2.2.1 Macronutrients

Macronutrients are proteins, fats, and carbohydrates, along with water and ash. Proteins, carbohydrates, and fats are nutrients that give the body energy. One gram of protein and carbohydrate give 4 kcal each, but one gram of fat gives 9 kcal (Insel, Ross, McMahon, & Bernstein, 2011). Bovine whole milk contains approximately 87.4% water, 3.4% protein, 4% fat, 4.7% carbohydrates, and 0.7% ash (Månsson, 2008). Milk is known to contain relatively high amount of saturated fatty acids (SFAs), but approximately 60% of milk fat is saturated (Haug, Høstmark, & Harstad, 2007).

2.2.1.1 Proteins

Proteins are organic compounds and serve multiple purposes in the human body. They are first and foremost building blocks for the body. Proteins are made from sequences of amino acids, and the body can choose from 20 different amino acids when building the sequences (Insel et al., 2016). Amino acid is a compound that consists of a central carbon atom, bonded to one hydrogen atom, one carboxylic group, one amino group, and one side group (R-group). They are generally divided into two groups: the essential amino acids, and the non-essential amino acids. The body can synthesize the non-essential amino acids, but it cannot make enough of the essential amino acids to maintain growth and nitrogen balance, so they must come from the diet (Insel et al., 2016; Watford, 2015).

The main sources of dietary proteins are foods of animal origin, such as fish, meat, eggs, and milk products. They contain high quality protein, i.e. proteins that provide amino acids in the right amount to satisfy the demands for protein synthesis measured by nitrogen balance. Protein is a major component in milk, and milk proteins can be divided into two major groups, i.e. casein complexes, and whey protein fractions. Milk with higher content of casein is usually referred to as “casein milk”, and is produced by ruminants. Milk with higher content of whey protein (as in lower casein/whey ratio) is usually referred to as “albumin milk”, and is produced by non-ruminants and humans. Bovine milk consists of about 80% of casein complexes, while human milk proteins consist of less than 50% casein complexes. The nutritional value of milk proteins depends mostly on the essential amino acids present. Human milk is considerably low in protein compared to bovine milk, and therefore also in total amino acids (both essential and non-essential) (Table 4). However, human milk contains more of free amino acids (FAA) than bovine milk (Table 4), and FAA are more easily absorbed than protein-bound amino acids, which makes the human milk a better nutritional source for infants than other milk (Chuang et al., 2005; Claeys et al., 2014; Zhang, Adelman, Rai, Boettcher, & Lőnnerdal, 2013).

Table 4. Amount of casein and whey proteins and amount of essential amino acids, non-essential amino acids and free amino acids in human and bovine milk (Claeys et al., 2014).

	Human	Bovine
Total casein (g/l)	2.4-4.2	24.6-28.0
Total whey (g/l)	6.2-8.3	5.5-7.0
Casein/whey ratio	0.4-0.5	4.7
Total essential amino acids (mg/100 g milk)	558	1380
Total non-essential amino acids (mg/100 g milk)	1854	4710
Free amino acids (µmol/l)	3020	578

Milk also has a large content of enzymes, but their biological function is less well known. The enzymes are either secreted by the mammary gland of the animals, or are of microbial origin. Some of these enzymes are also believed to have antimicrobial functions (Claeys et al., 2014), but they are destroyed during pasteurization (Fox & Kelly, 2006).

Certain tissues and organs, such as the skin, brain, heart and liver, rely on a constant supply of amino acids via the blood for the synthesis of new proteins. Protein is required for tissue replacement, deposition of lean body mass, and growth of infants during their rapid growth. The protein requirements are proportionally higher for infants than for older children and adults. For adults in general, 0.8 grams of protein per kg bodyweight per day (g/kg/day) should meet the protein requirements, compared to 1.1 g/kg/day for infants less than 12 months of age (Nordic Council, 2012). However, too high protein intake in infancy leads to faster growth (Thorsdottir et al., 2008), which has in turn been associated with higher BMI in 6 years old children. High consumption of bovine milk in the first year of life has been associated with overweight later in childhood (Hornell, Lagstrom, Lande, & Thorsdottir, 2013; Thorisdottir, Gunnarsdottir, Palsson, et al., 2014; Thorsdottir & Thorisdottir, 2011). Milk proteins increase the insulin flow as seen by the low glycaemic index of milk. A high protein intake, in general, seems to increase the insulin secretion, and the insulin growth factor 1 (IGF1) triggers multiplication and specialization among adipocyte precursors. This process has been suggested to contribute to overweight among children (Hoppe, Molgaard, Juul, & Michaelsen, 2004; Thorsdottir & Thorisdottir, 2011).

The protein proportion of the total energy intake has decreased since 1995-1997 but is still at the upper limit of the recommendations. Protein consumption in Iceland is in general rather high, and infants are no exception there. Infants get most of their protein from milk (Thorsdottir & Thorisdottir, 2011; Thorsdottir et al., 2008). However, a decrease in protein intake in infants has also been seen in recent years, which can mostly be explained by a shift from regular bovine milk intake to Support milk when breastfeeding ceases (Thorsdottir & Thorisdottir, 2011).

2.2.1.2 Lipids

Lipids are organic molecules that dissolve easily in organic solvents, such as alcohol, ether or acetone, but do not dissolve in water. Fat tissue accounts for about 15-30% of an average person's body weight. Fatty acids are the building blocks of lipids, and they determine the characteristics of the

lipid, e.g. whether it is solid or liquid at room temperature etc. Fatty acids (FA) differ in chain length (number of carbons), and are grouped as short-chain (less than six carbons), medium-chain (6-10 carbons), and long-chain (12 or more carbons) fatty acids. Short chain FAs have a higher melting point than longer chains. Fatty acids can be saturated, monounsaturated (MUFA), or polyunsaturated (PUFA), where saturated fatty acids do not contain any double bonds, monounsaturated fatty acids contain one double bond, and polyunsaturated fatty acids contain more than one double bond between carbons (Insel et al., 2016). Most of the fatty acids in bovine milk are bound in triglycerides, which account for 98% of the total lipids in milk. They consist of a glycerol molecule that has three FAs attached to it. Triglycerides in milk are synthesized from all the 406 FAs present (Månsson, 2008). Bovine milk lipids consist of approximately 1% of phospholipids (Månsson, 2008). Phospholipids are synthesized in the body and they are a major component of cell membranes. Phospholipids have the same structure as triglyceride, with the exception of the presence of a phosphate group instead of one of the FAs. Cholesterol accounts for less than 0.5% of cow milk lipids (Månsson, 2008). Although bovine milk lipids contain 406 different fatty acids, as previously mentioned, most of them account for less than 1% of the total lipids. Only 12 fatty acids in milk are present in larger amounts than 1% and they are listed in Table 5, where they are compared to the fatty acid composition of human milk from Brazilian women (German & Dillard, 2006; Månsson, 2008).

Table 5. Main fatty acids in bovine milk (German & Dillard, 2006; Nishimura, de Castro, Jordão Junior, & Sartorelli, 2013).

Fatty acid	Carbon number	Bovine milk average (%wt)	Human milk
Butyric	4:0	3.4	-
Caproic	6:0	2.1	0.1
Caprylic	8:0	1.2	0.3
Capric	10:0	2.6	2.0
Lauric	12:0	3.0	7.5
Myristic	14:0	10.6	6.8
Pentadecanoic	15:0	1.5	0.2
Palmitic	16:0	27.7	19.5
Palmitoleic	16:1	2.0	2.1
Stearic	18:0	12.8	5.8
Oleic	18:1	26.6	26.5
Linoleic (LA)	18:2	2.3	21.0
α-Linolenic (ALA)	18:3	1.6	1.5

Main sources of lipids in the diet are butter and other fatty dairy products, as well as oils, fatty fish, fatty meat, nuts, and seeds (Thorgeirsdottir et al., 2012). Lipids are the major milk component defining the energy value of the milk. Milk from different animals differs in fatty acid composition, which is also reflected in the total energy value of the milk. Ruminant milk contains one-third more SFAs than human milk, and human milk contains nine times more of PUFAs than ruminant milk (Barłowska, Szwajkowska, Litwińczuk, & Król, 2011). These differences can partially be explained by differences in feed, but also by differences in fatty acid synthesis between animals. The fat composition of milk from non-ruminants mostly reflects their feed, while the composition of human milk depends on the infant's

needs (Claeys et al., 2014; Hassiotou et al., 2013; Twigger et al., 2015). Ruminants, however, have ruminal microbes in their rumen that hydrogenate MUFAs and PUFAs to saturated fatty acids before absorption, which has an effect on the nutritional composition of the milk, such as the low PUFA content (German & Dillard, 2006). In this process, some trans-fatty acids are formed as well, including conjugated linoleic acid (CLA), which is believed to have health benefits in humans, e.g. lowering the risk of cardiovascular diseases (Claeys et al., 2014; Månsson, 2008).

Dietary fat is important for the body as a source of energy and vitamins, as well as for transporting fat-soluble vitamins in the bloodstream. All body cells contain fat as an essential part of cell membranes, mostly in the form of phospholipids, but also in the form of cholesterol and glycolipids. Fat is also important for the function of the brain, which consists of 60% fat, making it the fattiest organ in the body (Insel et al., 2016). The recommended intake of fat for infants less than 12 months of age is 30-45% of the total energy intake, and 30-40% for small children from 12 months and up to two years of age (Nordic Council, 2012).

2.2.1.3 Carbohydrates

Carbohydrates are organic compounds that contain carbon, hydrogen and oxygen in the ratio of 1:2:1 (Damodaran, Parkin, & Fennema, 2008). Carbohydrates can be defined as either simple or complex. Simple carbohydrates can be divided into two main groups: monosaccharides and disaccharides (Nordic Council, 2012). Monosaccharides consist of a single sugar molecule and the most common are: glucose (glu), fructose (fru) and galactose (gal). Glucose is the type of carbohydrate that is used for energy in the body. Fructose occurs naturally in fruits, and gives a more sweet taste than glucose. Galactose is usually bonded with glucose to form lactose, and rarely occurs as a monosaccharide in food. Both fructose and galactose are transformed to glucose in the liver. Disaccharides consist of two sugar molecules and the most common are: sucrose (fru-glu), maltose (glu-glu) and lactose (glu-gal). Lactose gives dairy products a slightly sweet taste. Complex carbohydrates are chains of more than two sugar molecules. Oligosaccharides contain 3-10 sugar molecules, and polysaccharides contain over ten molecules, and may consist of thousands of sugar molecules (Insel et al., 2016). This is how glucose is stored in the body, both in liver and muscles.

Main sources of carbohydrates are foods of plant origin, such as vegetables, fruits, grains and legumes. Milk and dairy products contain lactose as the main carbohydrate, and small amounts of oligosaccharides (Insel et al., 2016). Human milk thus contains over 100 different oligosaccharides, but in very small amounts. These are then broken down in the digestive tract by intestinal bacteria (Laurentin & Edwards, 2005), but not by enzymes like other sugars. Oligosaccharides contribute to a healthy gut flora and are important for infants. They provide protection against bacterial and viral infections by modulating the intestinal flora and influencing different gastro-intestinal and inflammatory processes (Kunz & Rudloff, 2006). Human, horse, and donkey milk contain a higher amount of lactose than ruminant milk, and human milk contains higher amount of oligosaccharides than milk from other animal species (Claeys et al., 2014). Most of the oligosaccharides are present in the colostrum, both in human and animal milk. Human colostrum contains 22-24 g/L of oligosaccharides, and human mature milk contains 12-13 g/L of oligosaccharides. These amounts are 20 times higher than what is present

in bovine colostrum and mature milk (Asakuma et al., 2008). Human milk oligosaccharides (HMOs) contain sialic acid, which is believed to play an important role in the expression and development of the brain and central nervous system functions in infants (Mehra & Kelly, 2006). The main difference between HMOs and oligosaccharides from bovine milk is the predominance of neutral oligosaccharides in human milk, whereas in bovine milk the oligosaccharides mainly consist of acidic components (mostly 3' sialyl-lactose) (Kunz & Rudloff, 2006). The neutral oligosaccharides are believed to play a preventive role against many pathogens. For example, they are believed to inhibit diarrhoea caused by some of the heat stable toxins of *Escherichia coli*, *Campylobacter jejuni*, *Salmonella fyris* etc. (Asakuma et al., 2008). Less is known about oligosaccharides in bovine milk, but recent studies indicate that they have a potential as a source of microbiotic modulators, like the HMOs (Aldredge et al., 2013; Zivkovic & Barile, 2011).

According to the Nordic nutrition recommendations, carbohydrates should provide 45-60% of the total energy intake for all age groups, and less than 10% should come from added sugars (Nordic Council, 2012). Carbohydrates are important for numerous body processes, mostly in the form of glucose, which has one major role: to supply the body with energy. For infants and young children, carbohydrates are a very important source of energy for brain development and in infancy, the brain consumes around 60% of the dietary intake. It has been suggested that cognition in 6-7 year old children may be influenced by glucose intake early in life, which underlines the importance of carbohydrates for infants (Stephen et al., 2012).

2.2.1.4 Water

Water has many roles in the body and is the major component of all body fluids. These fluids are essential for mechanical functions, such as shock absorption, lubrication, cleansing and protection. Water is, furthermore, the highway that moves nutrients and waste between cells and organs (Insel et al., 2016).

Water is also an important component in foods. All types of food contain some water, and loss of water in foods may cause undesirable changes in texture and mouth-feel (Damodaran et al., 2008). Dairy products in liquid form (i.e. not cheeses, butters, etc.) contain a relatively high proportion of water, generally over 80%. The proportion of water depends on whether or not the product is skimmed, fermented, flavoured, etc. Protein and fat content are also factors that affect the water content of dairy products. Skimmed milk contains e.g. higher amount of water than whole milk, which in turn contains a higher amount of water than flavoured, fermented milk (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015).

2.2.1.5 Ash

Ash in food refers to any inorganic material present in the food, and mainly consists of salts and minerals. It is determined by weighing the residue left after incineration, which removes water and organic compounds from the food. Typical ashing temperatures are in the range from 450-550°C (Damodaran et al., 2008). Individual minerals in the ash are determined by dissolving the ash in acid. For example, sulphuric acid is an optimal acid to dissolve the ash in. Phosphorus is readily dissolved in sulphuric acid, and a better dissolution is obtained with higher acid concentration (Cohen, 2009).

The mineral composition in plant foods depends on the soil's fertility, the environment in which the plant grows, and on the plant's genetics. There can be a great variety between plants as well as within the same species. The mineral composition of animal foods varies less than in plant foods. The reason for this is homeostatic mechanisms in animals that operate to regulate tissue concentrations of essential nutrients (Damodaran et al., 2008).

2.2.2 Micronutrients – vitamins and minerals

The main micronutrients in dairy products are calcium, magnesium, selenium, phosphorus, vitamin-B_2, B_5, and B_{12} (Muehlhoff, Bennett, & McMahon, 2013; Reykdal, Rabieh, Steingrimsdottir, & Gunnlaugsdottir, 2011). Support milk is enriched with vitamin A, C, D, and E, calcium, iron, and copper to be in line with regulations regarding FOF from EFSA (European Food Safety Authority, 2014). Milk products are a very important source of calcium, phosphorus and iodine, and besides these, Support milk is a very important source of iron for infants (Thorsdottir et al., 2008). However, calcium has been known to interfere with iron absorption, and it is generally not recommended to consume iron supplements along with milk products. Therefore, milk products would not seem to be the ideal products to enrich with iron (Abbaspour, Hurrell, & Kelishadi, 2014). Studies have, however, shown that the iron that is added to IF and FOF (iron sulphate) is highly bioavailable, or approximately 20% (compared to 15-35% bioavailability of heme iron) (Hurrell & Egli, 2010; Pizarro et al., 2015), which indicates that lower bioavailability can be overcome by higher levels of iron in the product. The calcium absorption does not seem to be affected by the higher iron content. World Health Organization recommends using ferrous sulphate for the enrichment of IF and FOF, but it is not convenient in liquids, thus instead, using ferric ammonium citrate is a possibility (WHO, 2006). Below is an overview of the four most abundant vitamins and minerals in Support milk.

2.2.2.1 Vitamin-B_2

Vitamin-B_2 is an important factor in number of oxidative enzyme systems as a precursor for the coenzymes flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD), and as a covalently bound flavin. Vitamin-B_2 also participates in electron transport pathways (Institute of Medicine, 1998).

Main sources of vitamin-B_2 are foods of animal origin, such as meat and meat products, as well as milk and dairy products. Dairy products are the major sources of vitamin-B_2 in the Nordic countries (Nordic Council, 2012). Support milk contributes to 28% of the vitamin-B_2 in the diet of one-year-old infants in Iceland, and milk and dairy products contribute to 35% of vitamin-B_2 in their diet. Therefore, in total, milk products and Support milk currently contribute to 63% of the vitamin-B_2 in the diet of one year old infants (Thorsdottir et al., 2008). The RDI for vitamin-B_2 for infants under 12 months of age is 0.5 mg/day, and 0.6 mg/day for children between 12 and 24 months of age (Directorate of Health, 2013). One carton of Support milk (0.5 L), which is the recommended intake of milk or milk products for infants, contains 0.8 mg of vitamin-B_2.

2.2.2.2 Vitamin-D

Vitamin-D is essential for bone health, and one of its primary functions in the body is to regulate calcium levels in the blood. Vitamin-D promotes bone development and growth in children. Insufficient

vitamin-D intake in infancy can cause rickets, which is a childhood bone disease where the bones weaken, and the skeleton fails to harden (Holick, 2006; Nordic Council, 2012).

If the body gets sufficient exposure to sunlight, it can synthesize all the vitamin-D it needs. In the Nordic countries, where there is a lack of sunlight for long periods of the year, the vitamin-D needs to come from the diet and/or supplements (Thorisdottir, Gunnarsdottir, Steingrimsdottir, Palsson, & Thorsdottir, 2014). However, there are not many foods that naturally contain vitamin-D. The major dietary sources for vitamin-D are fatty fish and fish oil supplements that are naturally rich of vitamin-D, as well as enriched products, such as some dairy products, e.g. butter, and breakfast cereals. Egg yolks and liver can also supply some vitamin-D (Nordic Council, 2012; Insel et al., 2016). Therefore, it is important to take vitamin-D supplements. Studies have shown that the best way to prevent vitamin-D deficiency in infants, with little or no sun exposure is to give them vitamin-D supplements or vitamin-D enriched products all year round, regardless of breastfeeding (Thorisdottir, Gunnarsdottir, Steingrimsdottir, et al., 2014). Supplements (both fish oil and vitamin-D drops) contribute to 65% of the vitamin-D in the diet of one year old Icelandic infants, while Support milk contributes for 21% of it (Thorsdottir et al., 2008).

The RDI for vitamin-D is 10 µg/day for children under 10 years of age (Directorate of Health, 2013). Recent studies show that most Icelandic infants (over 90%) are vitamin-D sufficient and none have intake below the lower level increasing risk of rickets (Thorisdottir et al., 2016; Thorisdottir, Gunnarsdottir, Steingrimsdottir, et al., 2014). In a follow up study on six years old children who had participated in a study on vitamin-D status when they were one year old, results indicated that vitamin-D intake decreased from 1-6 years of age, and at six years of age only 64% of the children were vitamin-D sufficient. Similar results were found in the national dietary survey among 6 years old children (Gunnarsdottir, Helgadottir, Thorisdottir, & Thorsdottir, 2013; Thorisdottir et al., 2016). However, one carton of Support milk contains 6 µg of vitamin-D, and thus contains 60% of the RDI of vitamin-D for children and infants.

2.2.2.3 Iron

Iron is a trace mineral and only small amounts reside in the body. In food, iron exists in two forms: as heme iron and non-heme iron. Iron is required for oxygen transport, and transports it in the body as a component of two heme proteins: haemoglobin, and myoglobin. Hemoglobin transports oxygen in the blood in red blood cells, delivering it through capillary beds to the tissues. Myoglobin facilitates the transport of oxygen into muscle cells. Iron also acts as a constituent or cofactor for many enzymes in reactions, and is important for the immune system and brain function (Nordic Council, 2012; Insel et al., 2016). Iron deficiency (ID) is an important public health problem and the most common nutritional deficiency worldwide, especially in the developing countries. ID is defined as reduced body iron stores. A worsening of ID is called iron deficiency anaemia (IDA). The most common causes of ID is insufficient intake of iron and rapid growth (Ozdemir, 2015). The need for iron is most during rapid growth, i.e. in infancy and adolescent, and also during pregnancy (Insel et al., 2016). Infants are considered to have adequate stores of iron up to 4-6 months of age, but after that, when complementary feeding starts, it is important for infants to get enough iron from the diet (Mahan,

Escott-Stump, & Raymond, 2012). Studies have shown that children who are iron depleted at 12 months of age often have lower self-help scores, and lower combined motor scores at the age of six years (Gunnarsson, Thorsdottir, Palsson, & Gretarsson, 2007; Thorisdottir, Gunnarsdottir, et al., 2013; Thorsdottir & Gunnarsson, 2006). These consequences are well known among anaemic children (Abbaspour et al., 2014). Prevention and treatment of ID and IDA has been shown to improve cognitive, motoric, and behavioural development in young children, and treatment of IDA can also improve attention and concentration in school children (Domellof, Thorsdottir, & Thorstensen, 2013). Up until 2003, the iron status in Icelandic children was poor. About 20% of children born in 1995-1997 were shown to be iron deficient, and 2.7% were anaemic (Thorsdottir, Gunnarsson, Atladottir, Michaelsen, & Palsson, 2003). This has been associated with high levels of bovine milk intake in late infancy (Thorisdottir, Ramel, Palsson, Tomassson, & Thorsdottir, 2013). Bovine milk is rich of proteins, and excessive protein intake in infancy has been associated with rapid growth (Thorsdottir et al., 2008), which is a common cause of ID (Ozdemir, 2015). However, after 2003, the iron status in Icelandic children has improved, and it is believed that it may partially be explained by the shift from using bovine milk to Support milk when breastfeeding decreased or ceased (Thorisdottir et al., 2011).

