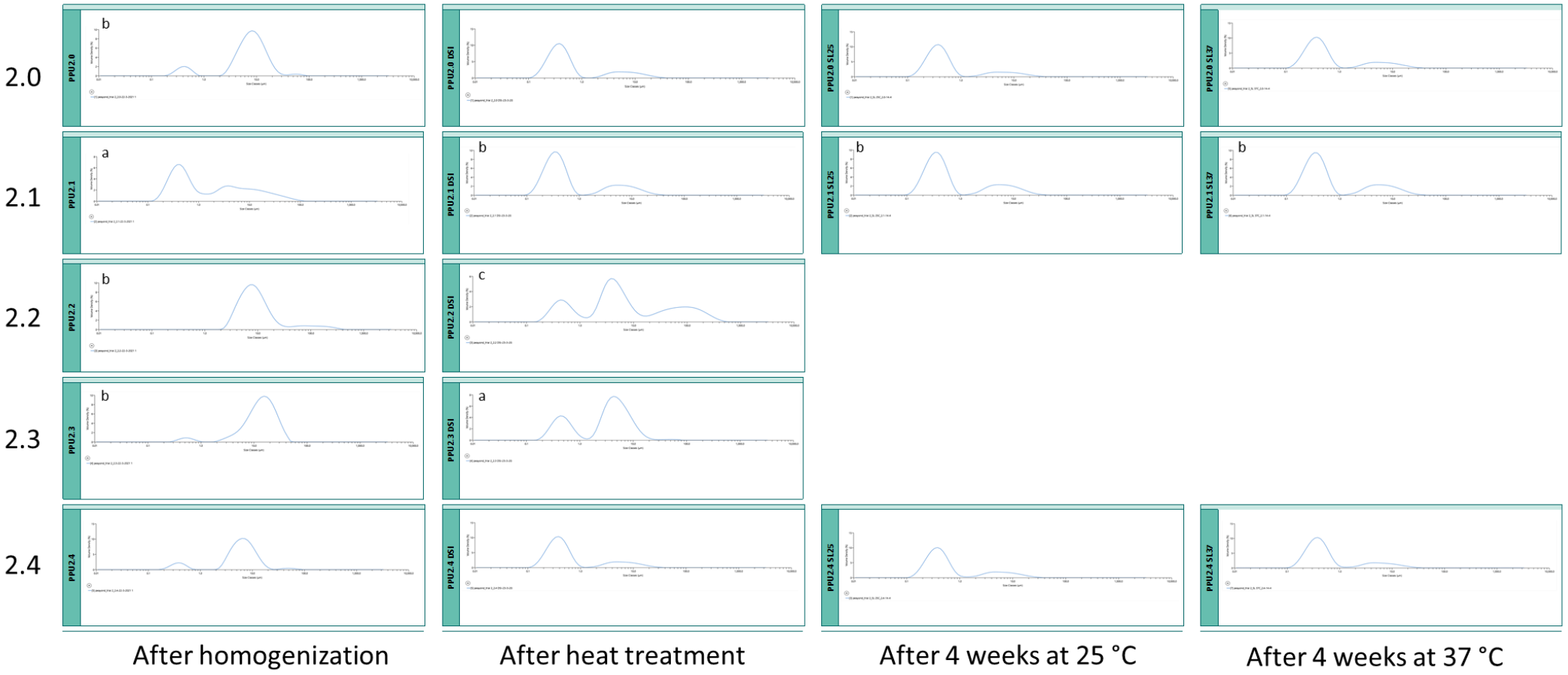

Figure 5 Particle size distribution of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 and 37 °C. X axis represents size classes (µm); 0.01, 0.1, 1, 10, 100, 1000, 10000. Y axis represents volume density (%); 0, 5, 10, 15.

[a] volume density (%); 0, 2, 4, 6, 8

[b] volume density (%); 0, 2, 4, 6, 8, 10

[c] volume density (%); 0, 2, 4, 6

Values of Samples 2.2 and 2.3 after four weeks of storage were not taken due to spoilage observed in the samples.

Compared to a study by Alonso-Miravalles et al. (2021) which examined reconstituted and heated spray-dried formulae of lentil, soy, and rice proteins, most of this study's samples were comparable to the values found at rice protein formula, which showed 90% of its particle size to be below 8.6 μm. Higher values were shown by soy and lentil protein formulae, with D [0.9] of 14 and 35 μm, respectively.

Over storage at the two temperatures, the particle size of Samples 2.0, 2.1, and 2.4 did not significantly increase as 90% of the samples' particle size distribution ranged at values below 7-8 μm. As seen in Figure 6, Sample 2.0 stored at 37 °C showed a minor increase in its particle size's peak between 1 to 100 μm, as compared to when stored at 25 °C.

Compared to a similar internal study in which the particle size of the sample had a particle size of 3 μm after heat treatment with an increase of 2 μm over storage, the current study's samples showed slightly higher values. Such values are expected to have arisen from Cereal 3 which was not evaluated previously. Additionally, as the control sample showed values alike the rest of the samples, it may lead to the conclusion that apart from Sample 2.3, the different carbohydrates may have not greatly contributed to the increased particle sizes. It may be suggested that homogenization pressure is increased during processing, as this may improve the solubility of particles in the matrices.

5.2.8 **Optical microstructure**

As seen in Figure 7, heat treatment decreased aggregates and assisted in homogeneity in all samples. Due to its high viscosity, Sample 2.3 showed the densest population of aggregates. Sample 2.2 presented the biggest size of aggregates, with most aggregates being at approximately 10 μm. Sample 2.4 showed comparable particle sizes to Sample 2.5, at around 5 μm, however, the former showed increased number of aggregates.

Over time, increased aggregation was observed in all samples, with samples 2.2 and 2.4 to have shown the biggest protein aggregates, with approximately 15 μm size. Samples 2.0 and 2.1 showed the smallest aggregates as compared to the rest of the samples, with the majority of the aggregates being below 5 μm. Lesser aggregates in the two samples may have been expected, as the former served as a control sample which has been proven to show acceptable physicochemical results. The latter, which was in a form of liquid, was expected to show decreased aggregation as compared to the rest of the carbohydrates which were in a powder form.