Red meat such as beef is an excellent source of iron, both in terms of amount and bioavailability. Fish, poultry and pork are also good sources and contain both heme and non-heme iron. Heme iron is better absorbable than non-heme. However, vitamin-C helps with the absorption of non-heme iron (Insel et al., 2016). Dairy products are not a good source of iron. Support milk, however, is enriched with iron, and contributes to 21% of the iron in the diet of one year old infants. Support milk is therefore an important source of iron for this age group (Thorsdottir et al., 2008). The RDI for iron is 8 mg/day for six months to two years old children (Directorate of Health, 2013). One carton of Support milk contains 3.75 mg of iron, which accounts for approximately half of the RDI for six months to two years old children.

2.2.2.4 Iodine

Iodine is an essential component of two thyroid hormones: triiodothyronine (T3) and thyroxine (T4). Thyroid hormones control the regulation of body temperature, basal metabolic rate, reproduction and growth. Iodine is present in two forms in food, as iodide or iodates, and the body absorbs almost all of it (Insel et al., 2016). Seafood is the best source of iodine, and salt-water species contain higher amounts of iodine than fresh water fish. Dairy products are also a good source because iodide is added to the cows' feed (Insel et al., 2016). Dairy products are the main source of iodine for Icelandic children (Gunnarsdottir et al., 2013). The RDI of iodine for 6-11 months old infants is 50 µg/day, and 70 µg/day for 12-23 months old infants (Directorate of Health, 2013). One carton of Support milk contains 60 µg of iodine.

2.4 Cultured dairy products

According to the Icelandic directive on milk and dairy products (Nyskopunarraduneyti, 2012), cultured dairy products are products made of pasteurized whole milk, low fat milk, non-fat milk, or recycled milk, that contain living bacteria. Several different types of cultured dairy products are available on the Icelandic market, such as buttermilk, yoghurt, skyr (an Icelandic fermented dairy product), AB-yoghurt, LGG, etc. According to the directive, products that are advertised based on their live cultures must contain at least one million of each species in one mL at the end of the shelf life (Nyskopunarraduneyti, 2012). The production of cultured dairy products is based on the bacterial fermentation of lactose by the starter culture, leading to the production of lactic acid instead (Lucey, 2004). The nutritional value, chemical composition and physicochemical properties of cultured dairy products depend on the milk, from which the products are processed, the cultures used, flavours added, chosen incubation process, etc. This process extends the shelf life of the milk from a few days up to about 3-4 weeks (Corrieu & Béal, 2016). Cultured dairy products are generally rich in proteins, and are generally considered an ideal carrier for probiotics, as they are considered to be healthy products, especially if they are without added sugar or sweeteners (Lourens-Hattingh & Viljoen, 2001).

2.4.1 Yoghurt culture and the production process

Yoghurt is, according to the Icelandic regulation on milk and dairy products, produced by fermenting milk with *Lactobacillus delbrueckii* subspecies (ssp.) *bulgaricus* (*L. bulgaricus*) and *Steptococcus salivarius* ssp*. thermophilus* (*S. thermophilus*)*.* Yoghurt should contain at least 8.5% of non-fat dry material from milk (Nyskopunarraduneyti, 2012). In some countries, a product must contain both *L. bulgaricus* and *S. thermophilus* to be allowed to bear the name “yoghurt”. However, both *L. bulgaricus* and *S. thermophilus* are able to ferment milk individually, but they complement each other in growth and acid production if they are used together (Sieuwerts, de Bok, Hugenholtz, & van Hylckama Vlieg, 2008). *L. bulgaricus* and *S. thermophilus* have not been considered as probiotics in the same sense as for example *L. acidophilus, B. bifidum* and *L. rhamnosus* GG. However, they are known to have high lactase activity, and yoghurt consumption may improve lactose digestion, and eliminate symptoms of lactose intolerance (Granato, Branco, Cruz, Faria, & Shah, 2010; Guarner et al., 2005).

The first step in the typical production of stirred yoghurt is to enrich the pasteurized milk with non-fat dry milk to increase the viscosity of the product. Generally, it is recommended to enrich the milk with 1-6% dry material. The viscosity of yoghurt depends mostly on the protein content of the milk, which means that a high protein content of the milk is important for a viscous yoghurt. Casein is the major contributor of viscosity, followed by the lipids and whey proteins. Stabilizers can also be used to influence the consistency of yoghurt (Corrieu & Béal, 2016). Figure 3 shows a flow diagram of a typical yoghurt production.

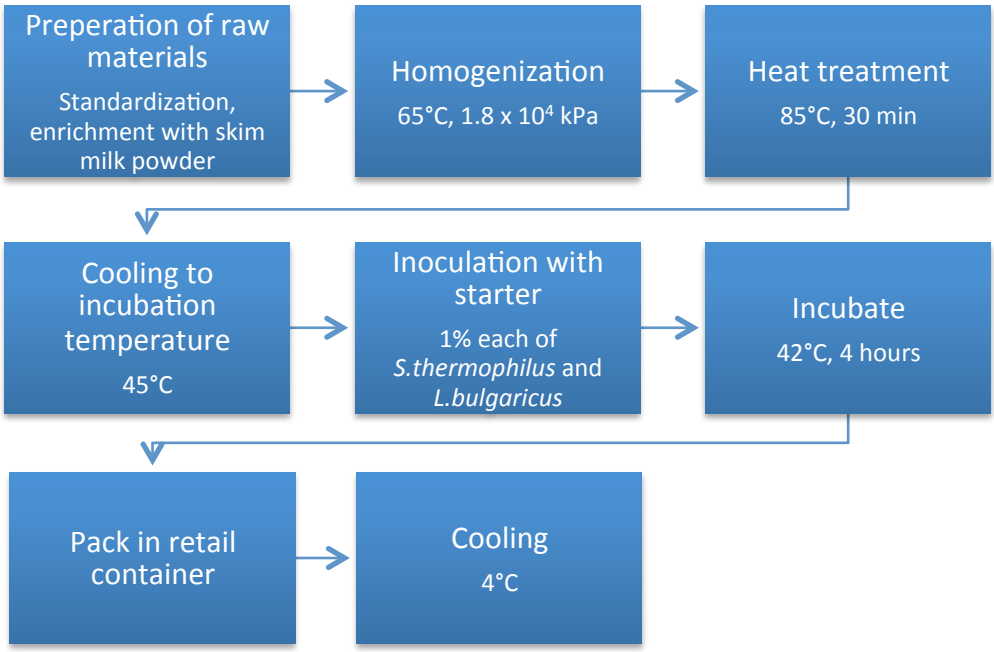


Figure 3. Flow diagram of a typical production process for stirred yoghurt (Corrieu & Béal, 2016)

When the raw material blend is ready, homogenization is performed. Homogenization is used to stabilize the lipid phase in the mixture. The lipids are lighter than the skim milk itself so they tend to separate from the skim milk and float on the surface. Homogenization prevents that from happening by reducing the diameter of the fat globules in the milk from 3-4 µm on average to 1-2 µm. The yoghurt mix is heated to 65°C, because the efficiency of homogenization is better at higher temperatures. The mixture is then forced at $1.8*10^4$ kPa through a small cavity, which causes the fat globules to reduce. This must be done after the milk is pasteurized because the reduced fat globules are more vulnerable to lipase degradation, but lipase is destroyed during pasteurization. Thus, if the homogenization is done before the pasteurization, the mixture must be pasteurized immediately to prevent lipolysis (Corrieu & Béal, 2016).

When the homogenization is finished, the mixture undergoes heat treatment. The yoghurt mixture is heated to 85°C for 30 minutes. The purpose of this step is mainly to denature the whey proteins, β-lactoglobulin and α-lactalbumin. The denatured β-lactoglobulin form a complex with κ-casein, which increases the hydrophilic properties of the casein, reduces the tendency of the gel to syneresis, and facilitates the formation of a stable coagulum. These results are maximal at 85°C but decrease at higher temperatures. The purpose with the heat treatment is also to destroy all pathogenic bacteria and most of the spoilage-causing bacteria, including thermodurics, and to inactivate all the enzymes that may be present, including lipase (Corrieu & Béal, 2016).

After the heat treatment, the mixture is cooled to 45°C and inoculated with 0.5-5% of a starter culture. Higher levels lead to more rapid acid production. The optimum level is 2%: 1% each of *L. bulgaricus* and *S. thermophilus*, respectively. *L. bulgaricus* hydrolyze the caseins, releasing essential amino acids, which stimulate the growth of *S. thermophilus.* Initially, *S. thermophilus* grows rapidly,

which reduces the pH to 5.4, thus stimulating the growth of *L. bulgaricus,* which is acid-tolerant and produces large amounts of lactic acid, which again reduces the pH (Corrieu & Béal, 2016; Lourens-Hattingh & Viljoen, 2001). The starter cultures are added to the mixture and distributed by stirring for 10-15 minutes. The mixture is incubated in bulk at 42°C for approximately four hours until the pH is 4.5. When the yoghurt has reached the desired pH value, it is stirred and cooled to 4°C, and packed in retail containers. The incubation temperature at 42°C is used because the optimal incubation temperature for *S. thermophilus* is 37°C, and 45°C for *L. bulgaricus.* Lower temperatures would favour the growth of *S. thermophilus*, but then the growth of *L. bulgaricus* would be poorer, which would affect the flavour of the product. The yoghurt cultures produce acetaldehyde, acetic acid and diacetyl (Corrieu & Béal, 2016; Routray & Mishra, 2011).

The cooling process of stirred yoghurt is performed in one phase. Once the desired pH value has been reached, the yoghurt is cooled to <10°C as quickly as possible. Then it is possible to add flavourings etc. During cold storage, it is very important to maintain the temperature below 5°C, and when transporting the products, the products must be handled gently, since shaking can lead to syneresis (Corrieu & Béal, 2016).

2.4.2 Buttermilk culture

Buttermilk is produced by fermenting milk with *Lactococcus lactis/cremoris (L. cremoris)*, *Lactococcus lactis* ssp. *diacetylactis (L. diacetylactis)* and *Leuconostoc citrovorum (L. citrovorum)*. A product is only allowed to be called “buttermilk” if it contains the before mentioned combination of starter cultures (Nyskopunarraduneyti, 2012). *L. cremoris* is used for its fermentation properties and is a mesophilic species, which allows the milk to be fermented at room temperature in the production of buttermilk. *L. diacetylactis* and *L. citrovorum* are, however, used for organoleptic purposes (Hill & Ross, 1998).

2.4.3 AB culture

Lactobacillus acidophilus (L. acidophilus) and *Bifidobacterium bifidum (B. bifidum)* are not used in cultured dairy products for their fermentation properties but for their health benefits. A typical yoghurt or buttermilk culture is used for the fermentation. *L. acidophilus* and *Bifidobacterium* subspecies are the most common bacteria used as probiotics (Shah, 2007; Sieuwerts et al., 2008). Both *L. acidophilus* and *B. bifidum* may be useful against diarrhoea caused by rotavirus (Marteau, de Vrese, Cellier, & Schrezenmeir, 2001; Saavedra et al., 1994) and diarrhoea caused by the use of antibiotics (Fox et al., 2015). Furthermore, *L. acidophilus* and *Bifidobacterium* may stimulate the immune system (Morita et al., 2002; Schiffrin, Rochat, Link-Amster, Aeschlimann, & Donnet-Hughes, 1995). They are also believed to produce organic acids that have anti-mutagenic functions against several mutagens and pro-mutagens, such as 2-nitroflourene, aflatoxin-B etc. (Lankaputhra & Shah, 1998).

2.4.4 LGG culture

Lactobacillus rhamnosus GG (*L. rhamnosus* GG) is, as *L. acidophilus* and *B. bifidum*, used in cultured dairy products for its health benefits, but not for its fermentation properties (Shah, 2007). *L. rhamnosus* GG may be effective against diarrhoea caused by rotavirus (Guandalini et al., 2000) and antibiotics (Fox et al., 2015; McFarland, 2006), and may reduce the risk and duration of diarrhoea in

children and infants (Chassard, de Wouters, & Lacroix, 2014; Gritz & Bhandari, 2015; Parker et al., 2013). However, no health claims are permitted for LGG according to EFSA (EFSA, 2011).

2.4.5 Gut flora and colonization

Initially, the gastrointestinal (GI) tract of normal foetuses was thought to be sterile, but recent studies have suggested otherwise. An infant's gut is believed to be exposed to a number of bacteria by swallowing of colonized amniotic fluid in the womb (Di Mauro et al., 2013; DiGiulio, 2012; DiGiulio et al., 2008; Underwood, German, Lebrilla, & Mills, 2015). Many factors affect the colonization in infants, such as vaginal birth, breastfeeding, antibiotics during birth, antibiotics during the first year of life etc. (Indrio & Neu, 2011; Westerbeek et al., 2006). Full-term, vaginally born infants are completely colonized by the first year of life, with a diverse array of bacterial families in clusters and species (>1000 species), with a reflection of the maternal vaginal flora (Di Mauro et al., 2013; Gritz & Bhandari, 2015; Wall et al., 2009; Weng & Walker, 2013)

The first bacterium believed to invade the infant's gut belongs to facultative anaerobic species, such as Enterobacteriaceae, *Streptococcus*, and *Staphylococcus*. These bacteria may not seem to be the best choice for the health of new-borns since they belong to species with a pathogenic potential. However, they are believed to have metabolisms that are good in preparing the path to a beneficial enteric flora in the gut of new-borns (Mikami, Kimura, & Takahashi, 2012; Morelli, 2008). A positive redox potential has been noticed in the intestines of new-borns right after birth, which is believed to be the reason for the colonization of Enterobacteriaceae, *Streptococcus* and *Staphylococcus*, but they lower the redox potential. These bacteria are soon replaced by obligate anaerobic bacteria, such as *Bifidobacterium*, *Bacteroides* and *Clostridium*, which later become the predominant bacteria residing in the gut, and approximately one month after birth, *Bifidobacteria* are the most predominant in infant's faeces. *Bifidobacteria* are considered to be the most important beneficial bacteria for infants, both physically and mentally (Mikami et al., 2012). Colonization with *Bifidobacterium longum* ssp. *infantis* (*B. infantis*) is furthermore believed to be associated with increased vaccine responses (Underwood et al., 2015). The growth of *Bifidobacteria* depends on the presence of oligosaccharides, which, as previously mentioned, human milk is rich of (Corrieu & Béal, 2016).

Lactobacillus and *Bifidobacterium* have commonly been given to infants as probiotics, and have been studied quite extensively in relation to infants (Chassard et al., 2014; Gritz & Bhandari, 2015). *Lactobacillus* and *Bifidobacterium* are believed to affect the intestinal epithelial barrier function directly by decreasing intestinal permeability, and improving intestinal epithelial resistance, thus inhibiting the growth of pathogenic organisms (Corrieu & Béal, 2016; Gritz & Bhandari, 2015). Some studies suggest that probiotics can decrease the risk and incidence of infections, diarrhoea and sepsis in preterm infants by decreasing the intestinal colonization rate of harmful bacteria (Indrio & Neu, 2011; Ren & Wang, 2010; Westerbeek et al., 2006). Regular consumption of yoghurt after the weaning period may therefore be beneficial and could be a part of a healthy infant's regular diet (Guerin-Danan et al., 1998). However, recent review studies have shown that there is not enough evidence to state that probiotics contribute to aforementioned health benefits (Braegger et al., 2011; Mugambi, Musekiwa, Lombard, Young, & Blaauw, 2012). Infant formulas are increasingly being supplemented

with probiotics (Braegger et al., 2011), but EFSA has stated that there are not enough evidence to advise or obligate supplementation of IF or FOF with probiotics, especially as the long-term effects of doing so are not well known (European Food Safety Authority, 2014).

3 Materials and methods

3.1 The survey

A net survey was made, using Google Forms, to obtain information about parents' interest in a new milk product made with infants' needs in mind. The net survey included 17 questions, and the form was sent to a Facebook group of 6000 mothers, called Tips for mothers, and then the form was shared further on Facebook. The survey was intended for parents of children 6 years old and younger. Initially, participants were asked about their background in four questions, i.e. gender, age, residence and education. One question was about number and age of participants' children. The next 11 questions were about participants' opinions on baby food products in general, their opinions on the Icelandic baby food market, their interest in the new product, and if it should be vitamin enriched or flavoured. For these questions, the Likert scale with 5-point was used, with neutral as the middle option. The next question was about what kind of qualities the new product should feature and the participants could choose more than one possibility. The final question was an empty text box where participants could write their comments about specific questions. The whole survey can be viewed in appendix 1.

A multivariate analysis was performed on the dataset in Matlab, version R2015a (The Mathworks Inc., Natick, Mass., USA) to see how the questioned parameters were related to each other. The answers were given characteristic numerical values that portrayed their distribution (i.e. a very positive attitude got a value of +2, a positive attitude the value +1, neutral the value 0, and a negative and very negative attitude the values -1 and -2 respectively). All variables were weighed with the inverse of their standard deviation to allow for different scales of the results and the data was centred prior to analysis. The data were then entered to the program IBM SPSS Statistics 22 (SPSS) to calculate the correlation coefficient.

3.2 Experimental design

The samples used in this project were provided and prepared by a dairy production company in Iceland, Mjólkursamsalan (MS). The experiment was divided into three parts: a pre-trial, a main experiment, and a final follow-up experiment.

3.2.1 Pre-trial

Two types of products were prepared for the pre-trial and three samples of approximately 500 g each were made for each type. The purpose of the pre-trial was to find out if Support milk was fermentable. Support milk was fermented with a typical yoghurt culture, *S. thermophilus* and *L. bulgaricus,* and a regular yoghurt was enriched with the vitamin blend used in the production of Support milk. In the pre-trial, the amounts of water, protein, fat, carbohydrates, and ash of the products were measured, along with water holding capacity (WHC), pH value, colour, viscosity, lactic acid bacteria, moulds, yeasts, nuclear magnetic resonance (NMR) and near infrared spectroscopy (NIR). Furthermore, the pH value, colour and NMR values of a regular yoghurt from the supermarket was compared to the support yoghurt and vitamin enriched yoghurt. Analyses of chemical and physical parameters in the pre-trial

were performed in triplicates and were only performed at one occasion, four days after the production date. The samples were stored at 0-2°C between production date and the date of measurements. An informal tasting was also performed at MS by the supervisor and student of this project, and by two staff members of MS, and the taste and texture of the products evaluated.

3.2.2 Main experiment

In the main experiment, four types of yoghurt were prepared and tested. Twelve samples of approximately 200 g were made for each product. Three types contained Support milk as the main raw material and the fourth type contained a regular whole milk as a main raw material and was used as a control sample. All products were fermented with *L. cremoris*, *L. Diacetyllactis* and *L. citrovorum,* along with *L. rhamnosus* GG. Table 6 shows the ingredients in each sample.

Table 6. Description of the support yoghurt formulations studied in the main trial

Sample	Raw material	Lactic acid bacteria	Vitamin enrichment	Fibres	Binding agent
1	Support milk	Buttermilk culture + lgg	-	Orafti HIS fibres 1%	0.2% gelatine
2	Support milk	Buttermilk culture + lgg	-	Orafti HIS fibres 1%	-
3	Support milk	Buttermilk culture + lgg	-	Orafti HIS fibres 1%	2.5% maize starch
4	Whole milk	Buttermilk culture + lgg	Vitamin blend for Support milk	Orafti HIS fibres 1%	-

All samples were pasteurized at 90°C for ten minutes and fermented at 20°C for 20 hours. The samples were measured regularly during the estimated shelf life and the planned end point of the experiment was set as five days after the best before date indicated by MS. The set best before date was based on known minimal shelf life of natural yoghurt produced by the company. A detailed chemical analysis of protein, fat, carbohydrates, and ash content was only performed in the beginning of the storage study, but water, WHC, pH, colour, lactic acid bacteria, moulds, yeasts, NMR and NIR were measured at four occasions over a storage period of approximately one month from production. The production date was December 20th. The measurements were performed at January 5th, 12th, 18th and 23rd. The expiration date was January 18th. The samples were stored at 0-2°C during the storage period. All analyses of physical and chemical parameters were performed in duplicates. An informal tasting of the products was performed by three students at the research lab of Matís and taste and texture of the products evaluated.

3.2.3 Final follow-up experiment

Two samples were chosen from the main experiment to research further. The support yoghurt containing only fibres was improved by increasing the protein amount up to 2.2% instead of the previous 1.8% to get a thicker consistency. The formulation of the Support yoghurt containing fibres and maize starch was kept the same as in the main experiment. Each product came in 15 samples of approximately 200 g. Chemical analysis was performed in the beginning, as well as measuring the amount of vitamin-B_2, vitamin-D, iron and iodine (samples were sent abroad and performed at a

Eurofins laboratory for these measurements). Water, WHC, pH, colour, lactic acid bacteria, moulds, yeasts, NMR and NIR were performed at three occasions over approximately one month period, March 9th, 21st and April 4th. The expiration date was March 31st. The samples were stored at 0-2°C during the storage period. Bligh and Dyer fat analysis with minor modifications was also performed for a more accurate fatty acid analysis. The samples were stored at 0-2°C during the storage time. All analyses of physical and chemical parameters were performed in duplicates.

3.3 Chemical analysis

Chemical analyses were performed by the Matís chemical lab as indicated in the experimental descriptions. Measurements were made for water (ISO 6496:1999), protein (ISO 5983-2, 2005), fat (AOCS Ba 3-38, 1997), and ash (ISO 5984, 2002). Carbohydrates were measured as a difference.

3.3.1 Water analysis

Approximately 5 g of the sample were weighed out and put in a ceramic bowl with sand. The bowl was put into an oven at 103±2°C for four hours and the sample dried. The water content was determined by weighing the sample before and after drying (ISO 6496, 1999).

3.3.2 Protein analysis

Approximately 1.5-2.0 g of protein was broken down and digested in concentrated H_2SO_4 with a $CuSO_4$ catalyst mixed with K_2SO_4. The carbon and hydrogen of the proteins oxidize partially to CO_2 and water, and the sulphuric acid redox to SO_2 but the amine redox to NH_3. After digestion, the nitrogen is in the form of NH_4^+ ions, and with the addition of NaOH, it transforms into NH_3, which is distilled into a boric acid solution, which is then titrated back with 0.2N H_2SO_4 (ISO 5983-2, 2005). The crude protein ratio was obtained by multiplying the amount of nitrogen with the conversion factor 6.25.

3.3.3 Fat analysis

The sample was dried at 103±2°C before the analysis. The fat was digested in acid and broken down and then extracted with petroleum ether with a boiling point range of 40-60°C. The ether was then removed and the fat weighed (AOCS Ba 3-38, 1997).

3.3.4 Fatty acid analysis

3.3.4.1 Lipid extraction

The lipid extraction of the samples was carried out using the method by Bligh and Dyer with adaptation (Bligh & Dyer, 1959). A ground sample of 25 g was weighed into a 250 mL centrifuge bottle. Then, 25 mL of chloroform, and 50 mL of methanol were added to the sample in the centrifuge bottle and homogenized for 2 minutes. Another 25 mL of chloroform was added to the mixture and mixed for 1 minute. Then, 25 mL of 0.88% KCl was added to the mixture and mixed for 1 minute and centrifuged at 2500 rounds per minute (rpm) for 20 minutes at 4°C. After centrifugation, a glass pipettes were used to absorb the lower chloroform phase containing the fat, and the chloroform phase was then filtrated on a glass microfiber filter under suction. The content in the suction flask was then poured into a 50 mL volumetric flask. Every trace of the upper phase was removed, and the 50 mL

volumetric flask was filled with chloroform to the mark. Then the chloroform phase was poured into a 50 mL test tube for the analysis of fatty acid composition.

3.3.4.2 Fatty acid composition

The methylation method used was based on AOCS official Method Ce 1b-89 with minor adjustments. First, the chloroform phase from the lipid extraction was evaporated in a round evaporation flask, and then in a screw cap glass tube by nitrogen jet at 55°C. The total amount of lipid was 60-90 mg in each sample. Then, 1.5 mL of 0.5N NaOH (in methanol) was added to the samples, mixed, and heated for 7 minutes at 100 °C. The test tubes were then cooled down to room temperature, and 2 mL of BCl_3 (12%) in methanol were added to the test tubes, which were heated for 30 min at 100°C. The test tubes were then cooled down to room temperature, and 1 mL of standard solution and 5 mL of concentrated NaCl solution were added to the test tubes. Then, the solution was placed on a vortex and mixed for half a minute in the test tubes. When the layer of isooctane separated from the aqueous layer, it was transferred to clean test tubes with a small amount of sodium sulfate. One mL of clean isooctane was then added to the solution and mixed again using a vortex. The isooctane layer (1.5 mL) was then moved to a small glass tube for gas chromatography fatty acid analysis.

3.3.5 Ash analysis

The ash content was determined by weighing out 2 g of the sample and ashing it at 550°C. Then the residue was weighed (ISO 5984, 2002).

3.3.6 Vitamins and minerals analysis

The vitamins and minerals analyses were performed by the Eurofins laboratory as described below.

3.3.6.1 Vitamin-B_2

Vitamin-B_2 was extracted from the sample in an autoclave using acid hydrolysis, and quantified by reverse phase high-performance liquid chromatography (HPLC) with fluorometric detection (ONORM EN 14152, 2006).

3.3.6.2 Vitamin-D

Vitamin-D_3 was saponified in the sample, using an alcoholic potassium hydroxide solution and extracted with a solution of hexane:ethylacetate. The extract was concentrated and cleaned up by a solid-phase extraction (SPE), followed by normal phase semi-preperative. The amount of vitamin-D_3 is determined by reverse phase HPLC with a diode array detector (DAD) at 265 nm (BS EN12821, 2009).

3.3.6.3 Iron

The sample was digested with nitric acid using microwave technique at temperatures up to 235°C. Then the iron was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (ISO 11885, 2007).

3.3.6.4 Iodine

The iodine content was determined by using the inductively coupled plasma mass spectrometry (ICP-MS) method (BS EN 15111, 2007).

3.4 Physicochemical analysis

3.4.1 Water holding capacity (WHC)

The WHC was determined by centrifuging 20 g of sample at 500 rpm for 5 minutes, weighing the supernatant, and then measuring the amount of supernatant recovered (%, v/w) (Panesar & Shinde, 2012). The percent syneresis was calculated as:

$$\% \textbf{ Syneresis} = \frac{\text{Volume of supernatant}}{\text{Weight of sample}} \text{ x } 100$$

The definition of WHC describes how well the sample retains the water during centrifugation. Syneresis, however, is defined by how much water is released proportionally during centrifugation.

3.4.2 pH

The pH of the samples was determined using a portable pH meter (Portamess 913, Knick, Germany) and a combination pH electrode. Each sample was measured twice. The pH meter was calibrated using buffer solutions with pH 4 and 7 before the use.

3.4.3 Viscosity

The viscosity of the samples was measured using a digital rotary viscometer (NDJ-8S, Graigar, China), with rotor no. 2 and a velocity of 30 rpm. The samples were taken out of a refrigerator with the temperature of 0-2°C, half an hour before the measurements were performed. The temperature of the samples was approximately 10°C when the viscosity was analysed. The viscosity was only analysed in the pre-trial. The viscometer was out of order when the main and follow-up experiments were performed.

3.4.4 Colour analysis

The colour of the surface of the samples was measured using a colour meter (Chroma meter CR-300, Konica Minolta, Tokyo, Japan). Each sample was measured twice. The colour was assessed by the CIA L*a*b colour scale, where L* describes lightness, from black to white (scale 0-100), a* describes redness (green to red), and b* describes yellowness (blue to yellow).

3.5 Microbiological analysis

3.5.1 Lactic acid bacteria

The lactic acid bacteria count was determined by preparing a dilution series of the sample according to general microbiological principles, followed by surface spreading with spiral plating on de Man,

Rogosa and Sharpe (MRS) agar. The plates were incubated in anaerobic jar and the incubation time was 72 hours at 30°C (NMKL 140, 2nd ed., 2007).

3.5.2 Moulds and yeasts

The colony-forming unit (cfu) level of moulds and yeasts was determined by preparing dilution series of the sample material according to the general microbiological principles, followed by spread plating onto specified agar medium in Petri dishes. The samples were incubated at 22.0±1.0°C for 5 days. Moulds and yeasts were counted separately. The number of colonies was counted on selected plates, and multiplied by the number of colonies as obtained using the dilution factor. Results were presented as cfu per gram of sample. Media: Dichloran Rose-Bengal Chloroamphenicol Agar (DRCB-Agar) (NMKL 98, 4th ed., 2005).

3.6 Near InfraRed (NIR)

NIR reflectance analysis was measured with a fibre probe using a Bruker Multi Purpose Analyser (Bruker Optics, Rheinstetten, Germany). The fibre probe was dug directly into the products. Each formulation was measured in duplicate and five scans were used to build each spectrum. All NIR spectra were maximum normalised prior to further comparison or analysis.

3.7 Nuclear Magnetic Resonance (NMR)

A Bruker Minispec mg 20 (Bruker Optics GmgH, Am Silberstreifen D-76287 Rheinstetten, Germany) was used to measure the proton distribution and characteristics in the samples. Approximately 1 g of sample was taken from each product and put in glass tubes using a pipette. The glasses were then put in the NMR instrument one by one and measured at room temperature. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958) was used for the transverse relaxation time analysis, using an interpulse time of 100 μs, 8 repetitive scans, and a recycle delay of 10 s. 8400 echoes were collected in each scan and no dummy shots were used. The obtained relaxation data was maximum normalized and fitted to a discrete exponential model with the Low field NMR Toolbox for Matlab (The Mathworks Inc., Natcick, Mass., USA), as described by Pedersen et al. (2002) (Pedersen, Bro, & Engelsen, 2002). Residual analysis was then used to assess the number of proton components in the samples.

3.8 Statistical analysis

Microsoft Excel 2011 was used to calculate means and standard deviations for the data in the pre-trial, main experiment and follow-up experiment and to create tables and graphs in the consumer survey and the experiments. Microsoft Excel 2011 was also used to perform a two-tailed, two-sample t-Test. Significance of differences was defined as the 5% level ($p<0.05$).

4 Results and discussion

4.1 The consumer survey

4.1.1 Background information of participants

Answers from 304 participants were obtained from the net survey, 289 women (95%) women and 15 men (5%). The age distribution of the participants was wide, but most of the participants were in the age group 21-30 years (77%). Only two participants were younger than 20 years old, and eight were over 40 years old. The majority of the participants lived in the capital area, or 71.3%, which is higher than the percentage of the population that resides in the capital area, which was 64% on January 1st, 2015 (Statistics Iceland, 2015a). Other participants were rather evenly distributed around the country. About half of the participants had finished a bachelor or higher degree at a university, which is rather high compared to the education level of the population, but according to Icelandic Statistics, 23% of Icelandic habitants aged 16-49 years had finished a degree at a university as of the year 2014 (Statistics Iceland, 2015b). The background data of the participants is compiled in figures 4-7.

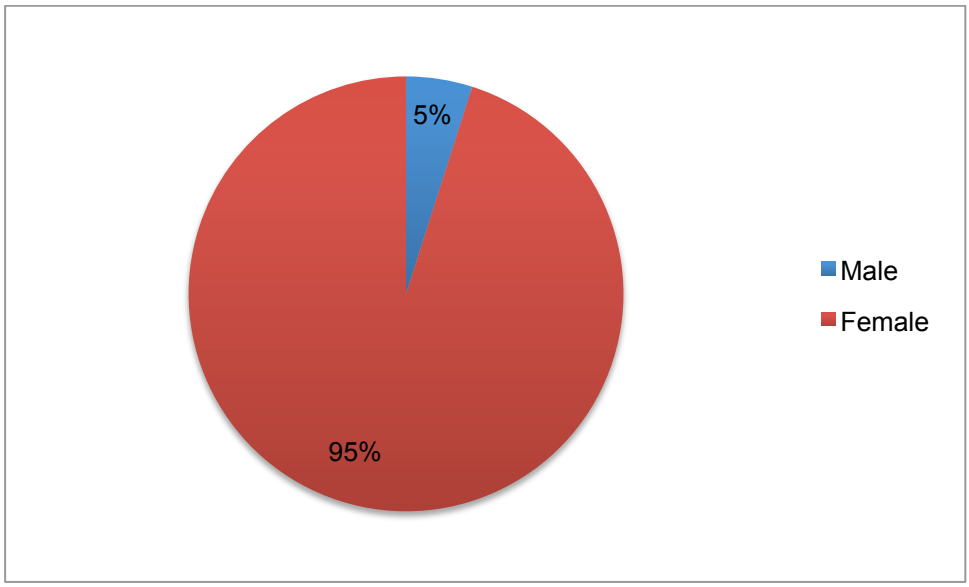


Figure 4. Gender distribution of the participants answering the survey.

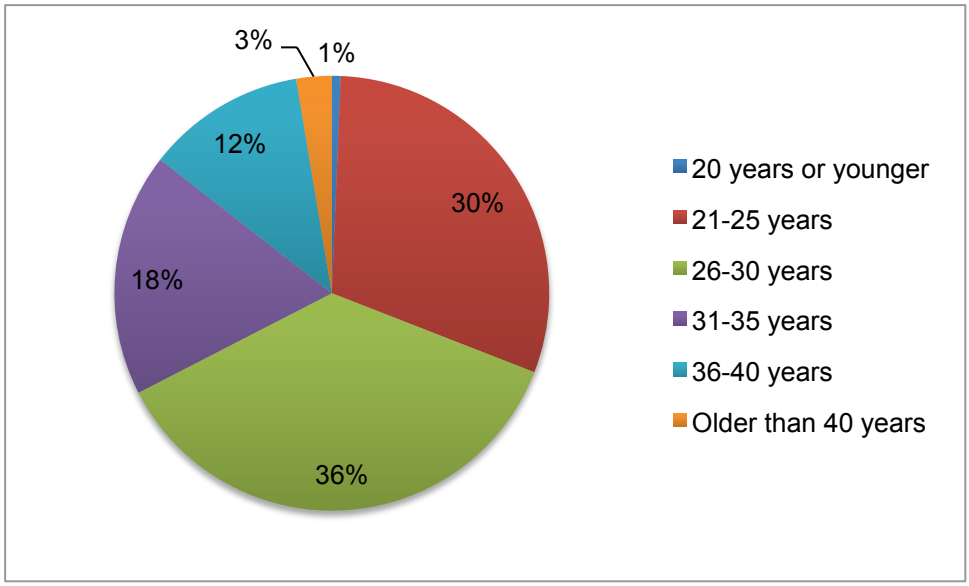


Figure 5. Age distribution of the participants answering the survey.

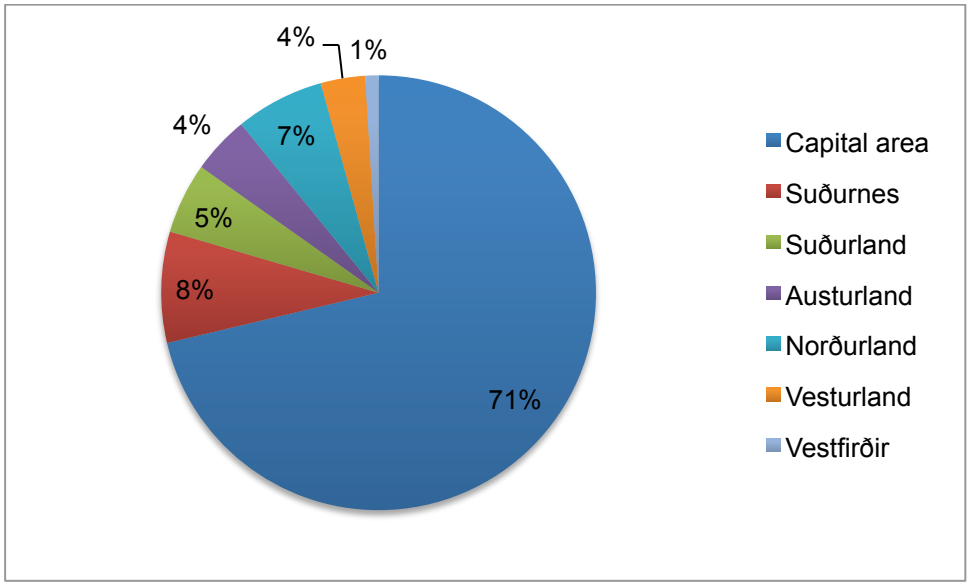


Figure 6. Residence of the participants answering the survey.

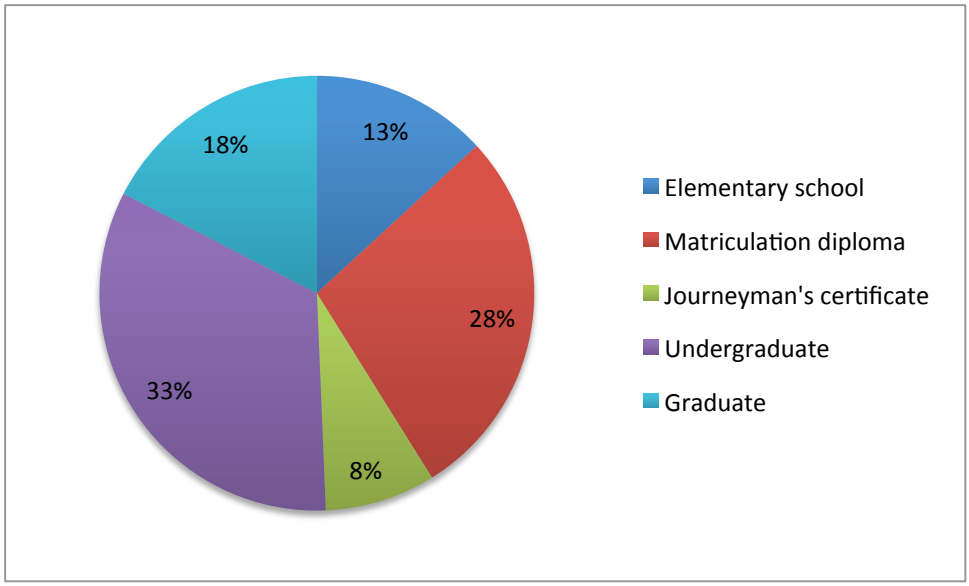


Figure 7. Education of the participants answering the survey.

In total, the participants reported to have 523 children (Table 7). Most of the children were between seven months to two years old (38%). Parents of these children were more positive towards a new product than parents of younger, and older children, respectively. Moreover, the age group seven months to two years is the main target group for the new product.

Table 7. Age of participants' children.

Age	Number of children	Percentage
0-6 months	65	12%
Seven months to two years	199	38%
3-6 years	150	29%
Older than 6 years	109	21%
Total	523	100%

4.1.2 Main results of the survey

Figures 9-10 show participants' attitude towards ready-to-eat baby food products on the market and how frequently they give their children such products. Over half of the participants (53%) were very or rather positive towards ready-to-eat baby products. The survey indicated that the participants who had a very or rather positive attitude towards ready-to-eat baby products also fed their children with them frequently, or at least once a week ($r=0.34$; $p<0.01$). It is interesting to see how many participants were positive towards ready-to-eat baby products; but only 20% were very or rather negative. The participants' negativity could be explained by two main reasons; either by the fact that the participants did not find the available products on the market versatile or interesting enough, or by the fact that some participants were not interested in such products at all. This might also be related to a growing organic trend amongst young parents, and the belief that young children should not be exposed to

processed foods but rather a “greener” alternative, like Lee and Yun (2015) showed in their review article about how consumers perceive organic food attributes (Lee & Yun, 2015). As shown in Figure 8, approximately one fourth of the participants never gave their children ready-to-eat baby food products, and the same goes for Support milk. However, by looking only at the age group seven months to two years, only 15% of the children were never given ready-to-eat baby products (Figure 9), which means that the age of the children did affect the results on this topic. This is in line with the recommendations from the Icelandic Directorate of Health; infants less than six months of age are advised not to be fed anything other than breast milk or infant formula, and children over the age of two years are advised to consume regular food (Directorate of Health, 2009). However, apart from this, the age did not have a great impact on the results.

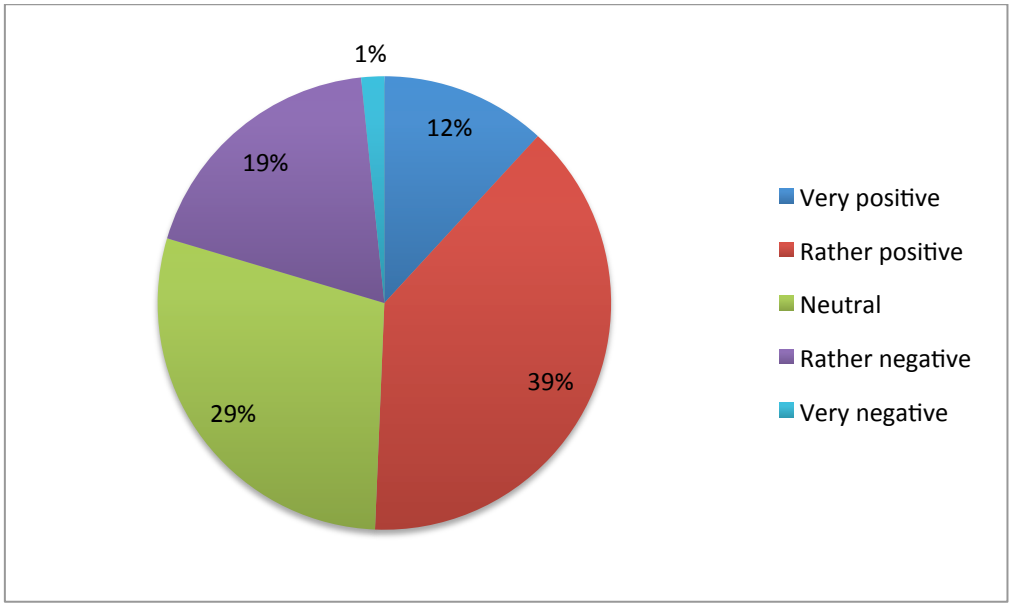


Figure 8. The attitude of the participants towards ready-to-eat baby food products.