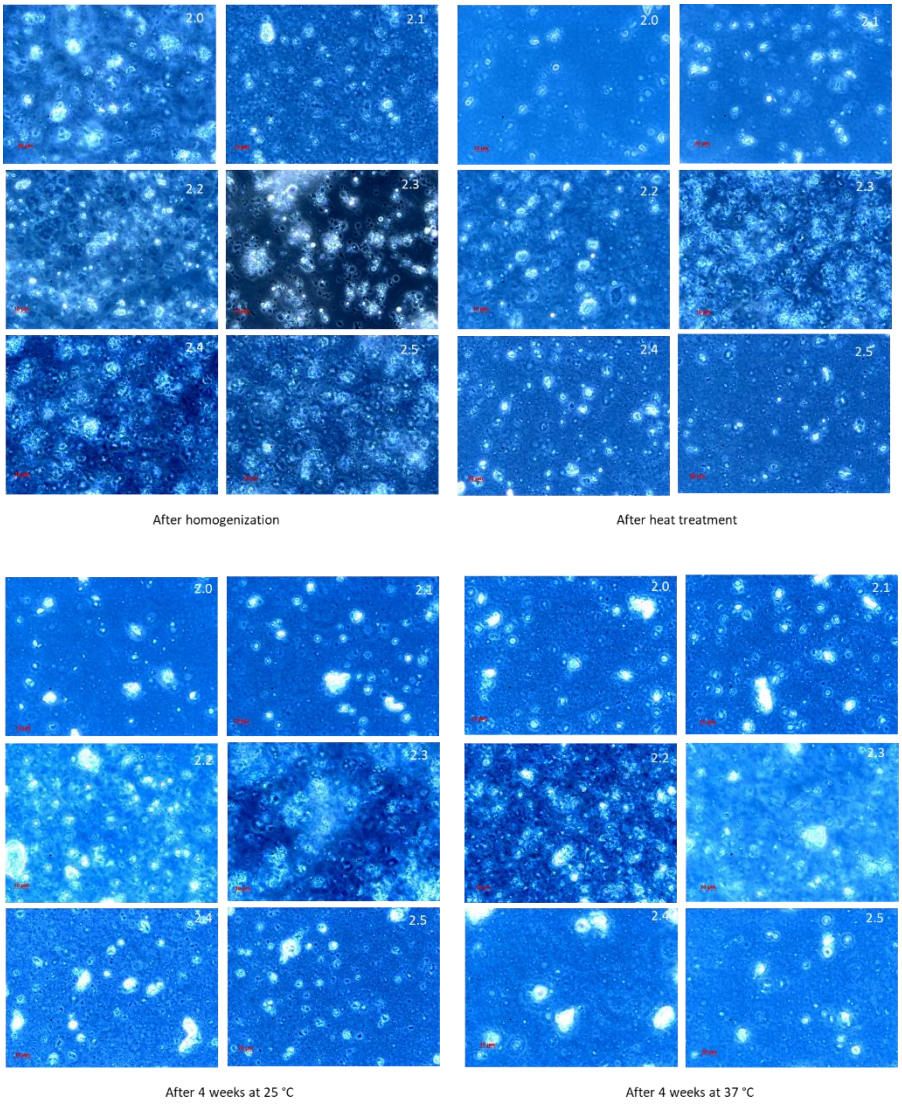

Figure 6 Optical microstructure of samples after homogenization, after heat treatment, after 4 weeks at 25°C, and after 4 weeks at 37°C. Scale indicates 10 µm.

5.2.9 **DE**

The samples' DE was evaluated based on information provided by the supplier. Sample 2.0 served as a control, showing a DE of 20. As noted in Table 18, Samples 2.1 and 2.4 showed DE lower than the control sample, with values 18 and 17, respectively. Information on the DE of Samples 2.2 and 2.3 was not available. It may be expected that Sample's 2.2 DE would be lower than 20, due to the

carbohydrate's unmodified form and high fibre containment. However, further information would be needed for the exact value to be obtained.

Table 18 Samples' dextrose equivalent (DE).

Sample	*Dextrose equivalent*
2.0	20
2.1	18
2.2	n/a
2.3	n/a
2.4	17

5.2.10 **PDCAAS**

As seen in Table 19, the PDCAAS of all samples except Sample 2.2 showed a value of 1. Sample 2.2 did not meet the PDCAAS requirement of at least 0.9, having exhibited a value of 0.85. This was due to the carbohydrate's high contribution to the protein content, which lowered the quality of the amino acid profile.

Table 19 PDCAAS value of the samples.

Sample	*PDCAAS*
2.0	1
2.1	1
2.2	0.85
2.3	1
2.4	1

5.2.11 **Conclusions**

All samples showed higher particle size compared to most of the samples of Trial 1. Such results may be expected to have occurred from Cereal 3. Sample 2.2 showed significantly higher viscosity and particle size compared to the rest of the samples, which was most likely due to the carbohydrate's wholegrain nature thus high insoluble fibres containment. Sample 2.3 showed also high viscosity, possibly due to its high fibre containment.

Visually, all samples showed sedimentation, with Samples 2.0 and 2.1 to had shown the lowest and Sample 2.2 to had shown the highest. Despite that, the latter exhibited the most acceptable organoleptic results from the two panellists, due to its increased creaminess and cereal flavour. Nevertheless, due to the poor physicochemical

properties of Sample 2.2, low PDCAAS (0.85), unknown DE, difficult commercial availability, and containment of flavour whose labelling requirements would needed to be examined, it was concluded that the sample may not fulfil the requirements for upscaling at this stage of the project.

Sample 2.1 containing a Liquid cereal carbohydrate exhibited the lowest sedimentation along with the control sample and had shown decreased aggregation under the microscope, possibly due to the carbohydrate's liquid nature. The sample was therefore selected for further examination. Despite that, considerations on the ingredient's labelling should be taken, as the carbohydrate received controversial comments by the consumers.

Sample 2.4 showed somewhat increased aggregation compared to the rest of the samples. Despite that, the rest of the results were considered as acceptable for the sample's further examination. Additionally, a coherent origin of the ingredients was considered as beneficial in terms of labelling and consumer acceptance. Similar to the above sample, considerations on the sample's labelling should be taken, as concerns may arise from the consumers due to the unfamiliarity of the ingredient's form.

Sample 2.3 showed phase separation thus its properties were not properly evaluated. The sample was not selected for further examination as the above two selected carbohydrates were assessed as more relevant for the overall recipe formulation, in contrast to the carbohydrate used in Sample 2.3. In addition, the DE of the sample was unknown and its sensorial analysis showed some undesirable attributes, which added another drawback.

5.3 Trial 3

Samples 2.0 and 2.5 were produced at a separate batch than Sample 3.0. Analyses of Sample 3.0 after homogenization and heat treatment were performed the same day as the trial, while analyses of the other two samples were done the same day and the day after the trial, for the treatments after homogenization and after heat treatment, respectively.

5.3.1 **Visual assessment**

The samples as seen in Figure 8 were visually assessed after homogenization, after heat treatment (day 0), and after four weeks of storage at 25, and 37 °C. After homogenization, all samples showed insoluble particles, which was improved after heat treatment, due to the additional homogenization step that occurs during heat treatment. After storage for four and twelve weeks, sedimentation of approximately 3 mm in Sample 2.0, and 1 mm in Samples 2.5 and 3.0 was observed.

Figure 7 Visual assessment of the samples. Samples from left to the right; 2.0, 2.5 and 3.0.

5.3.2 **Organoleptic properties**

A sensorial description of the samples was performed on the samples' production day by three non-trained panellists. Further examination on Samples 2.0 and 2.5 was not done due to the samples' spoilage due to error occurred at the pilot plant. The samples exhibited some similar organoleptic attributes, as described in Table 20, including neutral, watery, creamy, and nutty.

Table 20 Organoleptic attributes of the samples.

Sample	*Appearance*	*Smell*	*Flavour*	*Mouthfeel and afterfeel*
2.0	Whitest	Beany Pea Oat	Watery Oily Creamy Neutral	Astringent
2.5	-	Walnut Neutral	Nutty	Astringent Creamy Watery
3.0	-	Cereal/oat	Milky Nutty Hay/grain	Astringent Herbal Slightly bitter Slightly oxidized

Overall, the samples did not perceive a negative acceptance by the panellists, while the described attributes were considered of low intensity, indicating that the addition of different lipids did not significantly affect the organoleptic properties of the samples.

5.3.3 **Heat stability**

As noted in Table 21, all samples showed no gelling after ten minutes of heating. Samples 2.5 showed the earliest aggregation after 1.5 minutes, compared to Samples 2.0 and 3.0 which showed aggregation at 4 and 4.5 minutes, respectively.

Table 21 Aggregation and gelling points (in minutes) of the samples during heat stability test.