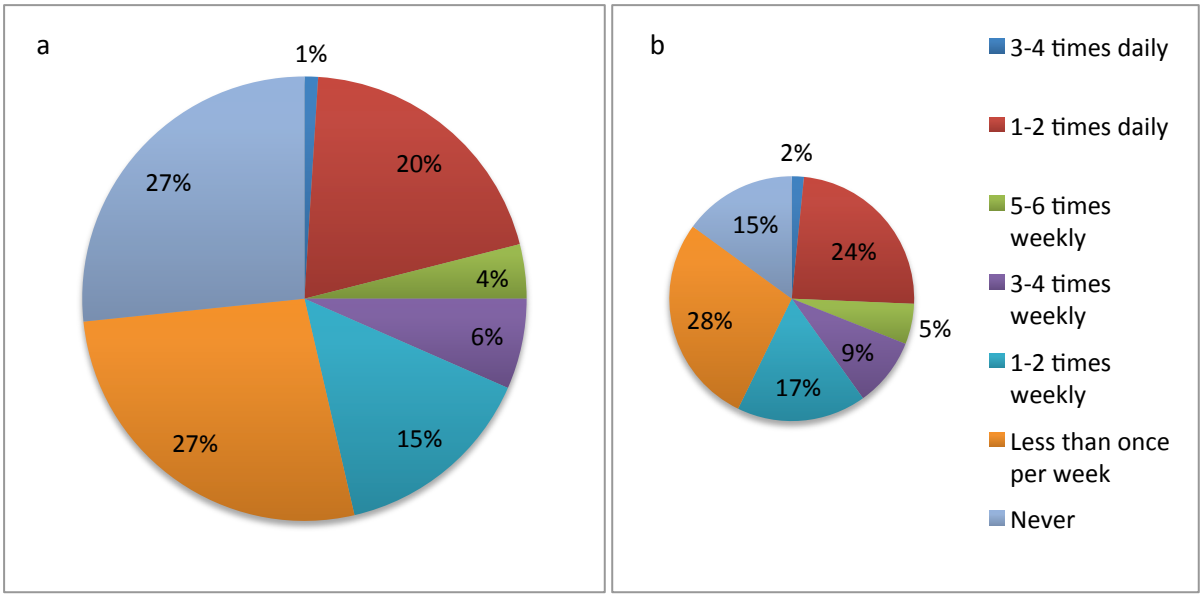


Figure 9. Figure a shows the number of ready-to-eat baby products that the children are given every day/week. Figure b shows the number of ready-to-eat baby products that only 7-24 months old children are given every day/week.

In figure 10, the participants' opinions on the variety of baby food products available on the Icelandic consumer market are summoned. It was interesting to see that only 4% of the participants found the variety to be very good. However, approximately one third of the participants were rather satisfied with the variety. A considerably high proportion (32%) of the participants found the baby food variety on the Icelandic consumer market rather or very poor. This indicates that although large improvements have been made in the last few years, there is still a room for improvement on the market for ready-to-eat baby products.

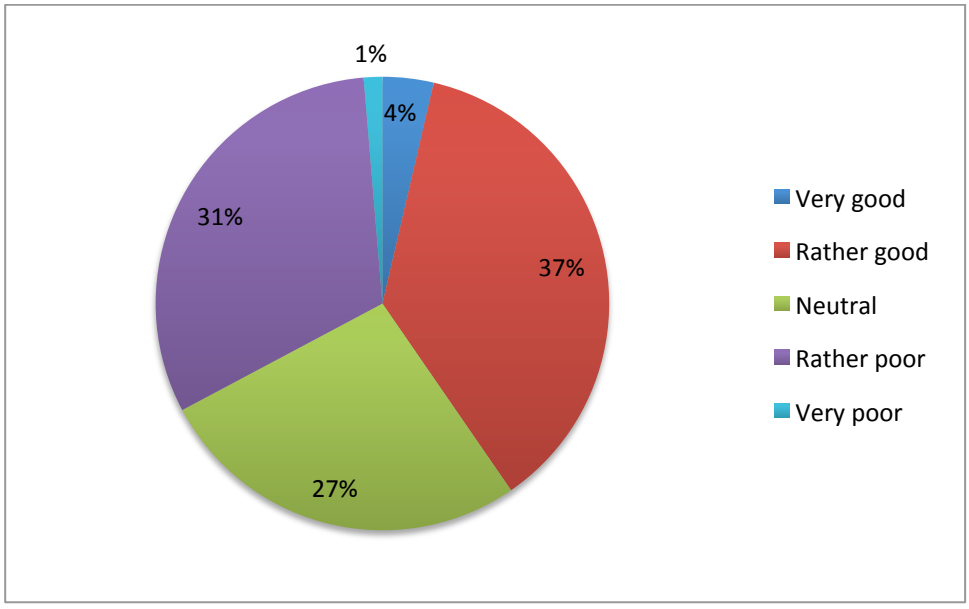


Figure 10. The participants' opinions on the variety of baby food products on the Icelandic consumer market.

As Figure 11 demonstrates, only 18% of the participants waited the first year to give their children fermented milk products. That was very interesting seeing that according to the Icelandic recommendations on infants nutrition, parents should wait until their children are at least one year old before feeding them yoghurt or other fermented milk products, due to the high protein content in these products (Directorate of Health, 2009). However, almost half of the participants (47%) gave their children fermented milk products between 6-10 months age. These results indicate that there is a need of a fermented milk product that satisfies the needs of children less than one year of age.

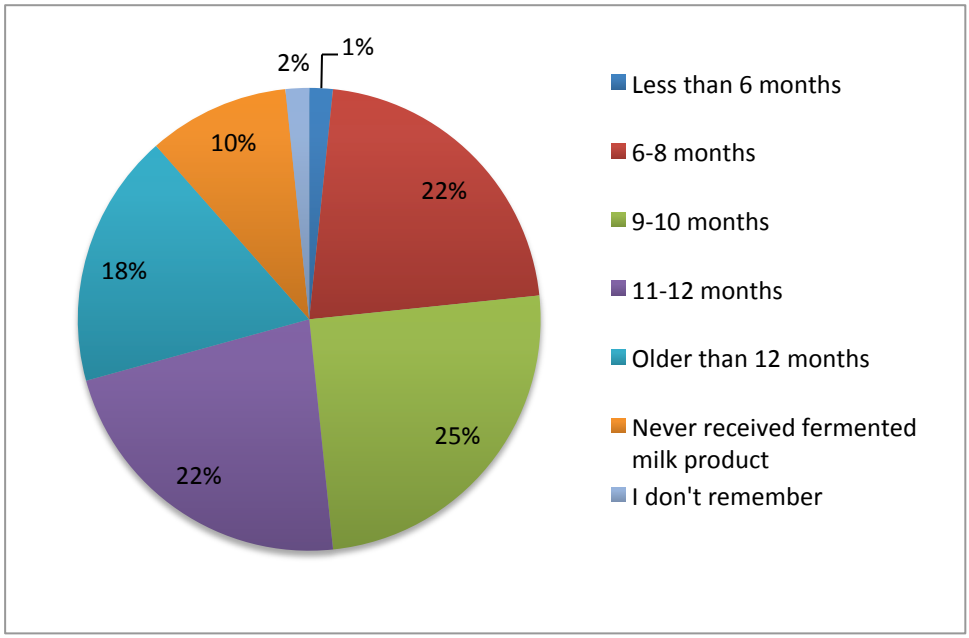


Figure 11. Age of children at the start of consumption of fermented milk products like yoghurt.

The majority of the participants had a very or rather high interest in the new product, or 83% (Figure 12). Participants who were interested in the new product were more likely to feel like the product should be enriched with vitamins ($r=0.31$; $p<0.01$), and were also more likely to pay more for the new product than other similar milk products on the current market ($r=0.38$; $p<0.01$).

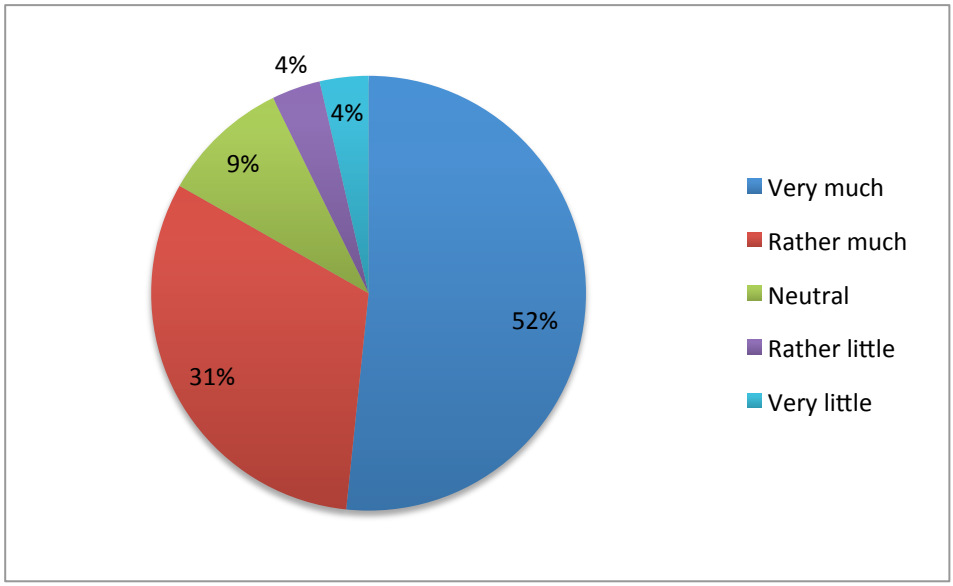


Figure 12. The figure shows the interest of all the participants in the product to be made.

Figure 13 shows what qualities the product should feature according to the participants of the survey. It was possible to select more than one feature and there was also an "other" possibility, where participants could write their opinion about what other qualities the product should feature. The "other" possibility got 29 marks, but none of the participants wrote a more detailed description in the provided comments field of what qualities this would apply to. About 95% of the participants wanted the product to be nutritious. About 74% wanted the product to have a good taste. About 57% wanted the product to be an Icelandic production, and approximately 53% of the participants wanted it to be cheap. Approximately 47% of the participants wanted the product to be organic, but only 8% wanted the product to have added flavour (e.g. of fruits or berries).

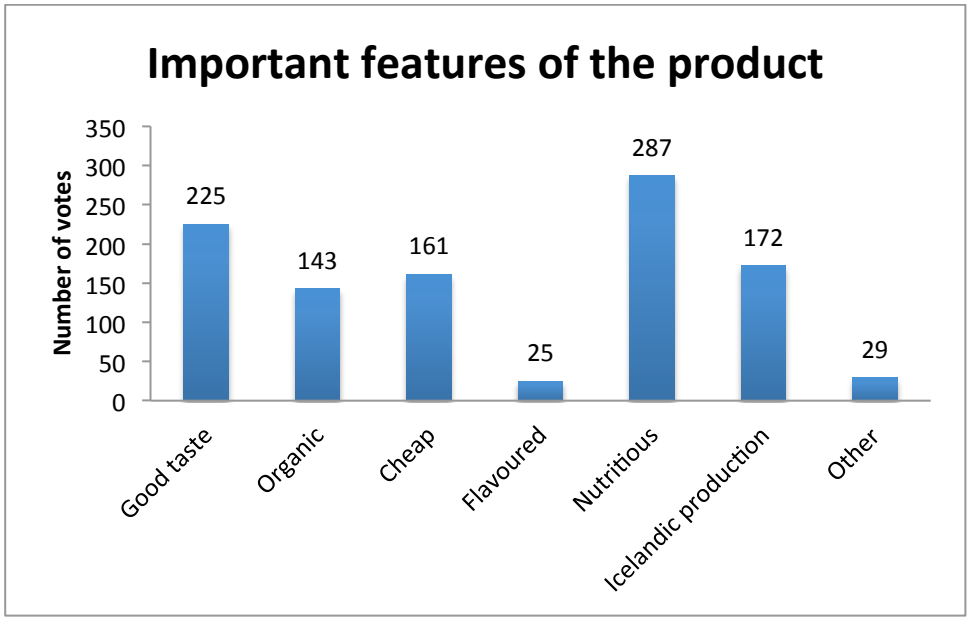


Figure 13. The participants' opinions on what features are important for the product.

When asked about an appropriate price for the product, answers showed that a similar price as currently available milk products should be appropriate, which is approximately 105-114 ISK, but the price ranges from 85-149 ISK depending on if the product is organic, or with added lactase, flavours etc. However, when people were asked how likely they were to pay more for the product than other currently available milk product, approximately 80% said that it was rather or very likely that they would pay more for this product than the milk products currently on the market.

The last question in the survey was an empty text box where people could leave their comments about single questions. In total, 20 people left comments, many quite interesting. Some of them were about how some questions were irrelevant for some of the participants. For example, some questions unintentionally were more relevant for parents of children within a certain age group than others. Some questions could thus have been phrased in a better way so they were suitable for everyone, or the survey could have been put forward only for parents of children 7-24 months, i.e. narrower group of participants. The comments can be viewed in appendix 2.

4.1.3 Multivariate analysis

The data from the survey was also analysed using a multivariate analysis to give an overall view of how the questioned parameters related to each other. Figure 14 shows the results from the analysis and indicates a wide distribution in the data, and that many factors influenced the way the participants answered the questions. The multivariate analysis indicated that older parents were less interested in the product, which could partially be explained with the fact that the older parents tended to have older children as well. It could be that younger children of older parents have a similar diet as their older siblings, and the parents are consequently less interested in a new product that only serves the dietary needs of a younger child. However, parents interested in nutrition can be considered more likely to buy healthy products even though they only serve the dietary needs of one child. Nutritional literacy was researched in the United States and it appeared to be low, which affects the health (Carbone & Zoellner, 2012). It is possible that this could affect the results of this survey as well.

Furthermore, the analysis indicated that parents that had not introduced Support milk to their children were generally parents of children that had not yet reached the intended age for use of Support milk (children less than six months), or had children who were older than two years of age and thus too old to benefit from it (Directorate of Health, 2009), which would have supported focusing only on the target group, i.e. parents of 7-24 months old children. Some of them had never been given milk products at all. The Support milk was introduced to the Icelandic market in 2003 and is intended for infants from six months to two years, as stated previously (Mjolkursamsalan, 2003). The fact that Support milk came on the market in 2003 could also be an influence on the attitude of older parents towards Support milk and the new product. However, no such notions can be made from the survey.

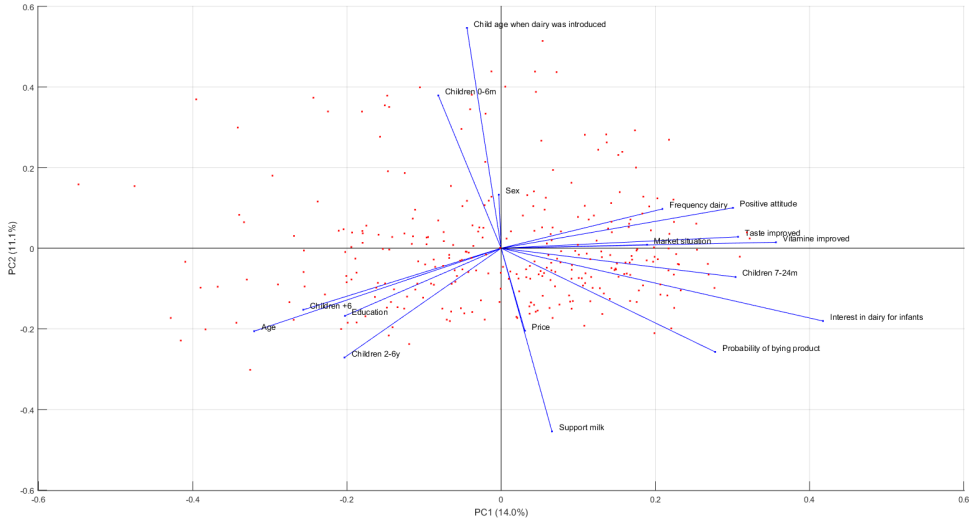


Figure 14. The figure shows the a PCA score plot with the results from a multivariate analysis with all the variables in the survey taken into account.

4.1.4 Survey summary

Parents of young children are a very interesting group to study. They have very strong opinions on everything regarding their children. Overall, the survey showed that participants were generally interested in having a new fermented product intended for children under the age of two years on the Icelandic market. Most of the parents that were interested in the product were the parents of children aged seven months to two years, which is the major consumer group that the new product would be intended for. The gender of the participants did not seem to have a major effect on participants' interest in the product. Education, however, was hard to estimate since the education level correlated with the age of the participants. Thus it is hard to know for sure whether it is the age or the level of education that had more effect on the participants' interest. Older and more educated parents seemed to be more sceptical towards such products than younger and less educated parents. Overall, the results of the survey indicated that dairy producers in Iceland might have an incentive in creating a product that appeals to younger parents in Iceland, and that there is a possibility of successfully producing, marketing and selling such a product on the Icelandic market.

4.2 Pre-trial

In the pre-trial, two types of samples were analysed; a yoghurt made from Support milk and a yoghurt made from whole milk and enriched with a vitamin-blend that is used in the production of Support milk. The physicochemical properties of a regular yoghurt, as available in the supermarket from the same producer, were also analysed for comparative reason. The following chapters compile the results of these experiments.

4.2.1 Chemical analysis

The samples of support yoghurt and regular yoghurt enriched with the Support milk vitamins were sent to chemical analysis in order to gain information about their chemical composition. The chemical analysis was performed on May 23rd 2016, 4 days after the production, and the results are compiled in Figure 15.

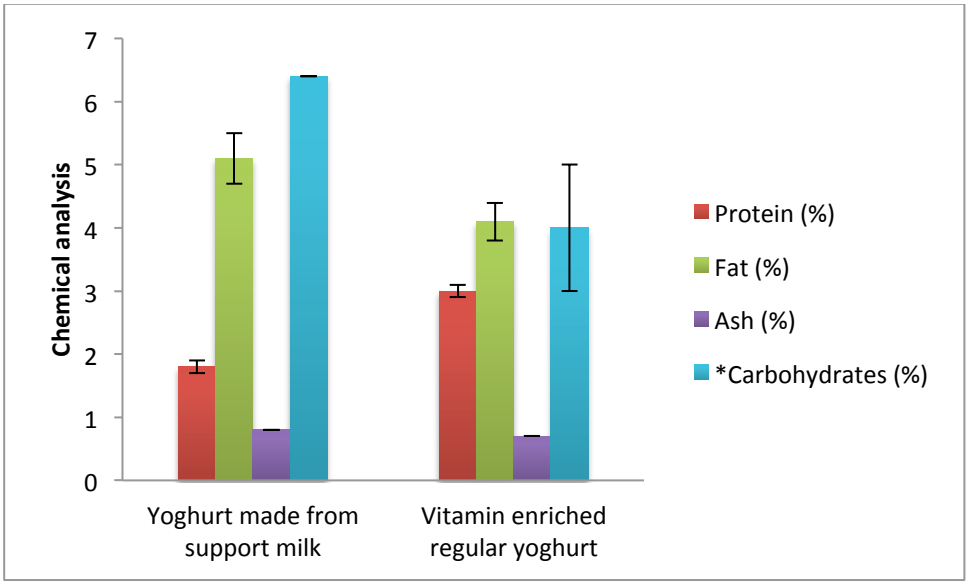


Figure 15. Chemical composition of the yoghurt products in the pre-trial.

*Carbohydrates of the formulations were determined by difference calculations.

As Figure 15 shows, there is a slight difference between the support yoghurt and the regular yoghurt enriched with the Support milk vitamins. The protein content was higher in the regular yoghurt than in the support yoghurt, explaining the thicker consistency observed in the regular vitamin enriched yoghurt. The higher protein content in the regular yoghurt could be explained by the difference in the protein content of the raw material used to produce these products. The regular yoghurt was made from whole milk (approximate protein content of 3,4%), while the support yoghurt was made from Support milk (approximately 1,8% protein). The support yoghurt had a slightly higher fat content than the regular yoghurt, as well as calculations of carbohydrates by difference indicated a higher carbohydrate content in the support yoghurt than in the vitamin enriched regular yoghurt. This might partly be explained by the differences in the composition of the raw materials used, Support milk and whole milk, respectively. Only a minor difference was observed in the ash content between the samples, indicating a similar amount of vitamins and minerals in the two products. However, no detailed assessment of the composition of these vitamins and minerals were performed in the pre-trial.

As seen in Figure 16, the water content was higher in the vitamin enriched regular yoghurt than in the support yoghurt but the difference was not significant.

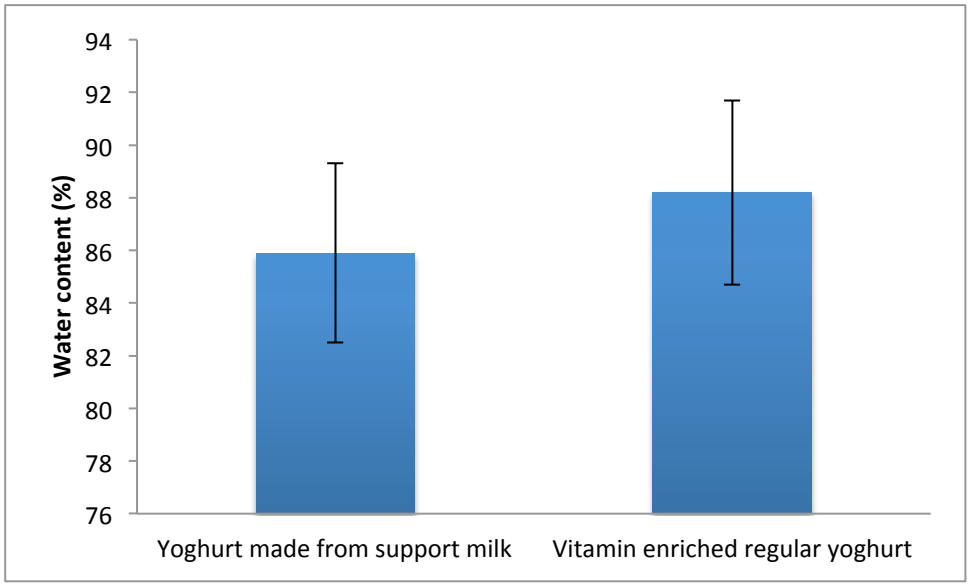


Figure 16. The water content in the yoghurt products in the pre-trial.

4.2.2 Physicochemical properties

4.2.2.1 WHC

During the WHC analysis the support yoghurt ran directly through the filter, leaving no remains in the filter after the centrifugation. This indicated that the formulation of the support yoghurt had very little to no ability to retain water (Table 8). However, the vitamin enriched regular yoghurt showed a very reasonable and high WHC of 82.7 ± 1.5 %. This indicated that if Support milk is to be used for the production, the formulation needs to be improved to yield a reasonable WHC of the product.

Table 8. The WHC of the yoghurt products in the pre-trial.

Analysis	Yoghurt made from support milk	Vitamin enriched regular yoghurt
Water holding capacity (%)	0.0 (0.0)	82.7 (1.5)

Results are presented as the average of duplicate measurements. Standard deviations of the results are given within brackets.

4.2.2.2 pH and viscosity

Since yoghurt is produced by fermentation of milk using live bacterial cultures, the pH is an important quality indicator for both the level of fermentation of the yoghurt, as well as changes in pH during storage, which can indicate the microbial stability of the product.

A slight difference was noticed in the pH values of the samples. As shown in Figure 17, the two regular yoghurts (both with and without vitamin enrichment) were more acidic than the support yoghurt. If the fermentation process is stopped either too early or too late, the pH of the yoghurt may become inadequate for optimal casein coagulation (Masulli, 2016). This can result in increased syneresis and lack of thickness in the yoghurt. The high pH observed in the support yoghurt may thus explain for the high syneresis (and non-existent WHC) observed in the support yoghurt in the pre-trial.

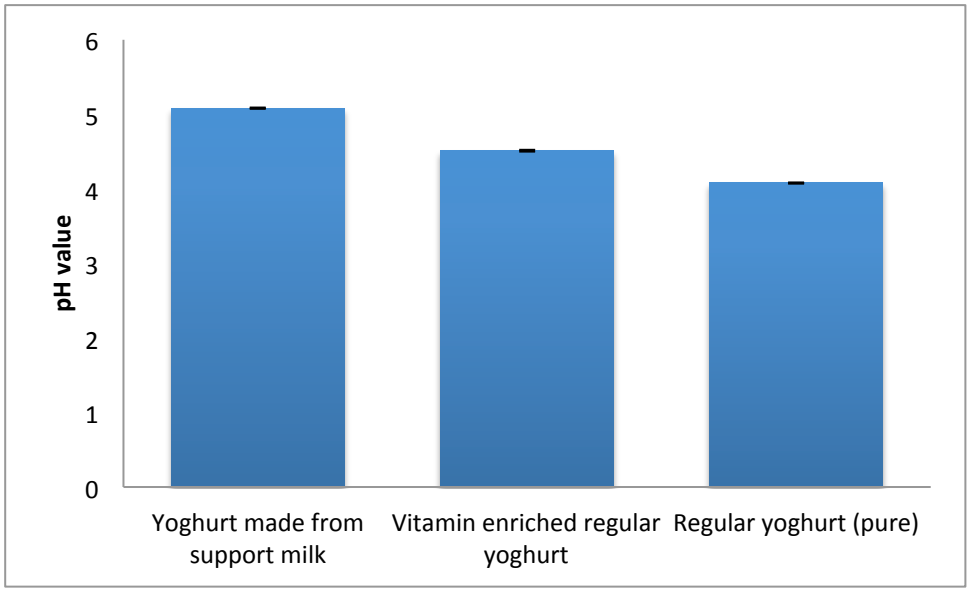


Figure 17. The pH value of the products in the pre-trial.

The viscosity was higher in the regular yoghurt than in the support yoghurt (Table 9) indicating a thicker consistency in the regular vitamin enriched yoghurt. This correlates with the protein amount being higher in the regular yoghurt than in the support yoghurt. Furthermore, this also confirms the inadequate level of the fermentation obtained in the support yoghurt.

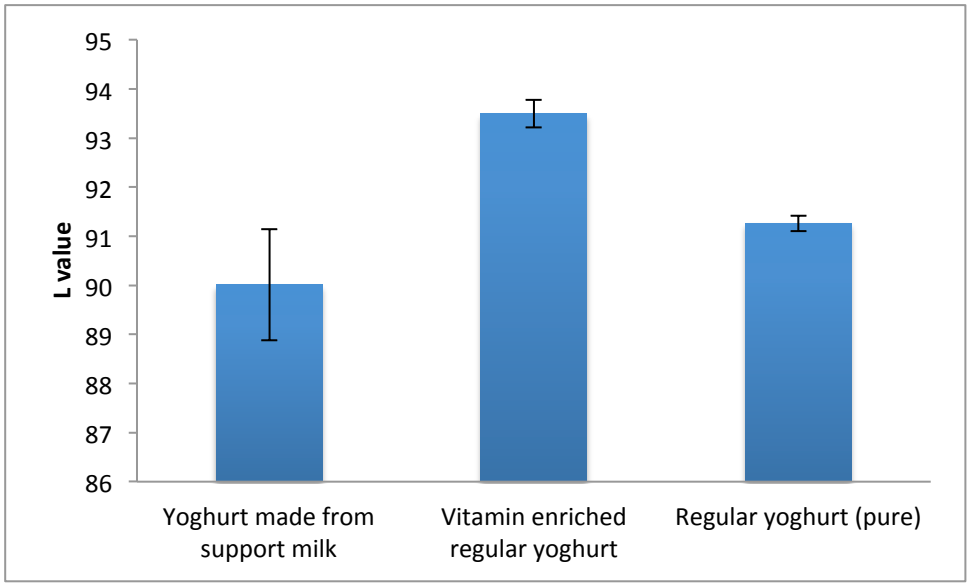
Table 9. The viscosity level in the yoghurt products in the pre-trial.

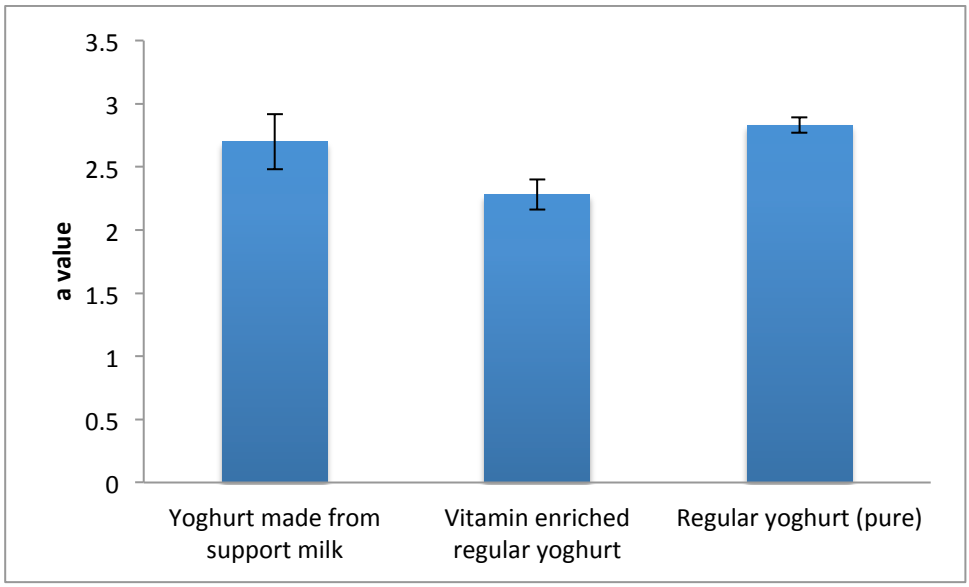
Analysis	Yoghurt made from Support milk	Vitamin enriched regular yoghurt
Viscosity (Pa*s)	116.3 (6.028)	958.3 (0.577)

Results are presented as the average of duplicate measurements. Standard deviations of the results are given within brackets.

4.2.2.3 Colour analysis

The L-value was higher in the vitamin-blend yoghurt than in the support yoghurt and the regular yoghurt (control) (Figure 18), indicating a lighter appearance of the vitamin enriched formulation compared to the other two. However, there was not a significant difference between the support yoghurt and the control. The regular yoghurt from the supermarket had a higher a-value than the vitamin enriched yoghurt, as shown in Figure 18. There was, however, no significant difference in the a-value between the support yoghurt and the other two products. A significant difference between the b-values was though observed in all samples. The support yoghurt had the highest b-value, and the vitamin enriched yoghurt the lowest.





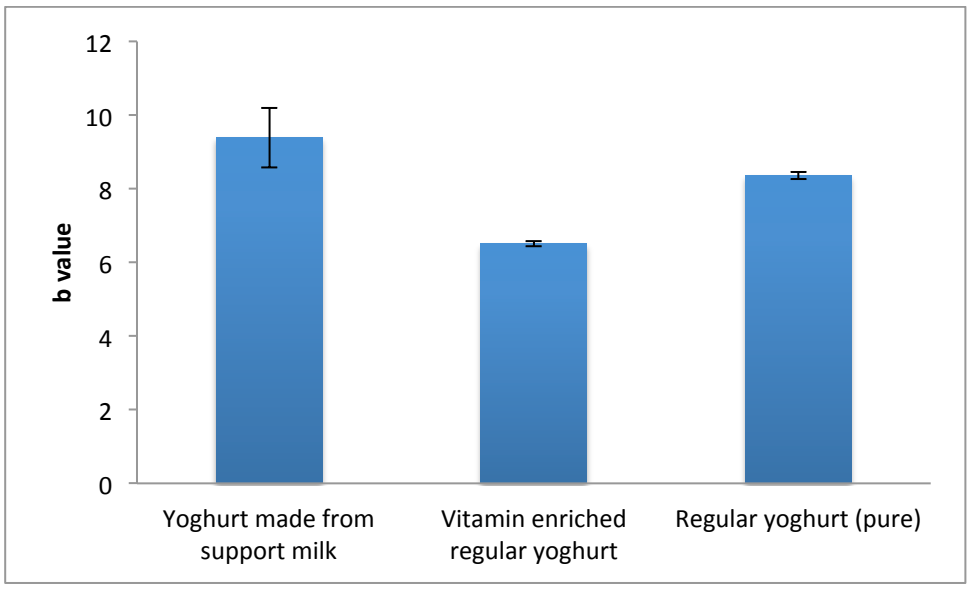


Figure 18. The colour L-, a- and b-values of the products in the pre-trial.

4.2.3 Microbiological analysis

The average amount of lactic acid bacteria was considerably higher in the regular yoghurt than in the support yoghurt (Table 10). However, the two duplicates of the regular yoghurt showed very different results, explaining the large standard variation observed in the regular yoghurt. Counts for both products still indicated a good survival rate of lactic acid bacteria in both products. Counts of moulds and yeasts were below 20 cfu/g, indicating that the products were both safe for consumption and were of good microbial quality.

Table 10. The lactic acid bacteria in the yoghurt products in the pre-trial.

Analysis	Yoghurt made from Support milk	Vitamin enriched regular yoghurt
Lactic acid bacteria (Log)	3.3 (0.0)	5.1 (5.0)

Results are presented as the average of duplicate measurements. Standard deviations of the results are given within brackets.

4.2.4 NIR

A Near infrared (NIR) spectroscopy analysis was performed on the samples in the pre-trial (Figure 19), revealing some minor differences in the composition of the two samples. The spectra revealed dominating peaks, referring to the stretching of the O-H bonds of water, at approximately 1500 and 1950 nm, respectively, as well as a smaller peak at approximately 1000 nm. Smaller peaks, due to the stretching of C-H in the lipids present, were also observed at approximately 1200 and 1800 nm, in agreement with the observations of Andersen et al. (2010) on the assessment of NIR absorption peaks in cream-cheeses. Some spectral differences were also observed in the spectral region between 1900 and 2200 nm, relating to the first overtones and combination bands of the nitrogen bonds in the present proteins, while combination bands from the CH, CH_2 and CH_3 bonds of the present fatty acids, and an effect of the carbohydrates in the samples were observed at higher wavelengths (2100-2400 nm).

Based on the spectra, the main differences between the samples were observed in the water O-H stretch peak at approximately 1450 nm, as well as in the spectral region above 2100, indicating differences in the protein, lipid or carbohydrate composition of the two samples. However, further analyses are needed to explain these differences in more detail.

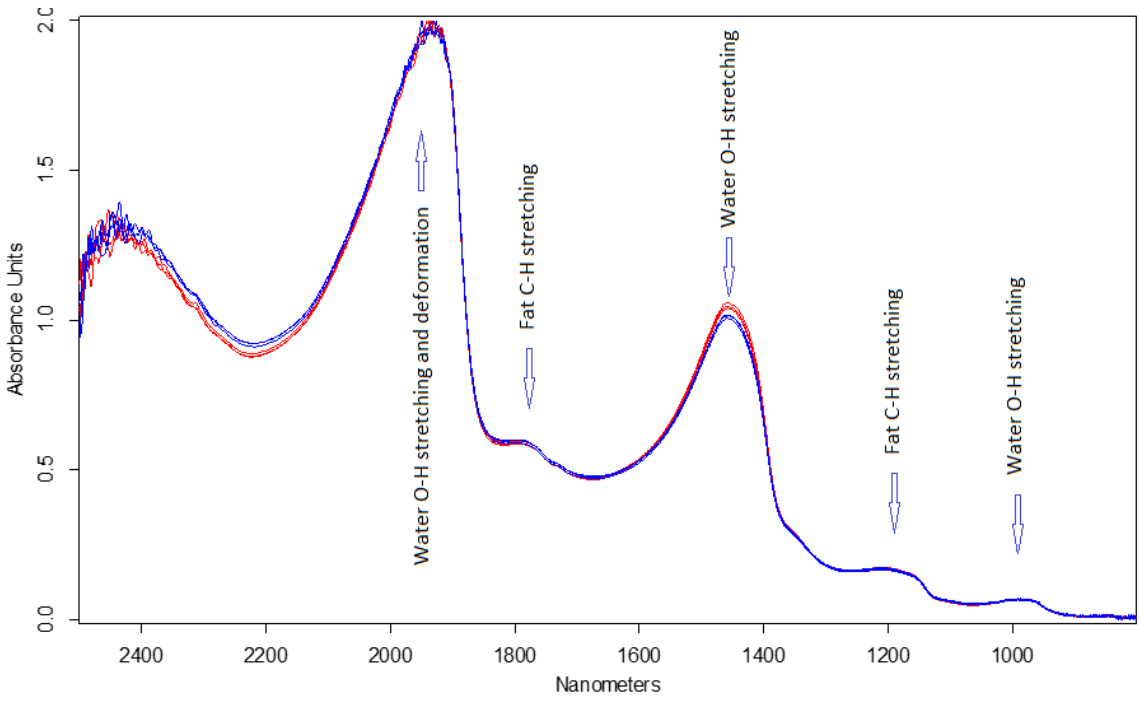


Figure 19. NIR spectra of samples in the pre-trial. Red spectra represents the vitamin enriched yoghurt, while the blue spectra represents the support yoghurt.

4.2.5 NMR

The transverse relaxation time analysis of the yoghurt samples indicated the presence of three proton populations in the three formulations. The fastest relaxing component T_{21}, representing 2.5-3.5% of the protons in the samples, had a relaxation time in the range from 23-38 ms (Figure 20). The intermediate relaxation time T_{22} was observed in the range from 156-238 ms, while the slowest relaxing component took values in the range from 411-610 ms (Figure 20). The T_{21} relaxation time, and corresponding population A_{21} are believed to mostly represent non-crystalized lipids in fat globules (Bertram, Wiking, Nielsen, & Adersen, 2005), but water in close association to the milk proteins might also contribute to some extent. This is in agreement with the observations of Andersen et al. (2010), who stated that a clear distinction between loosely bound fat and strongly restricted water can sometimes be challenging in the LF-NMR relaxation data. Bertram et al. (2005) furthermore observed a linear relationship between cream (lipid) content and a T_2 population with a relaxation time in the range from 10 to 100 ms. The fat in the yoghurt may therefore contribute to either the fast population A_{21} and/or to the intermediate relaxing population A_{22}. According to Bertram et al. (2005) the T_2 relaxation characteristics for the liquid fat populations (10 to 100 ms) was dependent on the lipid composition of the samples. Samples with a low content of long-chain fatty acids showed clear distinction between a minor population at 10 ms and a major population between 50 and 100 ms, while milk samples with a high content of long-chain fatty acids showed a broader distribution of relaxation times, where the peak could not be as clearly separated into two populations. This indicates that the relaxation times may also imply the degree of saturation of the present fatty acids, in agreement with the observations of Le Botlan and Helie (1994). Furthermore, according to Le Dean et

al. (2001, 2004), both the casein and lactose concentrations can have a predominant effect on the state of water in milk protein dispersions.

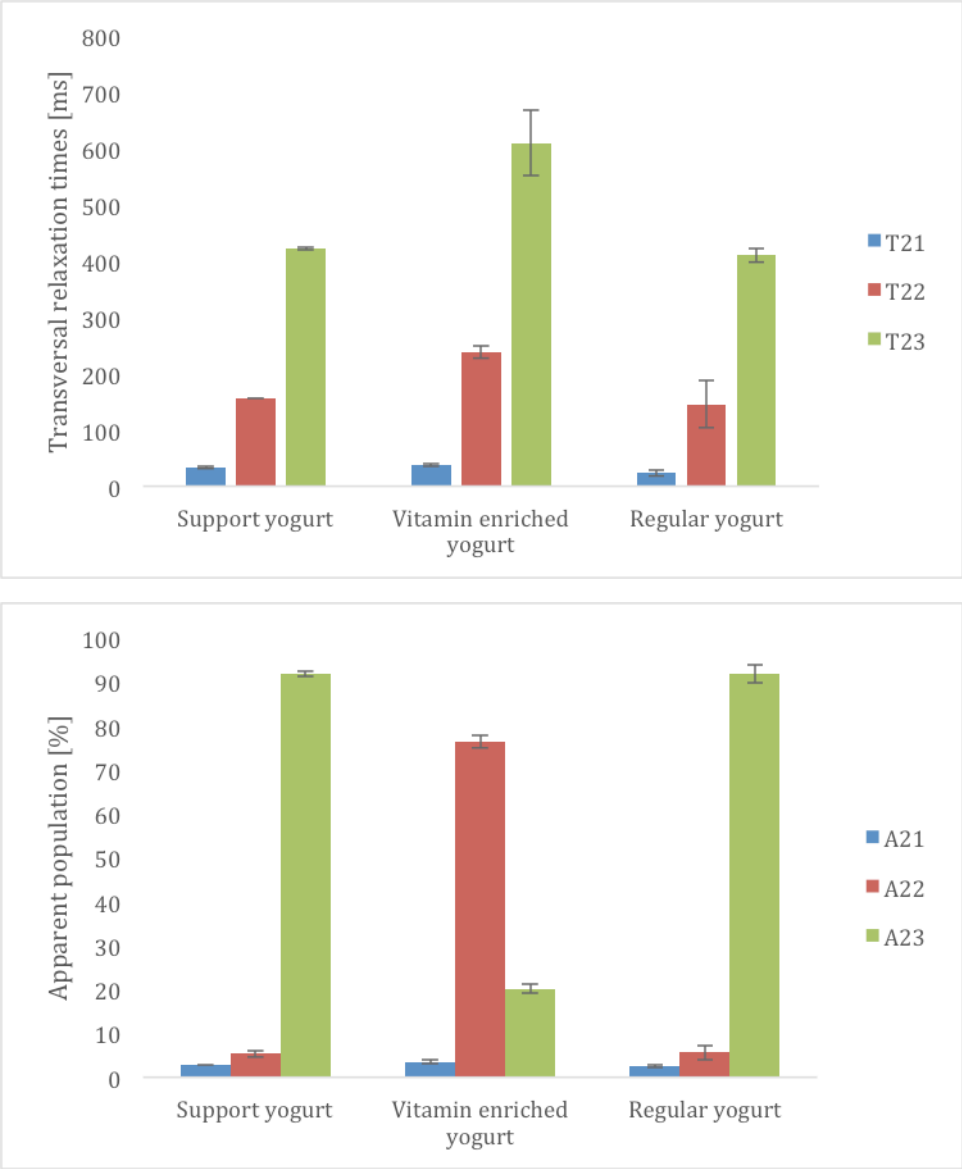


Figure 20. Transversal relaxation times (T_{21}, T_{22} and T_{23}) and their apparent populations (A_{21}, A_{22} and A_{23}) for the studied yoghurt formulations in the pre-trial.

However, since a very large variation was observed in the A_{22} population it is clear that this population cannot be explained by the fat content alone. It is therefore assumed that this component also represents restrained water within the coagulated casein network, while the longest relaxation time corresponds to more freely moving water. An obviously higher amount of water in the intermediate relaxation population was observed in the vitamin-enriched yoghurt compared to the other two products. This is in good agreement with the significantly higher WHC observed in the vitamin-enriched yoghurt. This may also indicate the level of effective casein coagulation, which in turn affects the ability of the casein network to retain water in the yoghurt. The support yoghurt on the other

hand contained a very large amount of more freely moving water, while limited coagulation effects and water retention were observed in the T_2 data for this sample, in agreement with the obtained WHC results. Interestingly, the NMR results of the regular yoghurt from the supermarket showed a more similar proton distribution and characteristics to the support yoghurt than the vitamin-enriched yoghurt.

4.2.6 Pre-trial summary

Overall, the results from the pre-trial gave a good impression of the products and indicated that there is a possibility of making a yoghurt with a low protein content. An informal tasting of the products was, furthermore, very promising. A slightly sweet taste was noted in the support yoghurt, which was anticipated as it was made from Support milk, which has higher carbohydrate content than whole milk. A hint of iron was also noted in both the support yoghurt and the vitamin-blend yoghurt, which indicated that the iron remained in the products during the production process. However, the thin consistency, and low WHC of the support yoghurt, gave an indication that the formulations required optimization towards these parameters, which led to the set-up of the main experiment.

4.3 Main experiment

The main experiment was more extensive and the physicochemical properties of four formulations were assessed in this round. Furthermore, the shelf life and stability of the products were also analysed in the main experiment, by performing measurements at four storage points, with the starting point at two weeks from production.