Sample	*Aggregation point*	*Gelling point*
2.0	4	> 10
2.5	1.5	> 10
3.0	4.5	> 10

As expected from the samples' low protein and total solid content, no gelation occurred, indicating that the samples may be sufficiently processed in a factory-scale.

5.3.4 **pH**

As observed in Table 22, all samples showed reduction in pH over time, by maximum 0.5. All samples maintained values above 7 throughout a four-week storage.

Table 22 pH of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C.

	Sample		
Treatment	***2.0***	***2.5***	***3.0***
After homogenization	7,41	7,52	7,52
After heat treatment	7,35	7,38	7,43
After four weeks at 25 °C	7,28	7,13	7,37
After four weeks at 37 °C	7,01	7,03	7,26

In an internal study conducted on a similar product, the pH of the product displayed values between 7.1 and 7.3 after a 4 weeks storage, while the samples' pH was reduced to 6.9-7.2 after a 12 weeks storage. The values shown after four weeks of storage may be considered alike to the current study's results. As a 12-week storage at 37 °C indicates a year of shelf-life, it is recommended that the samples are examined after 12 weeks, for an indication of the products' stability throughout their shelf-life to be obtained.

5.3.5 **Calcium ion activity**

As seen in Table 23, calcium ion activity of all samples was decreased after heat treatment. the values of Samples 2.0 and 2.5 after homogenization may have shown inaccuracy due to the equipment's sensitivity.

Samples 2.0 and 2.5 after heat treatment showed values of 33 and 37 ppm, respectively. Sample 3.0 showed a lower value of 8 ppm. As the latter was examined on a different day, differences in values may have been occurred due to the sensitivity and inaccuracy of the equipment.

Over time, the samples' calcium ion activity was decreased by a maximum of 14 ppm in Samples 2.0 and 2.5, and increased by 14 ppm in Sample 3.0. Such deviations were not considered impactful as calcium ion activity was considered to be in moderately low levels.

In a previous internal study on a similar product, calcium ion activity exhibited values between 23 and 36 ppm throughout the sample's storage for four weeks. In the current study, under the same treatments all samples displayed values between

8 and 37 ppm, indicating similar results to the aforementioned study. However, it should to be noted that the form of calcium of the current study differed from the compared study, which may influence the results.

Table 23 Calcium ion activity of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C. Values in ppm.

	Sample		
Treatment	***2.0***	***2.5***	***3.0***
After homogenization	41,27	59,98	8,67
After heat treatment	33,56	37,29	7,72
After four weeks at 25 °C	25,26	29,24	21,88
After four weeks at 37 °C	19,30	27,01	17,98

Overall, the calcium activity values shown in the samples indicated no risk of increased aggregation due to calcium's binding with proteins. Nevertheless, for an indication on the calcium ion activity and its impact on the products' stability throughout a one-year shelf-life to be obtained, it is suggested that the samples are examined after 12 weeks of storage at 37 °C.

5.3.6 **Viscosity**

As it can be seen in Tables 24 and 25, the samples showed alike viscosity under the two rotation treatments. Viscosity of the samples was reduced after heat treatment to values close to 2 (±0.1). No great changes were observed in the samples' viscosity over a four-weeks storage period, as the viscosity of all samples remained stable or showed a minor increase of up to 0.2 mPa-s. As a result, there was an indication that increased aggregation did not occur.

Table 24 Average viscosity of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C, under 100s-1 rotation speed. Values in mPa-s.

	Sample		
Treatment	***2.0***	***2.5***	***3.0***
After homogenization	3,82	3,49	2,42
After heat treatment	2,13	2,01	1,93
After four weeks at 25 °C	2,20	2,16	1,84
After four weeks at 37 °C	2,22	2,20	1,80

Table 25 Average viscosity of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 °C and 37 °C, under 1000s-1 rotation speed. Values in mPa-s.

	Sample		
Treatment	***2.0***	***2.5***	***3.0***
After homogenization	3,30	3,07	2,30
After heat treatment	2,11	2,01	1,94
After four weeks at 25 °C	2,17	2,13	1,83
After four weeks at 37 °C	2,18	2,16	1,80

In a previous internal study on a similar product, the viscosity of the sample post heat treatment was 2.6, while over a 12-week time the viscosity showed a minor rise of 0.2 mPa-s. Despite that the values of the study were alike to the current study's, it should be noted that the former contained ingredients which may have influenced its viscosity. It is therefore recommended that the viscosity of the current's study samples is re-evaluated upon the addition of such additional ingredients. Nevertheless, based on the current results the viscosity of the samples is not expected to significantly rise throughout their shelf-life, therefore indicating that no coalescence or aggregation may be occurred.

5.3.7 **Particle size distribution**

As seen in Figure 8, the particle size was decreased in all samples after heat treatment. The three samples showed similar values, ranging from D [0.9] 6 to 8 μm, with Sample 2.5 showing the smallest size, and Sample 3.0 the largest. Similarity of particle size between the samples was expected as oils are less likely to contribute to particle size's increase. Such values are in line with a rice protein formula examined by Alonso-Miravalles et al. (2021), while the same study's lentil, and soy proteins formulae had shown higher values, of 35 and 14 μm, respectively.

Over storage for four weeks at 25 °C, samples maintained similar values as Samples 2.0 and 2.5 showed D [0.9] of 6 μm, while Sample 3.0 had 8.5 μm. No significant difference was observed in the particle size of the samples when stored for four weeks at 37 °C, as all samples sustained values of D [0.9] 7, 6, and 8.5 μm for Samples 2.0, 2.5, and 3.0, respectively. As also observed in Figure 8, samples maintained similar peaks between them after heat treatment, and over storage at the two temperatures.

A previous internal study on a similar product showed values alike the current study's. The particle size of the sample after heat treatment in the former study exhibited a value of 7.4 μm, which showed no great change (-0.3 μm) over a 12-weeks storage period.

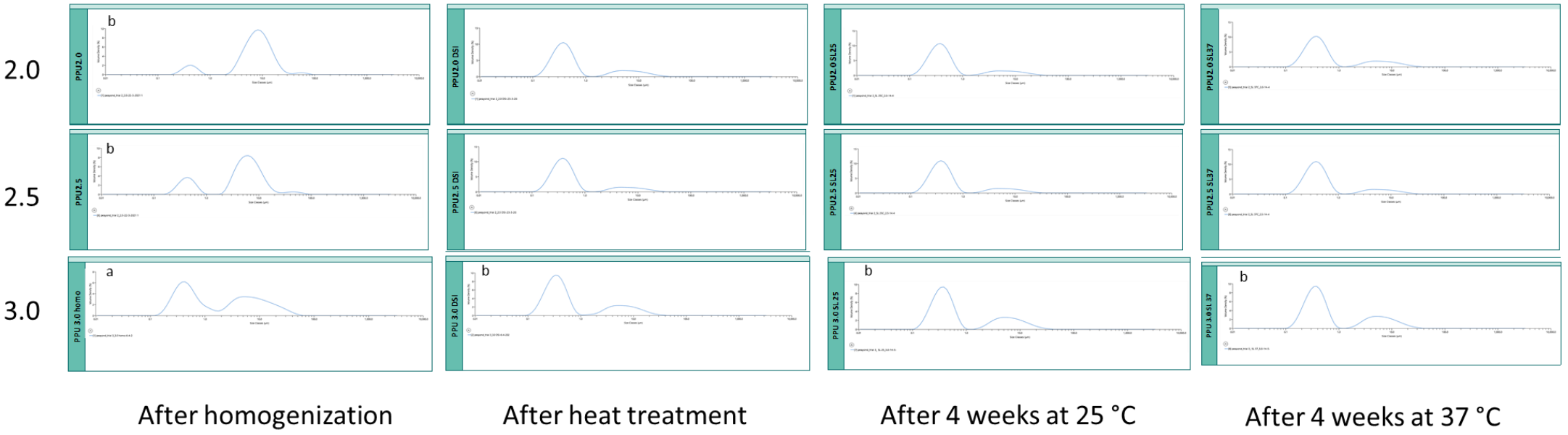

Figure 8 Particle size distribution of the samples after homogenization, after heat treatment, and after four weeks of storage at 25 and 37 °C. X axis represents size classes (µm); 0.01, 0.1, 1, 10, 100, 1000, 10000. Y axis represents volume density (%); 0, 5, 10, 15.

[a] volume density (%); 0, 2, 4, 6, 8

[b] volume density (%); 0, 2, 4, 6, 8, 10

5.3.8 **Optical microstructure**

As it may be seen in Figure 9, all samples showed decreased protein aggregation after heat treatment, with the majority of the aggregates being at approximately 5 μm. Over storage at 25 °C, in all samples the size of some aggregates was increased to around 10 μm. Further increase by approximately 5 μm was observed in some of the aggregates' size in Sample 2.0 and 3.0 when stored at 37 °C for a period of four weeks. Sample 2.5 maintained similar size of aggregates. Overall, the samples showed higher homogeneity compared to the previous trials of this study.

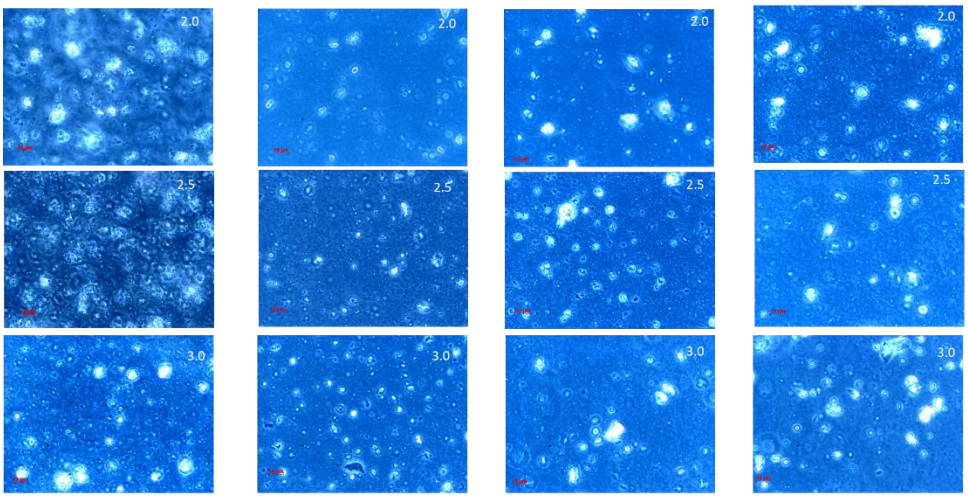

Figure 9 Optical microstructure of samples after homogenization, after heat treatment, after 4 weeks at 25°C, and after 4 weeks at 37°C. Scale indicates 10 μm.

5.3.9 **Conclusions**

The samples showed similar physicochemical and sensorial results. As additionally all three oils were positively perceived by the consumers, it was concluded that all oils may be used for further examination.

It should be noted for later stages that Nut oil may result to increased product cost due to its higher price compared to other oils. Additionally, allergenicity in relation to the raw material should be taken into consideration as it may receive negative consumer perception.

Lastly, as Seed oil 2 is highly prone to oxidation, handling in nitrogen-controlled atmosphere is suggested.

5.4 Trial 4

After the selection of the different raw materials; proteins, carbohydrates, and lipids, a brainstorming session took place for aligning the aforementioned raw materials with the different identified concepts. In addition to that, different flavours were matched with each concept. After that, Trial 4 was organized in a form of workshop at the company's Design Kitchen, during which a number of 13 flavours were infused in eight protein blends and were assessed for their sensorial attributes.

5.4.1 **Organoleptic properties**

A descriptive organoleptic analysis was conducted on the samples with seven non-trained panellists, all members of the project's team. The table with the identified attributes of each sample was not presented due to confidentiality reasons.

In terms of acceptance, all samples except 4.1 and 4.6 received a positive feedback and were selected for further examination. Samples 4.1 and 4.6 were not selected due to the undesirable attributes identified by at least two panellists.

5.4.2 **Conclusions**

Overall, as expected the addition flavours assisted in increased viscosity and creaminess, contributing therefore to the improvement of the watery attributes which were identified in previous trials. Also, the addition of flavours influenced positively the overall flavour of the samples, by masking undesired attributes observed in the samples of the previous trials. Nevertheless, it should be noted that flavours were added using a kitchen mixer, which may have influenced differently the viscosity, as more air was incorporated.

For enabling the inclusion of such flavours in the products further adjustments needed to be taken into account; reduction of flavours' quantities for nutritional compliance to be ensured as some of them contributed to increase of sugar or carbohydrate content, or their carbohydrate carrier had DE higher than 20 which is considered as added sugar. Additionally, exclusion of some flavours was needed due to unsuitability because of contaminants' presence or compounds of concern when used above certain quantities for YCF. Quantity reduction of some flavours was also necessary for safety reasons. Lastly, commercial availability was another consideration for ensuring the flavours' adequate supply upon the products' scale-up. Following the above considerations, 11 flavours were selected, out of which some replaced previously evaluated flavours, due to quality and safety considerations.

5.5 Packaging and labelling

5.5.1 **Ingredients' perception and labelling ideation workshop**

Initially, the consumers were asked to link foods and drinks they believe that aid to products properties. Furthermore, a number of raw materials were presented to the consumers for being assessed in terms of perception. The most well-perceived raw materials included seeds, cereals, legumes, and pseudocereals. The participants were then asked to draw a packaging which would include any of the previously presented ingredients or additional ones which they wish to have in a liquid PB YCF. The most common ingredients mentioned included Seed 2, Seed 1, Legume 4, and Legume 2. Additionally, consumers considered the addition of fruits and vegetables in the products, with the most common being Flavours A, B, and C, as these raw materials were believed to contribute to fruity and sweet taste or to the addition of vitamins and minerals.

Additional information desired by the consumers based on their drawings included locally-sourced raw materials as an aim to reduced carbon footprint, and raw materials derived from organic sources. Moreover, information was obtained in terms of packaging, as consumers expressed their interest in seeing recyclability information on the packaging, different packaging sizes; large for being stored in the fridge and being shared with the family as a PB milk, and small to have on the go and be consumed in a short period of time. Packaging materials showed in the drawings included pouches, cartons, and glass.

5.5.2 **Concepts and labelling perception study**

Based on the results, Concepts 1 and 2 received the highest acceptance. The former gained the highest acceptance as it was considered the most innovative idea, due to its potential availability in different flavours, and because it was found to fit in different feeding moments within a day. Concept 1, which also found a fit in different moments of a day, showed good potential in replacing cow's milk due to its improved nutritional composition and so-believed easier digestion. As previously expressed by the consumers, protein, vitamin D, and calcium are the nutrients that cow's milk is believed to be most beneficial. Therefore, labelling communication in addition to health claims, may further convince consumers who show preference in cow's milk.

In terms of labelling, the consumers expressed their concern when seeing oils as a major ingredient in the list, as they were unaware of their functionality and their effect in taste. As a result, the reason of the ingredients' presence may need to be communicated through the label. Some labelling was perceived negatively, despite

that others were well-perceived. Thus, communication on these ingredients may also need to be considered.