4.3.1 Chemical analysis

Protein, ash and fat content of the formulations were only measured at the starting point, but water was measured throughout the storage time, since it (along with WHC) was assessed to be the most sensitive towards any degradation changes during storage. Figure 21 shows the results from the chemical analysis.

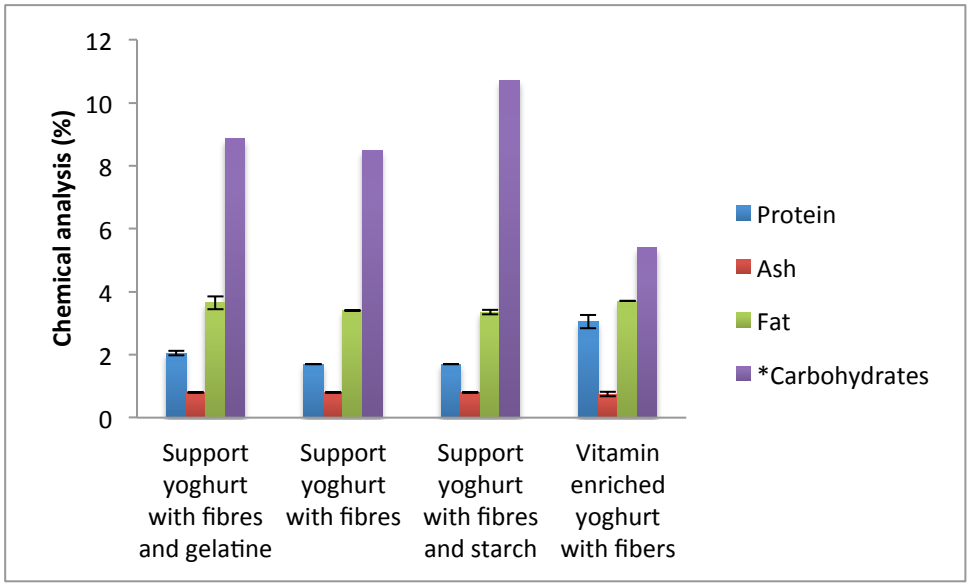


Figure 21. The amount of protein, fat and ash in the samples in the main experiment.

*Carbohydrates of the formulations were determined by difference calculations.

The support yoghurt formulation containing gelatine had a higher protein content than both the support yoghurt containing maize starch, and the support yoghurt without any thickening agent. This caught attention, since they were all made from the same dairy raw material. The reason for this may be the fact that it contained gelatine, which is a protein, and therefore adds to the protein amount in the product, while the other formulations did not contain any protein-containing additives. As expected, the regular vitamin enriched yoghurt had also a higher amount of protein than the other formulations, due to the higher amount of protein in the raw material it was made from (whole milk). The fat content of the formulation containing fibres and gelatine and the vitamin enriched yoghurt, were slightly higher than the fat content of the support yoghurt containing fibres and the support yoghurt containing fibres and maize starch. The amount of carbohydrates ranged from 5.4-10.7% in the formulations. The formulation containing maize starch had the highest amount of carbohydrates, which may be attributed to the maize starch. The vitamin enriched regular yoghurt had the lowest amount of carbohydrates, which was expected since it was made from whole milk, which contains a lower amount of carbohydrates than Support milk, which the other formulations were made from. The ash content was, however, very similar in all formulations.

As Figure 22 demonstrates, some changes were observed in the water content of the samples throughout the storage time. However, the changes were not significant ($p>0.05$), which indicated that all samples were relatively stable in terms of water content.

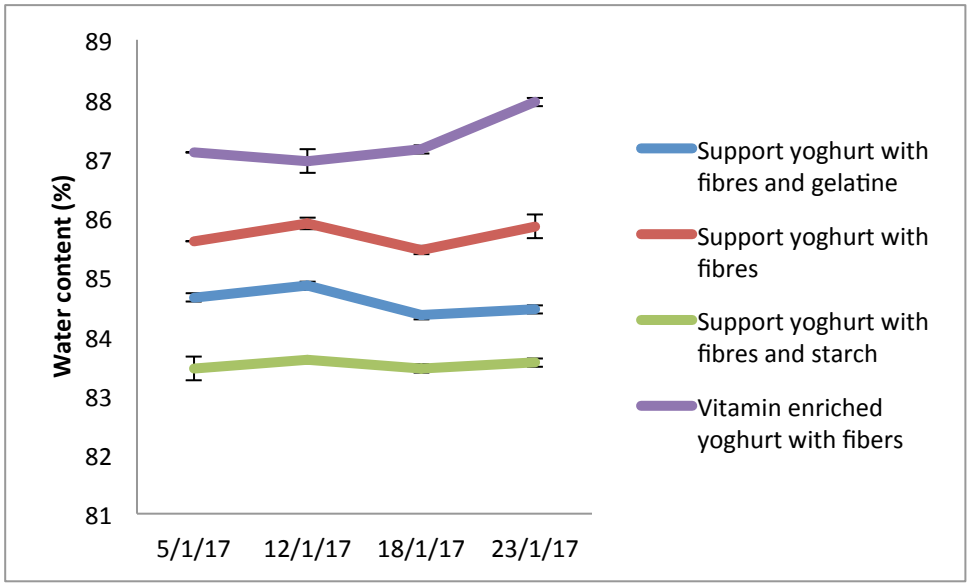


Figure 22. The water content in the samples throughout the storage time.

4.3.2 Physicochemical properties

4.3.2.1 WHC

Figure 23 shows the WHC of the formulations. The WHC of the support yoghurt containing fibres and maize starch, and the vitamin enriched regular yoghurt were very high at the beginning and significantly higher than in the support yoghurt containing fibres and gelatine or the support yoghurt only containing fibres. The ability to retain water decreased throughout the storage time in all formulations except the gelatine yoghurt, where it was slightly higher at the end of storage than at the beginning, but there was a problem with the measurement at third storage point in this formulation. All formulations still had a reasonable WHC at the endpoint except for the support yoghurt that only contained fibres, which ran directly through the filter, leaving no remains in the filter after centrifugation during the third and fourth storage points. This suggests that the fibres alone are not a sufficient stabilizer for this production and there is a need of using either maize starch or gelatine along with the fibres.

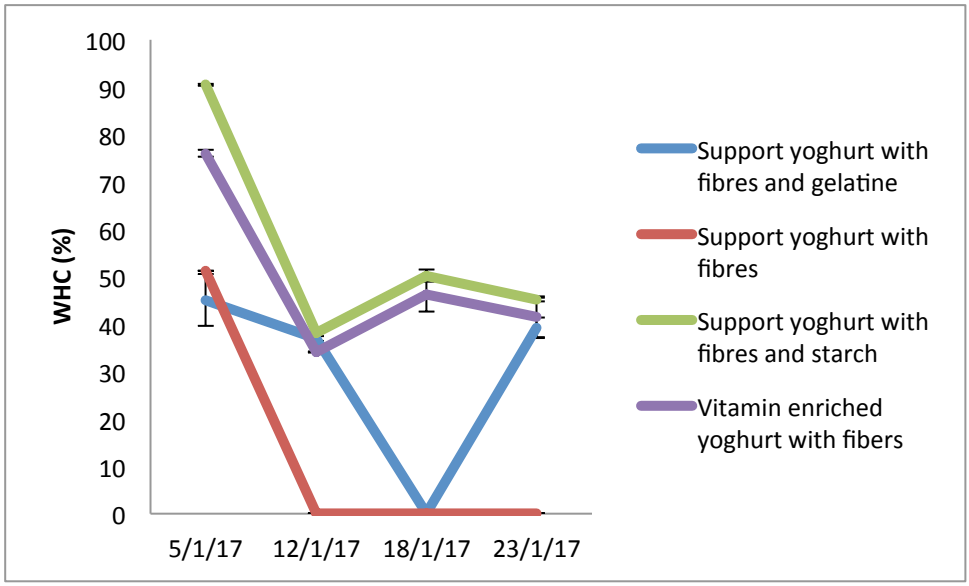


Figure 23. The water holding capacity of the samples throughout the storage time.

4.3.2.2 pH

The results from the pH measurements are shown in Figure 24. The figure shows how the pH value altered throughout the storage time. The vitamin-enriched yoghurt had a little higher pH value than the other products. The pH value decreased slightly throughout the storage time in all samples.

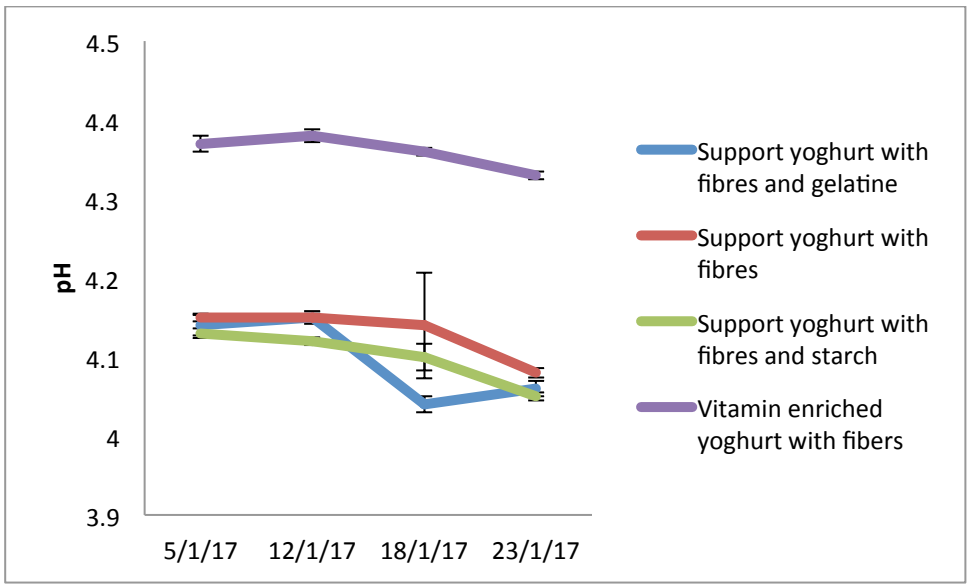
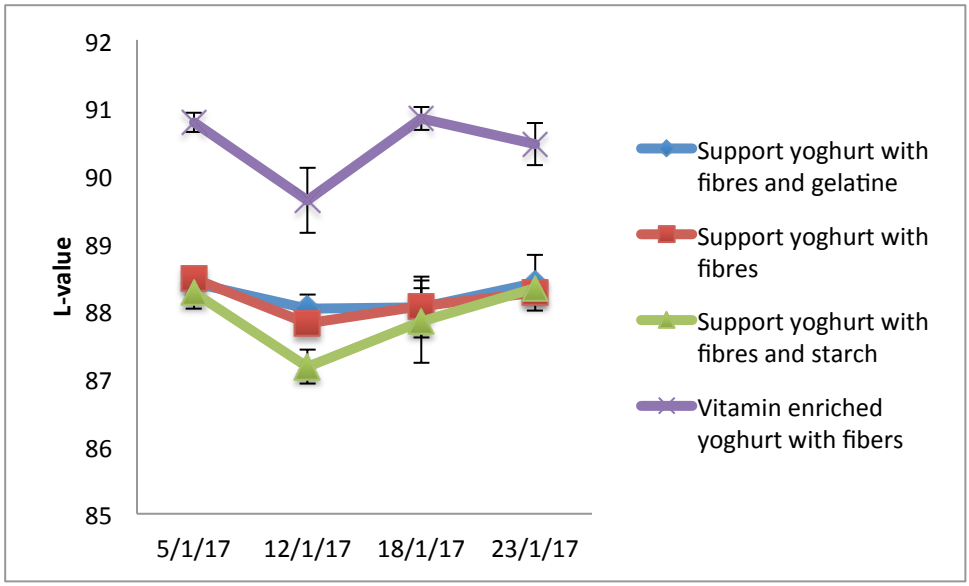


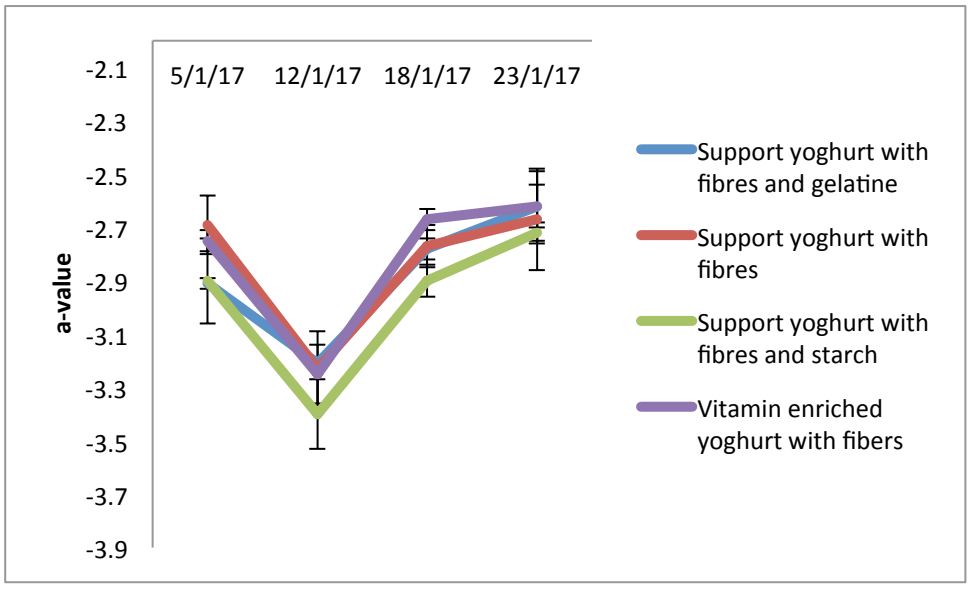
Figure 24. The pH value of the samples throughout the storage time.

4.3.2.3 Color analysis

As Figure 25 shows, there was not a significant difference between the samples in the a-value, but all samples had a negative a-value, indicating a greenish shade. All samples behaved in the same way

and took a sudden dip in the a-value at the second measurement point. This caught attention, but the difference was not significant though. However, in L- and b-values, the vitamin enriched regular yoghurt was different from the other samples. All samples had a high L-value, indicating a bright colour, though brighter in the vitamin enriched yoghurt. All formulations had a positive b-value, indicating a yellowish shade, but the support yoghurts had a slightly higher b-value than the vitamin enriched yoghurt. Overall, the colour analysis indicated that the colour of the formulations was rather stable throughout the storage time.





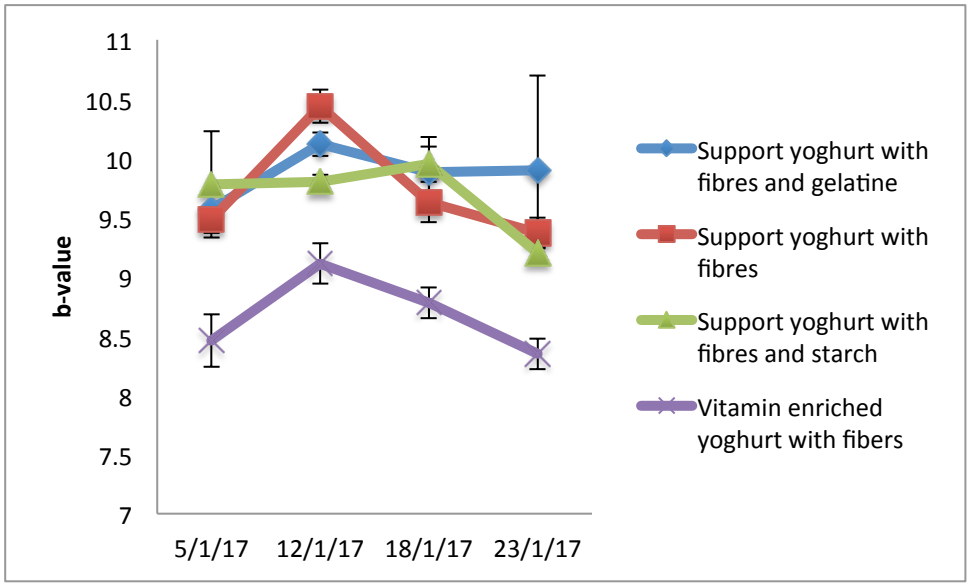


Figure 25. The colour L-, a- and b-values of the products in the main experiment.

4.3.3 Microbiological analysis

All formulations were very stable in terms of the lactic acid bacteria, and the survival rate of the bacteria was very high (Figure 26). The amount did not decrease significantly during storage. There was no statistical difference between the samples in lactic acid bacteria counts. The counts of moulds and yeasts were below 20 cfu/g in all samples throughout the storage time, which is in line with the survival rate of the lactic acid bacteria. Thus, the moulds and yeasts did not take over the survival of the lactic acid bacteria. In this experimental round, an optimal pH value of 4.0-4.5 was acquired, resulting in an optimal growth of lactic acid bacteria.

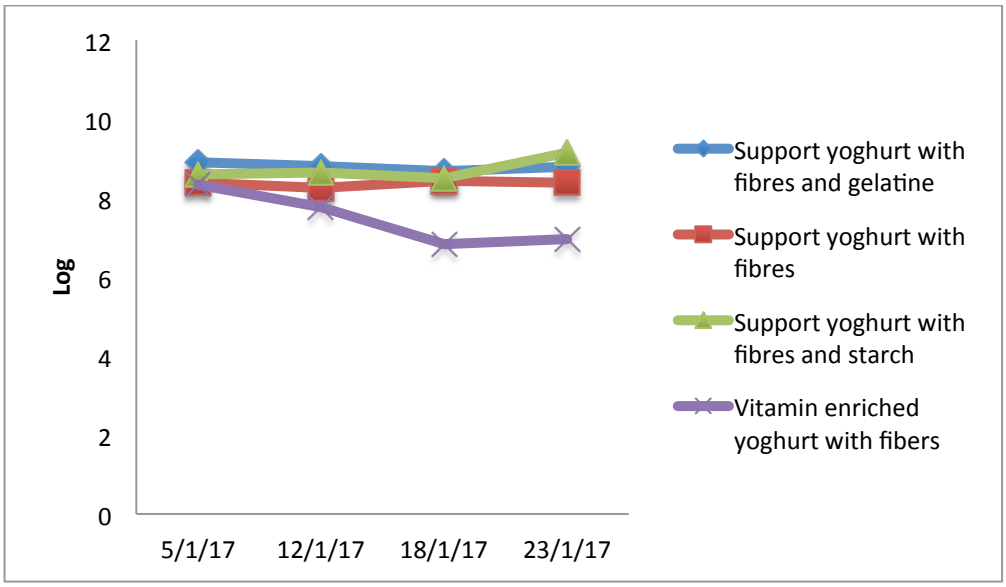


Figure 26. The amount of lactic acid bacteria in the products throughout the storage time, showed in log numbers.

4.3.4 NIR

NIR analysis of the samples in the main-trial revealed very similar spectra as observed in the pre-trial, and peaks could be assigned in a similar way (Figure 27). However, spectral differences were larger between the samples, as well as in a wider spectral range than before, indicating broader differences in the composition of the samples. To explain these differences further, a principal component analysis (PCA) was performed on the spectral data (Figure 28). The first two principal components (PC1 and 2) described 80% and 6% of the variation between samples, respectively. A clear distinction was observed between product 4 and the other products in PC1, which is in good agreement with the fact that product 4 was made from normal milk, while the other products were processed from Support milk. The distribution of the samples along PC1 is also in correlation with the water content of the samples, which was lowest in product 3, followed by product 1 and 2, and highest in product 4. It is therefore clear that the main differences between the samples are due to differences in water content in the samples. It is also interesting to see that a systematic effect was seen in the spectral characteristics based on the length of storage. This effect was mainly seen as variation in PC2, and is thus unlikely to be dominated by the water content of the samples, since the water content was mainly represented by PC1 and no major changes in water content were observed during the storage duration. Since spectral differences were observed in the C-H stretch peak at approximately 1800 nm it is likely that changes in the lipid content and/or composition are responsible for this storage effect. However, since no analysis of the lipids or proteins were performed through storage, it is difficult to confirm this hypothesis.

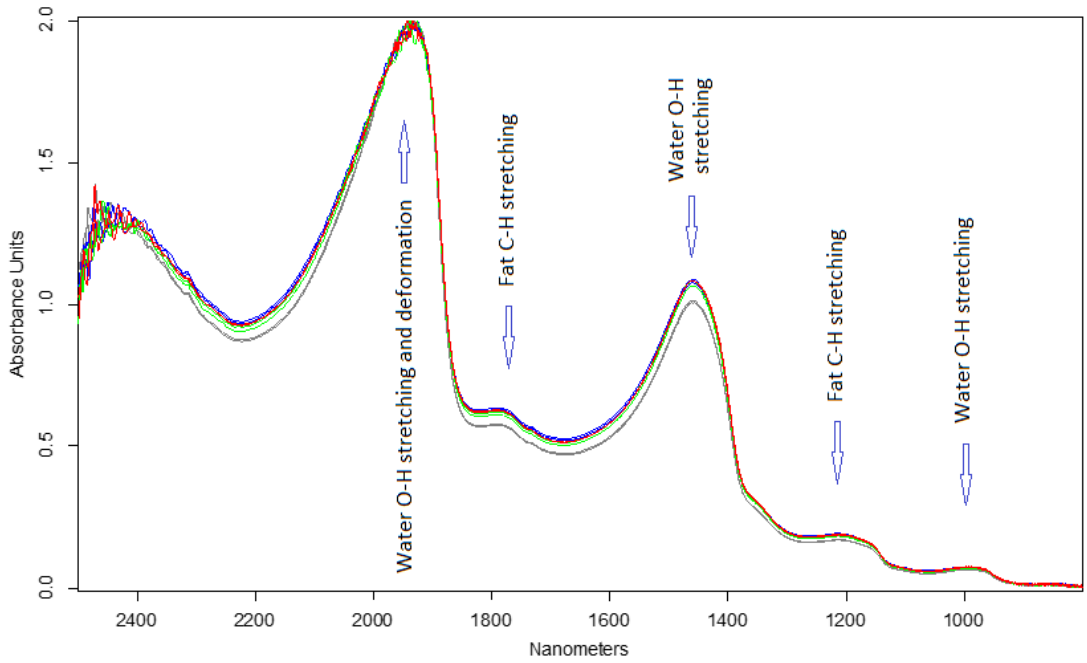


Figure 27. NIR spectra of the four products analysed in the main-trial. The red spectra refers to support yoghurt with fibres and gelatine (product 1), the green spectra to support yoghurt with fibres (product 2), the blue spectra to support yoghurt with fibres and starch (product 3), and the grey spectra to vitamin enriched yoghurt with fibres (product 4).