The addition of vitamins and minerals was well-accepted when the nutrients were written in different ways, more or less simple and extensive. In some wordings, the consumers believed that micronutrients were not present in the product. Labelling of ingredients is thus a key factor for conveying a message to consumers. Grouping of ingredients may also be considered, as it may provide convenience and better understanding when reading.

In relation to Liquid cereal carbohydrate, the ingredient was associated with sugar. Examination on the ingredient's labelling in different forms is suggested for preventing its association with sugar.

Communication on the functionality of other ingredients may additionally be needed due to unfamiliarity of the ingredients, and concerns whether the ingredients contribute to added sugar.

Moreover, consumers expected in some cases to see higher quantity of flavour-providing ingredients in the product. Communication may therefore be needed for providing knowledge that the ingredients are added in quantities that are considered safe and in nutritional compliance for the specific age group.

In terms of sustainability, Cereal 2 was considered as not a local source compared to other sources, which may indicate that the ingredient may need to have a lower focus in the front label, whilst focusing on local ingredients' communication may be beneficial. From the packaging aspect, all consumers expressed their desire in seeing more information on the sustainability of the product and packaging.

5.5.3 **Benchmark analysis**

A benchmark analysis on ten existing products of the same age category was performed. The analysis was not presented due to confidentiality reasons. The most common protein sources found in the products included soy, rice, and pea. All products examined were produced or launched in non-EU countries, including USA, Canada, Malaysia, Taiwan, and Australia, where legislations or the companies' approach are shown to be less strict than those of the EU and the industrial partner of this study. This is explained as in all cases the nutritional composition and the caloric content per serving of the identified products was poorer than the current study's products, while the front label included health claims which are not in compliance with the EU regulation on health claims (The European Commission, 2012). Additionally, ingredients including stabilizers, sweeteners, and salt were

found in some products, which may be considered unsuitable to this study's approach.

From all identified products, only one product was found in a liquid form (Ripple Foods, 2021). The specific product, although intended to kids, did not include an age category thus it may not be directly compared to YCF. All the other products were found in a powder form.

Various ingredients contained in the examined products were examined or were shown interest in this study, confirming therefore consumers' acceptance on such ingredients. Thus, it may be suggested that ingredients which gained interest in this study but were not examined due to availability, to be further examined in the future. Additionally, it may be recommended that probiotics, which were included in some of the products of the benchmark analysis in this study, are examined in the future as in addition to their health benefits, research has shown that probiotic supplementation may aid in better absorption of plant proteins (Jäger et al., 2020).

Further observations on similar products included the communication on the usage of specific raw materials, certain processing methods, the functionality of the ingredients used for preventing their negative perception, and the origin of the ingredients. It is therefore suggested that such observations may be useful to be examined for their inclusion in the current study's products in the future.

Lastly, the analysis' results demonstrated that most of the products were launched by start-up companies, which gives a further consideration to be examined on whether launching as a well-trusted brand or an ambitious new brand is preferred.

5.5.4 **Packaging and labelling design workshop**

Ideas from the workshop on packaging and labelling included story-telling and playful experiences which served as an incentive for the child to consume the product. Some ideas which can align with the developed concepts can be gained for their usage in the products' packaging and labelling for creating innovative experiences with the consumer.

5.6 Product concepts

The initial product concepts were formulated based on information gained by the consumer study. A number of six different product concepts were formulated, based on the different desired benefits that were identified during the consumer perception research. Each of the concepts differed in the benefits they provide.

Based on the physicochemical and sensorial results of trials 1-3, by following the additional insights obtained from the consumer perception study, and with further considerations on each raw material as described previously, a brainstorming session with four team members took place for linking the selected raw materials with the identified concepts.

Following from Trial 4, a second brainstorming session was organized with seven members of the project, in which the product concepts were reformulated. Considerations for the concepts' reformulation were taken based on consumer insights on the concepts with the highest acceptance and the most desired product attributes related to YCF, as explained in Chapter 5.5.2. Subsequently, further information on flavours as mentioned in the previous section was taken into consideration for the recipes' reformulation.

Based on the above, the flavours' composition and quantities in the samples was re-evaluated. As demonstrated in Table 26, the product concepts were finetuned, and were eventually merged into two categories, each of them including a product range. Each product concept's associated ingredients are shown in the table. The two main categories differed in their fitting during the different feeding moments within a day, while their product range targeted different benefits.

Table 26 Product concepts and their composition.

Product concept	***Protein***	***Carbohydrate***	***Lipid***	***Flavours***
Concept 1 - A	Legume 1, Cereal 3	Liquid Cereal carbohydrate	Nut oil	-
Concept 1 - B	Legume 1, Cereal 3	Liquid Cereal carbohydrate	Nut oil	Flavour 1 Flavour 2 Flavour 3
Concept 2 - A	Legume 1, Seeds 1, Cereal 2	Legume carbohydrate	Seed 2 oil	Flavour 4 Flavour 5 Flavour 3
Concept 2 - B	Legume 1, Seeds 1, Cereal 2	Legume carbohydrate	Seed 1 oil	Flavour 6 Flavour 7 Flavour 8
Concept 2 - C	Legume 1, Seeds 1, Cereal 2	Legume carbohydrate	Seed 1 oil	Flavour 9 Flavour 10 Flavour 11 Flavour 2

5.7 Suggestions for packaging and labelling solutions

Combining the information obtained from the different project phases, along with factory-scale considerations, packaging and front label suggestions were developed for the two product categories; Concept 1 and Concept 2.

Labels were designed with a consumer-friendly way, for easier communication and understanding of the ingredients and the claims, as seen in Figure 10.

Concept 2 was designed for a Tetra Prisma® Aseptic 500 Edge laminated with carton, low-density polypropylene (LDPE), aluminium, and a high-density polyethylene (HDPE) cap, while Concept 1 was designed for a laminated pouch made of Polyethylene terephthalate (PET), aluminium, polypropylene (PP) or LDPE, and a HDPE cap (Aluflexpack Group, 2021; Tetra Pak, 2021). Both products are sterilized thus the aforementioned packaging materials are considered suitable for ensuring maintenance of quality throughout the products' shelf-life. Recyclability of laminated packaging materials may currently be reduced, however, partnership with organizations such as TerraCycle or Gualapack, as currently done by other companies, may improve the packaging's recyclability by shifting to mono-materials with improved technologies, or by the packaging's usage for the production of non-packaging plastic products (Gualapack, 2019, 2021; TerraCycle, 2019).

Figure 10 Packaging and label of Concept 1 (left) and Concept 2 (right).

Further packaging and labelling considerations should be included when designing the back label, like suggestions on the products' fit during the different feeding moments within a day, or extra product features determined during the consumer study. Functional information may also be useful to be included, like providing guidance for optimal use of the product. Moreover, overall adjustments may need to be applied, for instance by mentioning only nutritional and health claims associated to children's needs, and the change of words normally used for dairy products, due to legal reasons.

6 Conclusions

This study aimed to the rapid-prototyping development of liquid plant-based young child formulae at a pilot scale, by examining various macronutrients and ingredients, including proteins, carbohydrates, lipids, and flavours. Seven protein blends; Cereal 1, Cereal 2, Legume 1, Legume 2, Seed 1, Pseudocereal 1, Legume 3, Legume 4, Cereal 3, Seed 2, and Legume 5, five carbohydrates; Control carbohydrate, Whole cereal carbohydrate, Liquid cereal carbohydrate, Legume carbohydrate, and Root carbohydrate, and three lipids; Seed oil 1, Nut oil, Seed oil 2 were developed, with a complete macronutrient content and key minerals.

The samples were examined for their sensorial, functional, and physicochemical properties, and nutritional compliance. Based on the aforementioned results, along with the consumer perception study's results, and the ingredients' commercial availability considerations, the macronutrients with the most promising results were determined; Legume 1, Seed 2, and Cereal 2 protein blend, Legume 1 and Cereal 3 protein blend, Liquid cereal carbohydrate, Legume carbohydrate, Seed oil 1, Seed oil 2, and Nut oil.

Each selected macronutrient was aligned with a number of product concepts identified during the consumer perception study. A number of 11 flavours was selected based on their sensorial properties when infused in protein blends, their nutritional and quality compliance, and their suitability to each of the identified product concepts.

Lastly, based on information obtained through workshops, draft packaging and labelling suggestions were developed for the product concepts. All elements collected during this study assisted in finetuning the final products propositions for the next steps of the project.

7 Recommendations for further steps

Further steps of this project should include a pilot scale development of the recipes as designed for each product concept, by combining all the selected raw materials in the respective matrices. This trial would be essential for examining the behaviour of the ingredients when combined. Additionally, flavours' colour and taste change over processing, and overall behaviour in the matrix would be determined upon this trial. Moreover, it is suggested that composition with complete nutritional compliance should be examined in this trial, by including additional ingredients as part of a complete product composition. In this way, the products' final composition can be adjusted and optimized, for the products to proceed to factory-scale development.

Improvements of the samples' physicochemical attributes like sedimentation may be examined by changes in processing settings or by incorporation of additives. In later steps of the project, it may be suggested that different suppliers for the same raw materials are examined, as ingredients with better physicochemical properties may be identified if needed.

Scaling-up considerations including quality and safety of raw materials such as baby food-grade compliance, safety dosage of flavours, and safe contaminants levels should be examined, as well as whether additional micronutrient supplementation is needed to compensate the eventual presence of antinutrients in the raw materials.

Nutritional and health claims as stated in the EU regulations 1924/2006 and 432/2012, respectively, are suggested to be adapted to those intended to children, due to the company's approach of following the follow-on formulae guidelines (European Parliament, 2006; The European Commission, 2012). As consumers were particularly interested in specific benefits, it is suggested that ingredients associated with these benefits can be included in the product.

In addition, consumers found that some micronutrients are lacking in the children's diet, while others are believed to be beneficial for cow's milk. Therefore, communication on ingredients associated with these nutrients or health claims may be important to be applied for the product to gain competitive advantage.

Exploration of different raw materials and processes is suggested, as consumers showed interest in specific raw materials or processing methods. Such raw materials may additionally provide a nutritional benefit to the product. In addition to that, addition of probiotics may be recommended to be examined, as studies have shown that such supplementation may aid in better absorption of plant proteins (Jäger et

al., 2020). Moreover, consumers expressed their preference in seeing raw materials which undergo processes that maintain the ingredients' beneficial compounds. Due to consumers' preference in seeing natural ingredients, sourcing of such ingredients with improved physicochemical characteristics may be beneficial. Considerations on usage of organic ingredients may additionally be taken into account, due to consumers' preference.

In terms of packaging and labelling, based on the results shown by the benchmark analysis and the primary consumer study, communication of the ingredients' functionality in the product may be considered essential, as otherwise consumer may develop negative perception on certain raw materials.

Lastly, sustainability aspects should also be taken into consideration as they have been shown to be highly valued by the consumers. Such considerations may include the usage of raw materials from specific origins, and sustainable harvesting or manufacturing methods. These aspects could also be communicated with the consumers through the label.

References

Alonso-Miravalles, L., Barone, G., Waldron, D., Bez, J., Joehnke, M. S., Petersen, I. L., Zannini, E., Arendt, E. K., & O'Mahony, J. A. (2021). Formulation, pilot-scale preparation, physicochemical characterization and digestibility of a lentil protein-based model infant formula powder. *Journal of the Science of Food and Agriculture*, *March*. https://doi.org/10.1002/jsfa.11199

Aluflexpack Group. (2021). *Pouches*. https://www.aluflexpack.com/product/pouches/

Badger, T. M., Gilchrist, J. M., Pivik, R. T., Andres, A., Shankar, K., Chen, J. R., & Ronis, M. J. (2009). The health implications of soy infant formula. *American Journal of Clinical Nutrition*, *89*(5), 1668–1672. https://doi.org/10.3945/ajcn.2009.26736U

Barać, M. B., Pešić, M. B., Stanojević, S. P., Kostić, A. Z., & Čabrilo, S. B. (2015). Techno-functional properties of pea (Pisum sativum) protein isolates-a review. *Acta Periodica Technologica*, *46*(November), 1–18. https://doi.org/10.2298/APT1546001B

Bhatia, J., & Greer, F. (2008). Use of soy protein-based formulas in infant feeding.

Pediatrics, *121*(5), 1062–1068. https://doi.org/10.1542/peds.2008-0564

Bogahawaththa, D., Hoang, N., Chau, B., Trivedi, J., & Dissanayake, M. (2019). LWT - Food Science and Technology Impact of selected process parameters on solubility and heat stability of pea protein isolate. *LWT - Food Science and Technology*, *102*(October 2018), 246–253. https://doi.org/10.1016/j.lwt.2018.12.034

Bos, S. (2019). *The effectiveness of sustainability labels in promoting sustainable food choices*. *July*. https://edepot.wur.nl/497077

Boye, J., Wijesinha-Bettoni, R., & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition*, *108*(SUPPL. 2). https://doi.org/10.1017/S0007114512002309

Boz, Z., Korhonen, V., & Sand, C. K. (2020). *Consumer Considerations for the Implementation of Sustainable Packaging : A Review*. 1–34.

Canadian Paediatric Society. (2009). *Concerns for the use of soy-based formulas in infant nutrition*. *14*(2), 109–113. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2661347/pdf/pch14109.pdf

Chan, E. S., Abrams, E. M., Hildebrand, K. J., & Watson, W. (2018). Early introduction of foods to prevent food allergy. *Allergy, Asthma & Clinical Immunology*, *14*(s2), 1–9. https://doi.org/10.1186/s13223-018-0286-1

Cherry, E. (2006). Veganism as a Cultural Movement: A Relational Approach. *Social Movement Studies*, *5*(2), 155–170. https://doi.org/10.1080/14742830600807543

Chouraqui, J.-P., Turck, D., Tavoularis, G., Ferry, C., & Dupont, C. (2019). *The Role of Young Child Formula in Ensuring a Balanced Diet in Young Children (1–3 Years Old)*. 1–17.