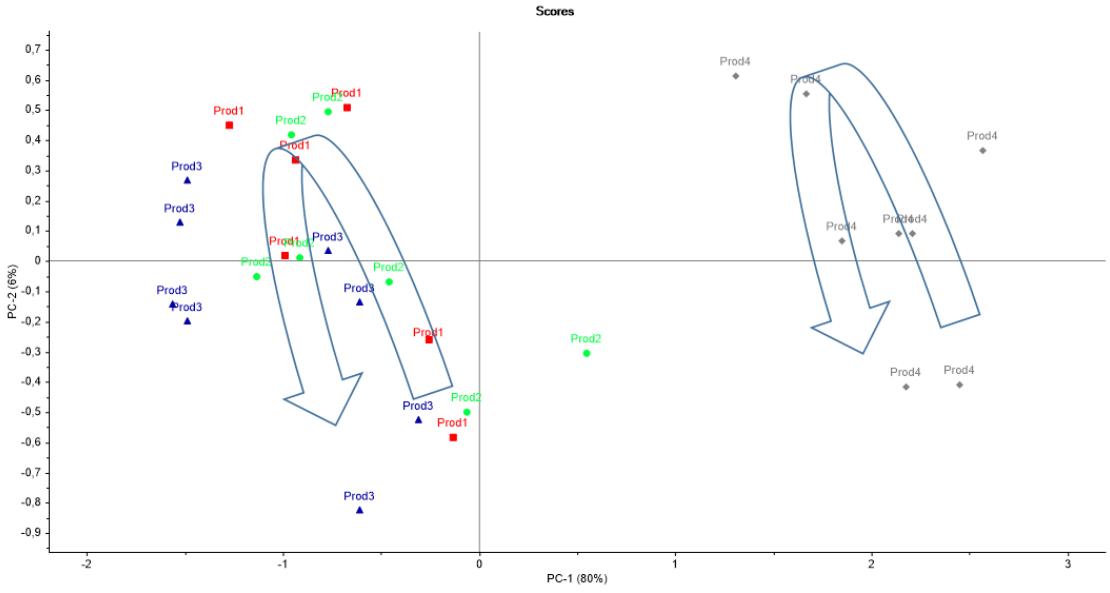


Figure 28. PCA score plot of NIR spectral data from the main trial. Arrows indicate the storage effect, but the arrow points towards longer storage. The red boxes marked Prod 1 refer to support yoghurt with fibres and gelatine, the green dots marked Prod 2 to support yoghurt with fibres, the blue triangles marked Prod 3 to support yoghurt with fibres and starch, and the grey diamonds marked Prod 4 to vitamin enriched yoghurt with fibres.

4.3.5 NMR

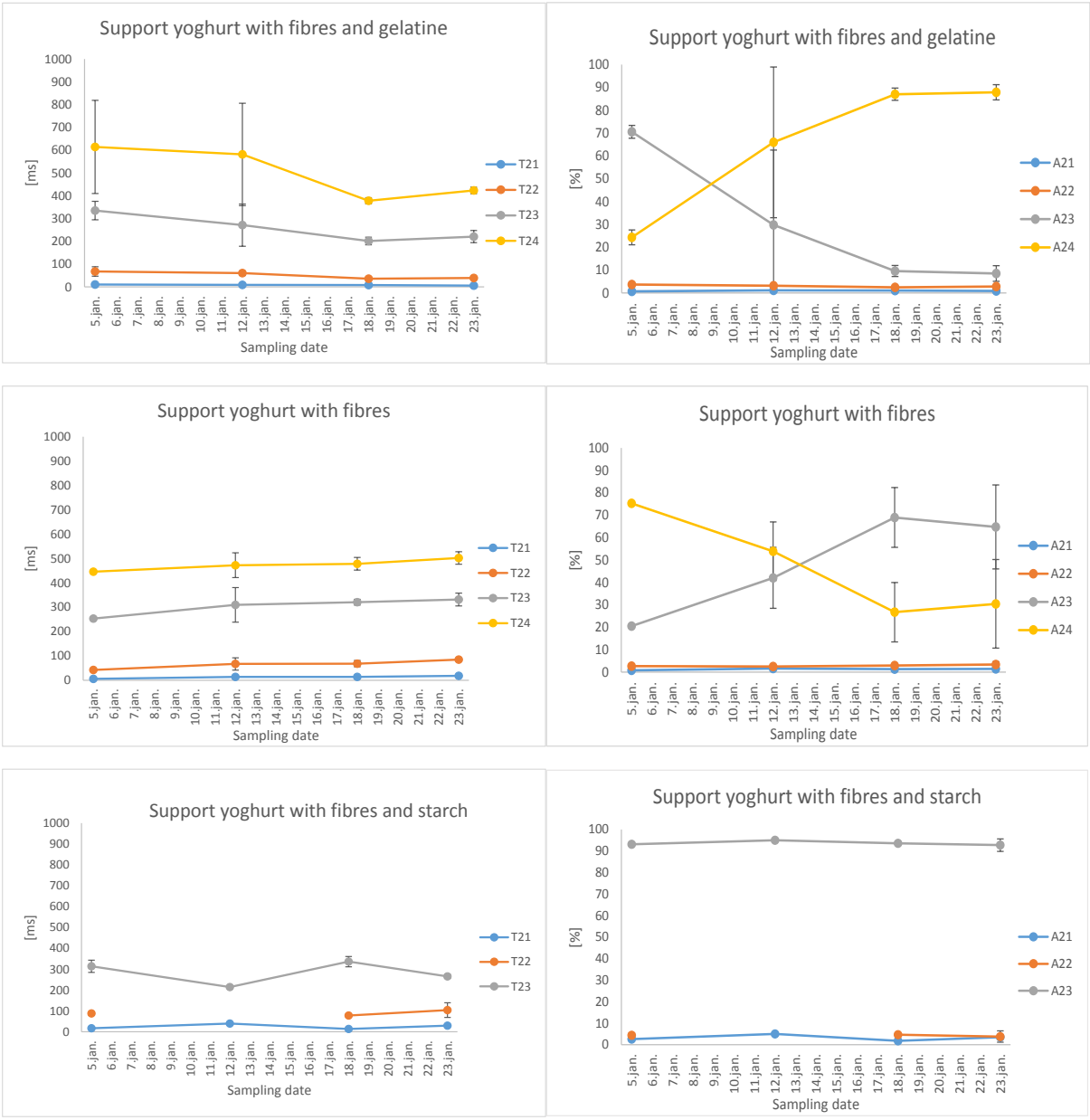
A similar transversal relaxation time analysis was performed on the products in the main trial as before. The analysis indicated the presence of 3-4 proton populations, depending on the type of formulation assessed. This was in agreement with the observations of Hinrichs et al. (2003) who observed four proton populations in yoghurt, and the two most mobile components included 80-90% of the protons. An even faster relaxing component T_{21} was observed in the samples in the main trial, compared to the pre-trial samples, which exhibited shorter relaxation times in the range from 5.8 to 18 ms, indicating increased interaction with water to the proteins in the yoghurt samples. The second fastest component T_{22}, had relaxation times in the range from 43 to 104 ms and is believed to mostly correspond to the lipid protons, while the intermediate T_{23} component, which had relaxation times in the range from 201 to 335 ms, and the slowest relaxing component $T_{24,}$ showing relaxation times in the range from 378 to 835 ms, are believed to correlate to immobilized and free water protons in the sample, respectively.

No major differences were observed between the two fastest relaxing components (T_{21} and T_{22}, and their respective apparent populations A_{21} and A_{22}) between the different samples, and no significant changes were observed in these parameters during storage. However, significant differences were observed in the slower relaxing water components T_{23} and T_{24} and/or their apparent populations A_{23} and A_{24} in two of the products.

In the support yoghurt with fibres and gelatine no significant changes were observed in the T_{23} or T_{24} relaxation times with increased storage time, but an effective exchange of water from the more restricted population A_{23} to the more freely moving population A_{24} was observed. Simultaneously, no significant difference was observed in the relaxation times (T_{21} to T_{24}) between this yoghurt and the support yoghurt containing only fibres, indicating that the gelatine did not provide any stronger binding of the water than the fibres alone. Furthermore, the transformation of water from the more freely moving water population A_{24} was observed towards the more restricted water population A_{23} with storage time in the support yoghurt with fibres only, indicating an increased water binding and thickening effect on this formulation. This observation, in combination to visual assessment of the support yoghurts indicated that both formulations had a very thin and runny consistency, and inadequate water binding properties. The use of fibres alone or fibres in combination with gelatine was therefore not considered for further analysis.

The support yoghurt with fibres and starch, did on the other hand, show significantly shorter water relaxation times than the other formulations, and the slowest component (T_{24}) was not detected in this sample, and in one of the samples (June 12th) only two water populations could be distinguished. These generally shorter relaxation times indicated more restriction of the water in this sample compared to the other formulations, which was in excellent agreement with a thicker consistency of the product. It can therefore be concluded that the starch in combination with fibres had an effective thickening effect on the support yoghurt. Furthermore, only minor changes were observed in all NMR parameters for this product with storage time, indicating that the product had excellent storage stability towards the water and fat distribution and characteristics.

The vitamin enriched yoghurt made from regular milk and using fibres had very similar relaxation times as the first two support yoghurt formulations, but no changes in neither the relaxation times nor the apparent populations indicated that this product had better storage stability than the support yoghurt products with fibres and/or gelatine. However, visual assessment of the consistency revealed a thin and runny consistency of the vitamin-enriched yoghurt, ruling it out as a viable option for further trials. Figure 29 summarises the results from the NMR analysis.



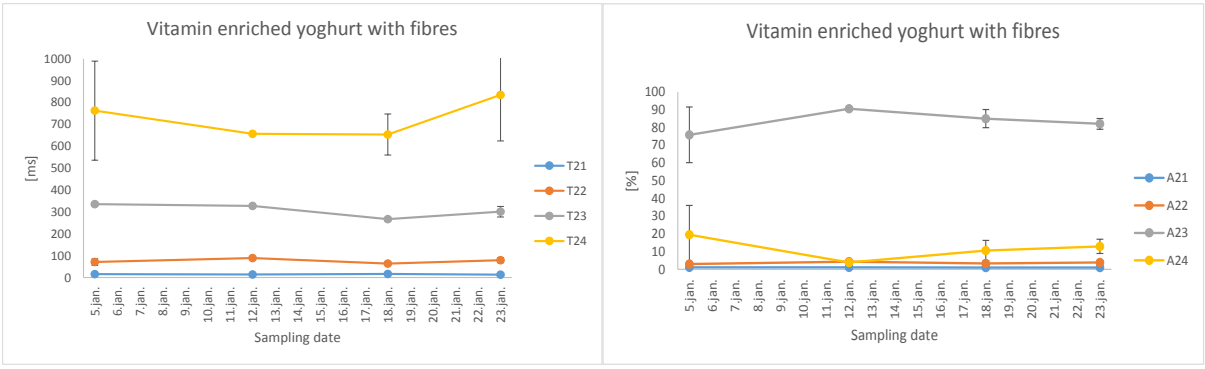


Figure 29. Transverse relaxation times (left) and their apparent populations (right) in the four products assessed in the main trial. These were support yoghurt with fibres and gelatine (top), support yoghurt with fibres (second from top), support yoghurt with fibres and starch (second from bottom), and vitamin enriched yoghurt with fibres (bottom).

4.3.6 Main experiment summary

The results from the main experiment indicated that the products were stable throughout the storage time of regular yoghurt (four weeks). The analysis indicated that there is a need for using a thickening agent in the support yoghurts, since the fibres alone were not enough to make the products thicker. The WHC of the products indicated that the support yoghurt containing fibres and starch had a better ability to retain water and the results from the NMR analysis supported this. An informal tasting suggested that the support yoghurt formulations had a sweeter taste than the vitamin-enriched yoghurt, and that there was still a hint of iron in all products. However, fibres and maize starch gave the support yoghurt a more desirable taste and texture than fibres alone, or the combination of fibres and gelatine. Thus, in the follow-up experiment it was decided to continue with the combination of fibres and maize starch, as well as using the formulation of support yoghurt with fibres for comparison, only in the follow-up experiment, the protein content of the support yoghurt with fibres was increased from 1.8% up to 2.2%.

4.4 Final follow-up experiment

In the final follow-up experiment, only two types of formulations were measured, support yoghurt containing fibres and maize starch, and support yoghurt containing fibres and an increased amount of protein. The same measurements were performed in this round as in the main experiment, but further analysis of the fatty acid composition was also performed as well as analysis of vitamin-B_2, vitamin-D, iron and iodine content.

4.4.1 Chemical analysis

Protein, ash, and fat were measured at the starting point and the results are shown in Figure 30. The protein content was higher in the support yoghurt with fibres and increased amount of protein, as expected. However, increasing the protein amount did not have the effects that were hoped for in the formulation, i.e. a thicker consistency. The carbohydrates were considerably higher in the support

yoghurt containing the maize starch, just like in the main experiment, which can be explained by the fact that it contained maize starch. The fat and ash contents were very similar between the samples.

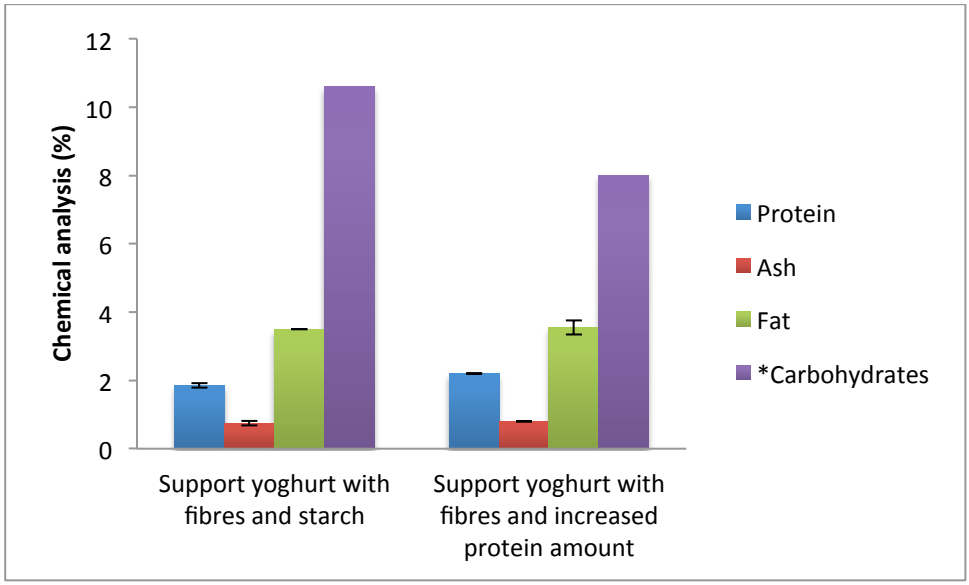


Figure 30. The amount of protein, ash and fat in the samples in the follow-up experiment.

*Carbohydrates of the formulations were determined by difference calculations.

The water content was considerably higher in the support yoghurt with fibres and increased amount of protein than in the support yoghurt containing fibres and starch (Figure 31). No significant changes were observed in the water content throughout the storage time of both samples, indicating very stable products.

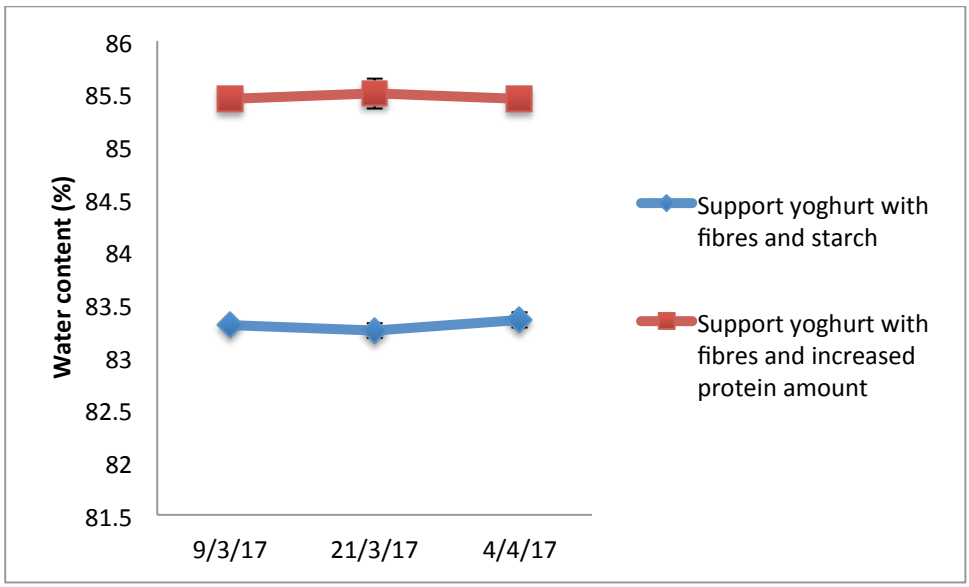


Figure 31. Water content of the samples in the follow-up experiment.

4.4.1.1 Fatty acid analysis

The fatty acid analysis revealed the fatty acid composition of the formulations. Figure 33 shows the proportion of SFA, MUFA and PUFA in the formulations. There was no difference between the samples in fatty acid composition. Table 11 shows more accurately the distribution of individual fatty acids in the samples. As Table 11 shows, palmitic acid was the most predominant of the fatty acids in both formulations, contributing to 37.7% of the lipids in the support yoghurt containing fibres and starch, and 37.3% in the support yoghurt containing fibres and protein. The formulations were also rich in oleic acid, which contributed to 19.8% and 19.7% of the lipids in the formulations, respectively.

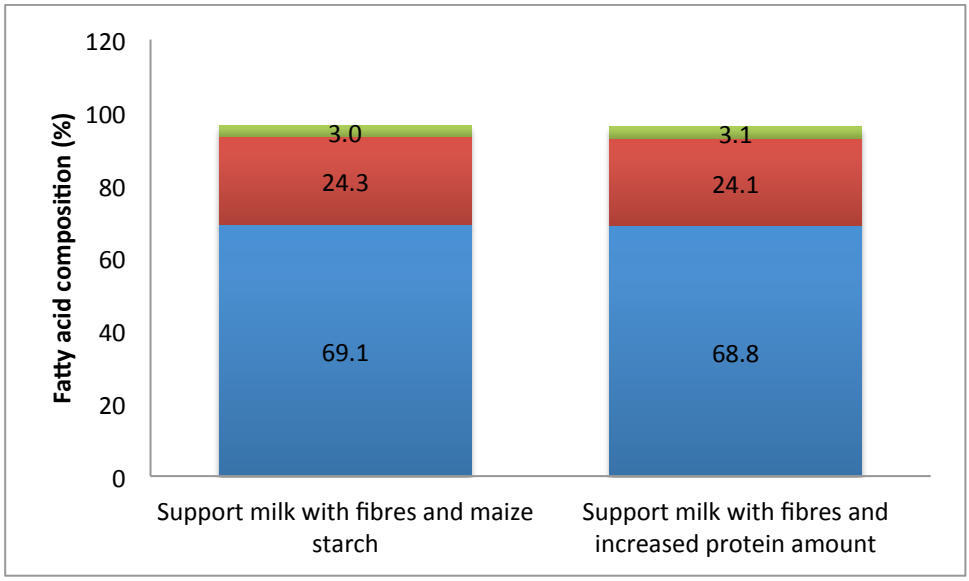


Figure 32. Fatty acid composition of the formulations.

Table 11. Fatty acids in the formulation.

Fatty acid	Carbon number	Support milk with fibres and maize starch (%)	Support milk with fibres and increased protein amount (%)
*Caproic	C6:0	1.2 (0.1)	1.3 (0.1)
Caprylic	C8:0	0.9 (0.0)	1.0 (0.0)
*Capric	C10:0	2.6 (0.1)	2.6 (0.1)
*Lauric	C12:0	3.4 (0.1)	3.4 (0.1)
Trydecylic	C13:0	0.1 (0.0)	0.1 (0.0)
*Myristic	C14:0	12.0 (0.1)	12.0 (0.1)
Myristoleic	C14:1	0.8 (0.0)	0.8 (0.0)
Pentadecanoic	C15:0	0.9 (0.0)	0.9 (0.0)
*Palmitic	C16:0	37.7 (0.4)	37.3 (0.4)
*Palmitoleic	C16:1n7	1.8 (0.0)	1.8 (0.0)
Hexadecadienoic	C16:2n4	0.3 (0.0)	0.3 (0.0)
Margaric	C17:0	0.6 (0.0)	0.6 (0.0)
Heptadecenoic	C17:1	0.2 (0.0)	0.2 (0.0)
*Stearic	C18:0	9.6 (0.1)	9.5 (0.1)
*Oleic	C18:1n9	19.8 (0.2)	19.7 (0.1)
*Vaccenic	C18:1n7	1.7 (0.0)	1.7 (0.0)
*Linoleic (LA)	C18:2n6	1.4 (0.0)	1.4 (0.0)
γ-Linoleic (GLA)	C18:3n6	0.2 (0.0)	0.2 (0.0)
α-Linolenic (ALA)	C18:3n3	0.5 (0.1)	0.6 (0.1)
Stearidonic	C18:4n3	0.6 (0.0)	0.5 (0.0)
Arachidic	C20:0	0.1 (0.0)	0.1 (0.0)

Results are presented as the average of duplicate measurements. Standard deviations of the results are given within brackets.