Chouraqui, J. P., Tavoularis, G., Simeoni, U., Ferry, C., & Turck, D. (2020). Food, water, energy, and macronutrient intake of non-breastfed infants and young children (0–3 years). *European Journal of Nutrition*, *59*(1), 67–80. https://doi.org/10.1007/s00394-018-1883-y

Codex Alimentarius. (2007). Standard for infant formula and formulas for special medical purposes intended for infants. *Codex Stan*, *72–1981*, 1–21. http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:STANDARD+FOR+INFANT+FORMULA+AND+FORMULAS+FOR+SPECIAL+MEDICAL+PURPOSES+INTENDED+FOR+INFANTS#1%5Cnhttp://www.codexalimentarius.org/standards/en/

Cooke, L. J., Wardle, J., Gibson, E., Sapochnik, M., Sheiham, A., & Lawson, M. (2004). Demographic, familial and trait predictors of fruit and vegetable consumption by pre-school children. *Public Health Nutrition*, *7*(2), 295–302.

https://doi.org/10.1079/phn2003527

Cui, Z., Chen, Y., Kong, X., Zhang, C., & Hua, Y. (2014). Emulsifying properties and oil/water (O/W) interface adsorption behavior of heated soy proteins: Effects of heating concentration, homogenizer rotating speed, and salt addition level. *Journal of Agricultural and Food Chemistry*, *62*(7), 1634–1642. https://doi.org/10.1021/jf404464z

Drulyte, D., & Orlien, V. (2019). *The Effect of Processing on Digestion of Legume Proteins*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6616939/

EFSA. (2013). Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. *EFSA Journal*, *11*(10), 1–103. https://doi.org/10.2903/j.efsa.2013.3408

Elverum, C. W., Welo, T., & Tronvoll, S. (2016). Prototyping in new product development : Strategy considerations. *Procedia CIRP*, *50*, 117–122. https://doi.org/10.1016/j.procir.2016.05.010

European Bioplastics. (2018). *Bioplastics Facts and figures*.

European Comission. (2006). Commission Directive 2006/141/EC of 22 December on infant formulae and follow-on formulae and amending Directive 1999/21/EC. *Official Journal of the European Union*, *L 401*, 1–33. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:401:0001:0033:EN:PDF

European Commission. (2011). REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, an. *Official Journal of the European Union*, *1169*, 18–63. https://doi.org/2004R0726 - v.7 of 05.06.2013

European Parliament. (2006). REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL. *Official Journal of the European Union*, *3*(1924), 1–30. http://www.fsai.ie/uploadedFiles/Consol_Reg1924_2006.pdf

Eurostat. (2020a). *Agricultural production - livestock and meat*. https://ec.europa.eu/research/foresight/index.cfm

Eurostat. (2020b). *Packaging waste generated, recovered and recycled, EU-27, 2008-2018*. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Packaging_waste_statistics

Eussen, S. R. B. M., Pean, J., Olivier, L., Delaere, F., & Lluch, A. (2015). Theoretical Impact of Replacing Whole Cow's Milk by Young-Child Formula on Nutrient Intakes of UK Young Children: Results of a Simulation Study. *Annals of Nutrition and Metabolism*, *67*(4), 247–256.

https://doi.org/10.1159/000440682

Fahey, G. C. (2014). *Viscosity as Related to Dietary Fiber : A Review Critical Reviews in Food Science and Nutrition Viscosity as Related to Dietary Fiber : A Review*. *February 2006*. https://doi.org/10.1080/10408390500511862

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, uses, and fate of all plastics ever made. *Science Advances*, *3*(7), 5. https://doi.org/10.1126/sciadv.1700782

Gluckman, P. D., & Hanson, M. A. (2004). Living with the past: Evolution, development, and patterns of disease. *Science*, *305*(5691), 1733–1736. https://doi.org/10.1126/science.1095292

Godfrey, K. M., Lillycrop, K. A., Burdge, G. C., Gluckman, P. D., & Hanson, M. A. (2007). Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatric Research*, *61*(5 PART 2 SUPPL.), 31–36. https://doi.org/10.1203/pdr.0b013e318045bedb

Gorissen, S. H. M., Crombag, J. J. R., Senden, J. M. G., Waterval, W. A. H., & Bierau, J. (2018). Protein content and amino acid composition of commercially available plant - based protein isolates. *Amino Acids*, *50*(12), 1685–1695. https://doi.org/10.1007/s00726-018-2640-5

Gualapack. (2019). *Gualapack launches the first 100% monomaterial recyclable pouches*. https://www.gualapackgroup.com/gualapack-launches-the-first-100-monomaterial-recyclable-pouches/

Gualapack. (2021). *Kraft Heinz, with its brand Plasmon, launches Italy's first fully recyclable baby food pouches*. https://www.gualapackgroup.com/kraft-heinz-with-its-brand-plasmon-launches-italys-first-fully-recyclable-baby-food-pouches/

Hui, Y. H. (2005). *HANDBOOK OF FOOD SCIENCE, TECHNOLOGY, AND ENGINEERING*. https://www.academia.edu/10365690/LIVRO_Handbook_of_Food_science_technology_and_engineering_Part1

Jäger, R., Zaragoza, J., Purpura, M., Iametti, S., Marengo, M., Tinsley, G. M., Anzalone, A. J., Oliver, J. M., Fiore, W., Biffi, A., Urbina, S., & Taylor, L. (2020). Probiotic Administration Increases Amino Acid Absorption from Plant Protein: a Placebo-Controlled, Randomized, Double-Blind, Multicenter, Crossover Study. *Probiotics and Antimicrobial Proteins*, *12*(4), 1330–1339. https://doi.org/10.1007/s12602-020-09656-5

Jarpa-Parra, M. (2018). Lentil protein: a review of functional properties and food application. An overview of lentil protein functionality. *International Journal of Food Science and Technology*, *53*(4), 892–903. https://doi.org/10.1111/ijfs.13685

Jones, V. S., Drake, M. A., & Harding, R. (2007). Consumer Perception of Soy and Dairy Products : *Journal of Sensory Studies*, *23*(2008), 65–79.

Katz, Y., Gutierrez-Castrellon, P., González, M. G., Rivas, R., Lee, B. W., & Alarcon, P. (2014). A comprehensive review of sensitization and allergy to soy-based products. *Clinical Reviews in Allergy and Immunology*, *46*(3), 272–281. https://doi.org/10.1007/s12016-013-8404-9

Kramer, R. M., Shende, V. R., Motl, N., Pace, C. N., & Scholtz, J. M. (2012). Toward a molecular understanding of protein solubility: Increased negative surface charge correlates with increased solubility. *Biophysical Journal*, *102*(8), 1907–1915. https://doi.org/10.1016/j.bpj.2012.01.060

Le Roux, L., Mejean, S., Chacon, R., Lopez, C., Dupont, D., Deglaire, A., Nau, F., & Jeantet, R. (2020). Plant proteins partially replacing dairy proteins greatly influence infant formula functionalities. *Lwt*, *120*(November 2019), 108891. https://doi.org/10.1016/j.lwt.2019.108891

Lovell, A. L., Davies, P. S. W., Hill, R. J., Milne, T., Matsuyama, M., Jiang, Y., Chen, R. X., Wouldes, T. A., Heath, A. L. M., Grant, C. C., & Wall, C. R. (2018). Compared with Cow Milk, a Growing-Up Milk Increases Vitamin D and Iron Status in Healthy Children at 2 Years of Age: The Growing-Up Milk-Lite (GUMLi) Randomized Controlled Trial. *Journal of Nutrition*, *148*(10), 1570–1579. https://doi.org/10.1093/jn/nxy167

Mangels, A. R., & Messina, V. (2001). *Considerations in planning vegan diets: infants*.

Mangels, R., & Driggers, J. (2012). The Youngest Vegetarians: Vegetarian Infants and Toddlers. *ICAN: Infant, Child, & Adolescent Nutrition*, *4*(1), 8–20. https://doi.org/10.1177/1941406411428962

Maphosa, Y., & Jideani, V. A. (2018). *Factors Affecting the Stability of Emulsions Stabilised by Biopolymers*.

Marsh, K. A., Munn, E. A., & Baines, S. K. (2012). Protein and vegetarian diets. *Medical Journal of Australia*, *1*(June), 7–10. https://doi.org/10.5694/mjao11.11492

Messina, M., Erdman, J., & Setchell, K. D. R. (2004). Safety of Soy-Based Infant Formulas Containing Isoflavones:The Clinical Evidence. *Journal of Nutrition*, *134*(5), 1220–1224. https://doi.org/10.1093/jn/134.5.1205s

Navarrete, M. O. (2018). *Pre-treatment and digestion of plant proteins - The quinoa case*. https://doi.org/https://doi.org/10.18174/451326

Nehete, J., Bhambar, R., Narkhede, M., & Gawali, S. (2013). Natural proteins: Sources, isolation, characterization and applications. *Pharmacognosy Reviews*, *7*(14), 107–116. https://doi.org/10.4103/0973-7847.120508

NHS. (2018). *Prescribing Guidelines for Specialist Infant Formula Feeds in Cow ' s Milk Protein Allergy And Lactose Intolerance*. *April*. https://mm.wirral.nhs.uk/document_uploads/guidelines/WirralCMAguidelines-16-V3.0.pdf

NIH. (2010). *Guidelines for the Diagnosis and Management of Food Allergy in the United States: Summary of the NIAID-Sponsored Expert Panel Report*. https://doi.org/10.1038/jid.2014.371

Peng, W., Kong, X., Chen, Y., Zhang, C., Yang, Y., & Hua, Y. (2016). Effects of heat treatment on the emulsifying properties of pea proteins. *Food Hydrocolloids*, *52*, 301–310. https://doi.org/10.1016/j.foodhyd.2015.06.025

Ripple Foods. (2021). *Kids milk*. https://www.ripplefoods.com/kids/

Ritchie, H., & Roser, M. (2019). *Meat and Dairy Production*. https://ourworldindata.org/meat-production

Robertson, G. L. (2016). Packaging and food and beverage shelf life. *The Stability and Shelf Life of Food*, 77–106. https://doi.org/10.1016/B978-0-08-100435-7.00003-4

Rossen, L. M., Simon, A. E., & Herrick, K. A. (2016). Types of Infant Formulas Consumed in the United States. *Clinical Pediatrics*, *55*(3), 278–285. https://doi.org/10.1177/0009922815591881

Schyver, T., & Smith, C. (2005). Reported attitudes and beliefs toward soy food consumption of soy consumers versus nonconsumers in natural foods or mainstream grocery stores. *Journal of Nutrition Education and Behavior*, *37*(6), 292–299. https://doi.org/10.1016/S1499-4046(06)60159-0

Strom, B. L., Schinnar, R., Ziegler, E. E., Barnhart, K. T., Sammel, M. D., Macones, G. A., Stallings, V. A., Drulis, J. M., Nelson, S. E., Hanson, S. A., Strom, B. L., Barnhart, K. T., Sammel, M. D., Macones, G. A., Schinnar, R., Strom, B. L., Barnhart, K. T., Macones, G. A., Stallings, V. A., … Hanson, S. A. (2001). Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. *Journal of the American Medical Association*, *286*(7), 807–814. https://doi.org/10.1001/jama.286.7.807

TerraCycle. (2019). *Recycling in partnership with Gerber®*. https://www.terracycle.com/en-US/brigades/gerber

Testa, I., Salvatori, C., Di Cara, G., Latini, A., Frati, F., Troiani, S., Principi, N., & Esposito, S. (2018). Soy-Based Infant Formula: Are Phyto-Oestrogens Still in Doubt? *Frontiers in Nutrition*, *5*(November), 1–8. https://doi.org/10.3389/fnut.2018.00110

Tetra Pak. (2021). *Tetra Prisma® Aseptic 500 Edge*. https://productxplorer.tetrapak.com/packaging/package/tetra-prisma-aseptic-500-edge

The Business Research Company. (2021). *Vegan Food Global Market Report 2021: COVID 19 Growth And Change To 2030*. https://www.thebusinessresearchcompany.com/report/vegan-market-global-report-2020-30-covid-19-growth-and-change

The European Commission. (2012). Commission Regulation (EU) No 432/2012. *Official Journal of the European Union*, *13*(1924). http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0432&from=EN

The European Commission. (2016a). *COMMISSION DELEGATED REGULATION (EU) 2016/127*.

The European Commission. (2016b). *REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL on young-child formulae*.

Tilman, D., & Clark, M. (2014). Global diets link environmental sustainability and human health. *Nature*, *515*(7528), 518–522. https://doi.org/10.1038/nature13959

Tome, D., & Miller, G. D. (2000). Criteria and significance of dietary protein sources in humans. *Journal of Nutrition*, *130*(7), 1865–1867.

United Nations. (2019). *World Population Prospects 2019: Highlights*. https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html

Vandenplas, Y., De Ronne, N., Van De Sompel, A., Huysentruyt, K., Robert, M., Rigo, J., Scheers, I., Brasseur, D., & Goyens, P. (2014). A Belgian consensus-statement on growing-up milks for children 12–36 months old. *European Journal of Pediatrics*, *173*(10), 1365–1371. https://doi.org/10.1007/s00431-014-2321-7

Venlet, N. V., Hettinga, K. A., Schebesta, H., & Bernaz, N. (2021). *Perspective : A Legal and Nutritional Perspective on the Introduction of Quinoa-Based Infant and Follow-on Formula in the EU*. *7*, 1–8. https://academic.oup.com/advances/advance-article/doi/10.1093/advances/nmab041/6226904

Verger, E. O., Eussen, S., & Holmes, B. A. (2016). Evaluation of a nutrient-based diet quality index in UK young children and investigation into the diet quality of consumers of formula and infant foods. *Public Health Nutrition*, *19*(10), 1785–1794. https://doi.org/10.1017/S1368980015003134

Vickers, M. H., Breier, B. H., Cutfield, W. S., Hofman, P. L., & Gluckman, P. D. (2000). Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *American Journal of Physiology - Endocrinology and Metabolism*, *279*(1 42-1), 83–87.

https://doi.org/10.1152/ajpendo.2000.279.1.e83

Wandosell, G., Parra-meroño, M. C., & Alcayde, A. (2021). *Green Packaging from Consumer and Business Perspectives*. 1–19.

Westhoek, H. J., Rood, G. A., Berg, M. Van Den, & Janse, J. H. (2011). The Protein Puzzle : The Consumption and Production of Meat , Dairy and Fish in the European Union. In *European Journal of Food Research & Review* (Vol. 1, Issue 3). http://www.pbl.nl/sites/default/files/cms/publicaties/Protein_Puzzle_web_1.pdf.%5Cn%5Cnhttp://www.pbl.nl/sites/default/files/cms/publicaties/Protein_Puzzle_web_1.pdf.%5Cn;%5Cnfile:///Users/dtgrassian/Library/Application%5CnSupport/Mendeley%5CnDesktop/Down

WFP. (2020). *WFP shelf life study protocol – Processed food products*. *July*, 39–41. https://docs.wfp.org/api/documents/WFP-0000118387/download/

WHO. (2003). *Feeding and nutrition of infants and young children*. https://www.euro.who.int/__data/assets/pdf_file/0004/98302/WS_115_2000FE.pdf

WHO. (2009). Child Growth Standards. *WHO Library Cataloguing*.

WHO. (2018). *Guideline: counselling of women to improve breastfeeding practices*.

Wunsch, N.-G. (2020). *Number of vegans in Great Britain from 2014 to 2019*. https://www.statista.com/statistics/1062104/number-of-vegans-in-great-britain/

Zhang, J., Wang, D., & Zhang, Y. (2020). Patterns of the consumption of young children formula in chinese children aged 1–3 years and implications for nutrient intake. *Nutrients*, *12*(6), 1–10. https://doi.org/10.3390/nu12061672