*Fatty acids that are present in larger amounts than 1% of the total fatty acids in the formulations.

4.4.1.2 Vitamin and mineral analysis

There was no difference between the formulations in vitamin-B_2, vitamin-D, and iodine amounts. The iron amount was slightly higher in the support yoghurt with fibres and starch, but the difference was not significant. Table 12 shows the results from the analysis.

Table 12. Amount of the measured vitamins and minerals in the formulations.

Nutrient	Support yoghurt with fibres and maize starch	Support yoghurt with fibres and increased protein amount
Vitamin-B_2 (mg/100 g)	0.13 (0.0)	0.13 (0.0)
Vitamin-D (µg/100 g)	1.3 (0.0)	1.3 (0.1)
Iron (mg/kg)	6.3 (0.2)	5.9 (0.2)
Iodine (µg/kg)	40.0 (1.0)	40.0 (1.0)

As Table 13 shows, the vitamin-B_2, vitamin-D and iron seemed to be rather stable through the fermentation process, seeing that their amount was only a little bit lower in the support yoghurt than in the Support milk. However, the support yoghurt had a relatively higher amount of all the measured nutrients than regular yoghurt.

Table 13. The comparison of vitamin-B_2, vitamin-D, iron and iodine between Support milk, regular yoghurt and support yoghurt. The values for the Support milk and regular yoghurt were obtained from Table 3 and the values for the support yoghurt were converted into amount/100 kcal from table 12.

Nutrient	Support milk	Regular yoghurt	Support yoghurt
Vitamin-B_2 (mg/100 kcal)	0.24	0.03	0.16
Vitamin-D (µg/100 kcal)	1.8	0	1.6
Iron (mg/100 kcal)	1.1	0.1	0.8
Iodine (µg/100 kcal)	18	16	50

4.4.2 Physicochemical properties

4.4.2.1 WHC

The support yoghurt containing fibres and maize starch had a higher and more stable WHC throughout the storage time than the support yoghurt containing fibres and increased amount of protein (Figure 33). The latter seemed to lose all ability to retain water after less than two weeks of storage, which indicates that increasing the protein amount did not have the aspired effects. Maize starch was shown to attain the best ability to retain water, both in the main experiment and the follow-up experiment.

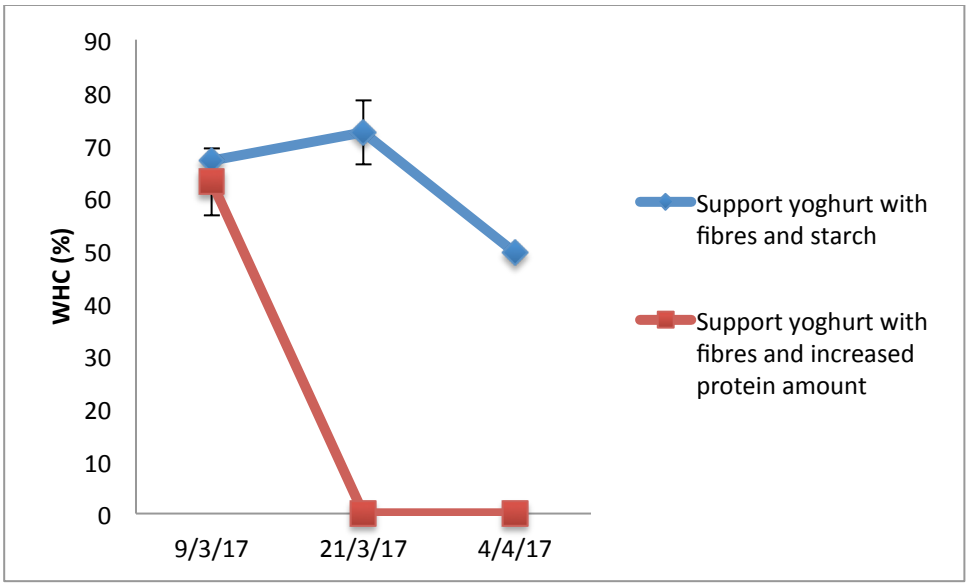


Figure 33. The WHC of the samples in the follow-up experiment.

4.4.2.3 pH

The support yoghurt containing fibres and starch was a little more acidic than the support yoghurt containing fibres and protein (Figure 34). Both products reached an optimal pH value during the production process, allowing the lactic acid bacteria to grow and thrive favourably.

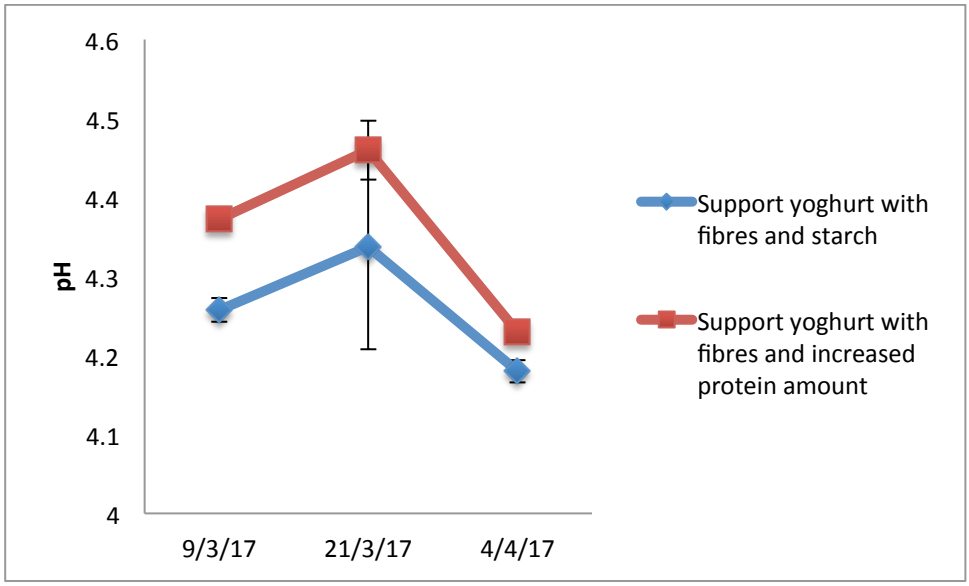
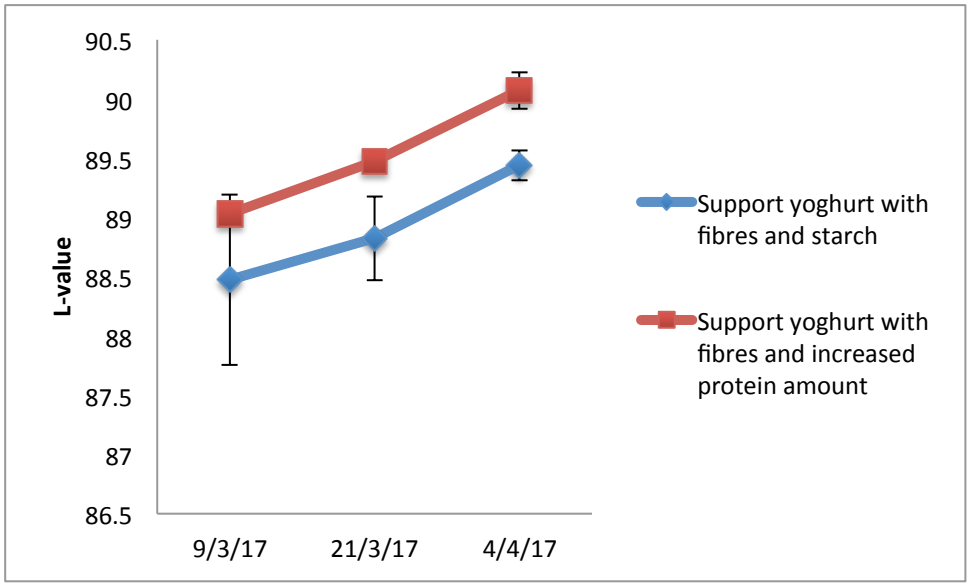
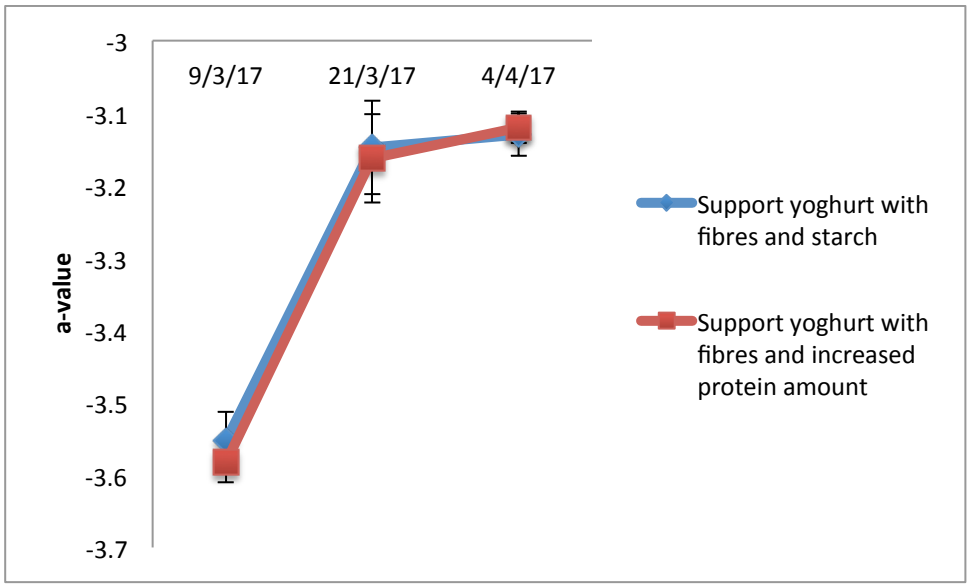


Figure 34. The pH value of the formulations throughout the storage time.

4.4.2.4 Colour analysis

As Figure 35 shows, the L-value was slightly higher in the support yoghurt with fibres and protein than in the support yoghurt with fibres and starch. Though both samples had a very high L-value, indicating a bright appearance of the samples. The L-value increased remotely during the storage time in both samples. No difference was observed between the samples in the a-value, but the negative values indicated a greenish shade, just like in the previous experiments. The a-value elevated somewhat throughout the storage time but remained greenish. The support yoghurt with fibres and starch had a little higher b-value than the formulation containing fibres and protein at the beginning, but the difference was not significant. At the end of the experiment, the b-value had decreased in both samples, and both had a very similar value at the endpoint. The positive value indicated a yellowish shade, like in the previous experiments.





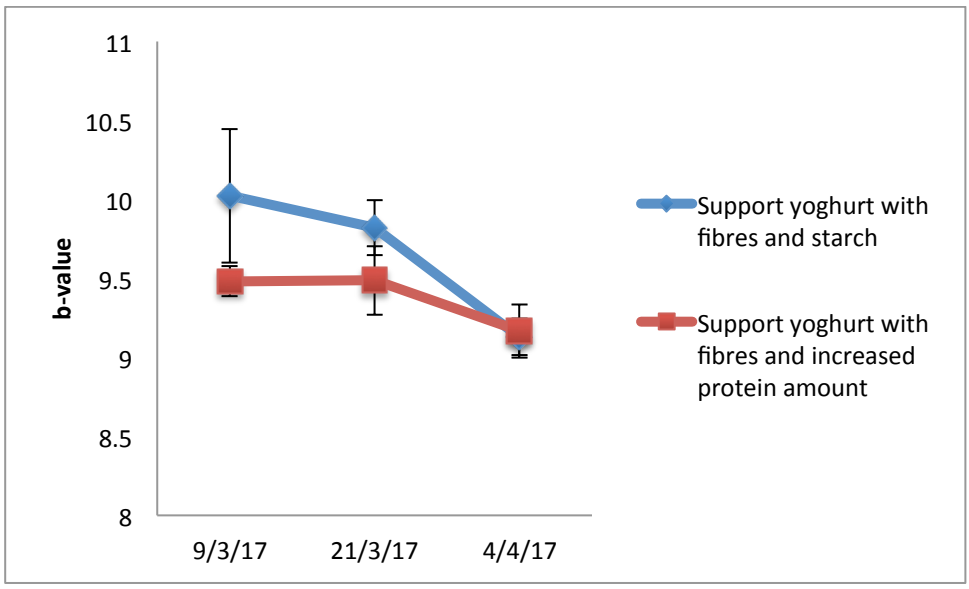


Figure 35. The L-, a- and b-value of the formulations in the final experiment.

4.4.3 Microbiological analysis

As Figure 36 shows, the survival rate of the lactic acid bacteria decreased a little throughout the storage time in both samples. The count was a little higher in the support yoghurt with fibres and protein than in the formulation containing fibres and starch. Though, there was not a statistical difference between the samples. Counts of moulds and yeasts were below 20 cfu/g at all measurement points, indicating stable products that are safe for consumption, even four days after the expiration date.

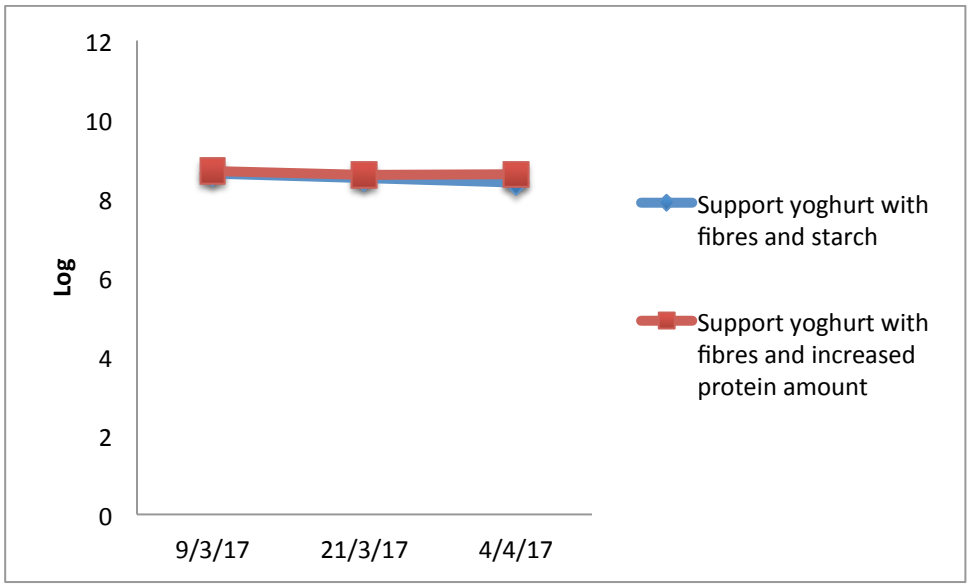


Figure 36. The amount of lactic acid bacteria in the formulations throughout the storage time, shown in log numbers.

4.4.4 NIR

NIR spectra of the products tested in the final trial can be seen in Figure 37. NIR spectra of Support milk and natural yoghurt available from the supermarket were analysed in comparison to the final products. The peaks could be assigned in the same way as in the earlier trials and little differences were seen between the two final products from the spectra alone. However, principal component analysis of the spectra indicated that variation within each product with storage time was greater than the variation between the two products. This was reflected in the PCA score plot (Figure 38), in which PC1, describing 80% of the overall variation between samples could mainly be attributed to the storage effect of the products, while distinction between the two products was only seen in PC2, which only described 7% of the overall variation. Interestingly, the storage effect on the spectra is very similar on the products in the final trial as in the main trial. As before, a more detailed analysis is, however, needed to provide adequate explanations to this trend.

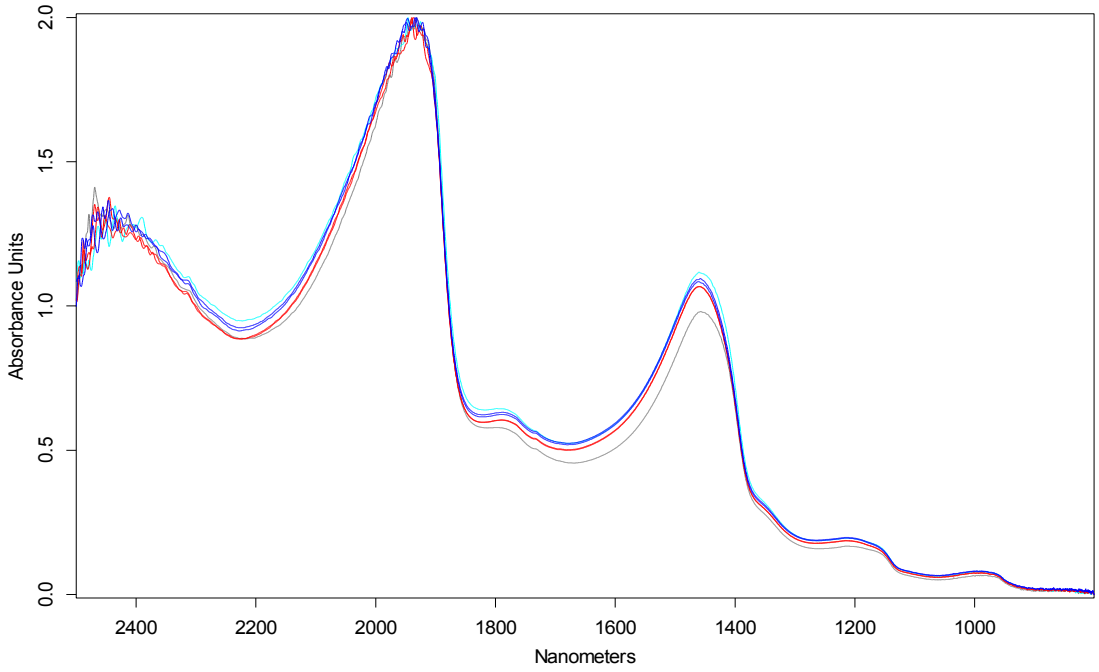


Figure 37. NIR spectra from final trial, where blue represents support yoghurt with fibres and starch (product 3) and red represents support yoghurt with fibres and increased protein content (product 5). For comparative reasons, the turquoise spectra, referring to support milk, and grey spectra referring to neutral yoghurt as available at the supermarket, are included in the figure as well.

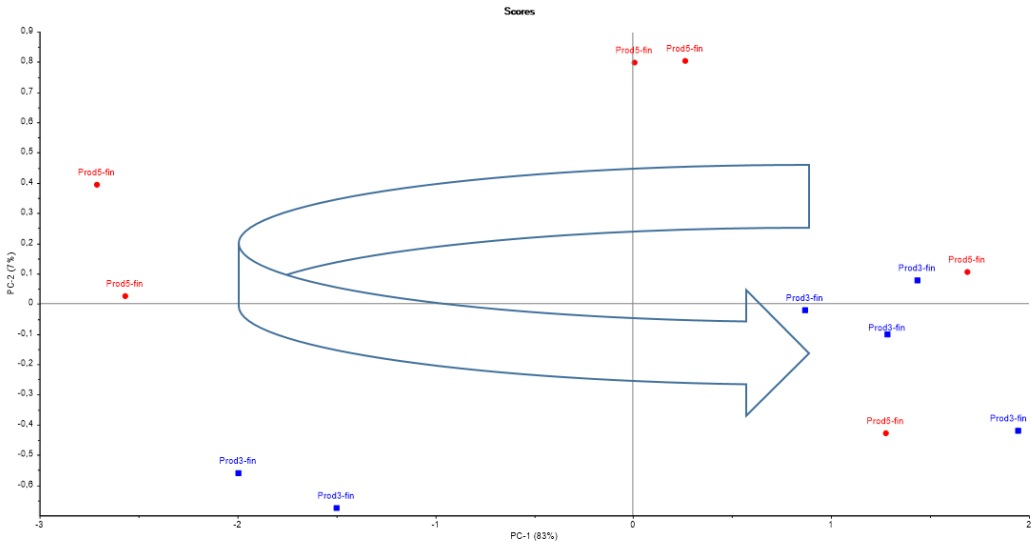


Figure 38. PCA score plot of NIR spectral data from products in the final trial. Blue boxes, marked Prod 3 refer to support yoghurt with fibres and starch, and red dots marked Prod 5 refer to support yoghurt with fibres and added protein content. Red ellipse marks samples analysed on March 9th, the blue ellipse marks samples analysed on March 21st, and the green ellipse marks samples analysed on April 4th. Arrow shows overall storage effect.

To set the analysis of the final products into perspective towards the products tested in the earlier trials an overall PCA score plot analysis was performed (Figure 39). A clear distinction was observed in PC1 (describing 74% of the variation between samples) between the yoghurts that were produced from Support milk, compared to those produced from regular milk, with the exception of the yoghurts tried in the pre-trial. An effect of the storage duration could also be seen in both PC1 and PC2 (7% of the variation described).

The study indicated that NIR spectroscopy is a very useful technique for fast evaluation of chemical differences due to variation in raw materials, handling, storage or other processing. The method is, furthermore, more sensitive and precise than many of the traditional physicochemical methods, since it provides a more holistic view of the sample by analysing multiple components simultaneously. However, extensive validation and correlation analyses to accepted and/or accredited methods are needed to allow a detailed and deep evaluation of the spectra, which limits the use of the method alone.

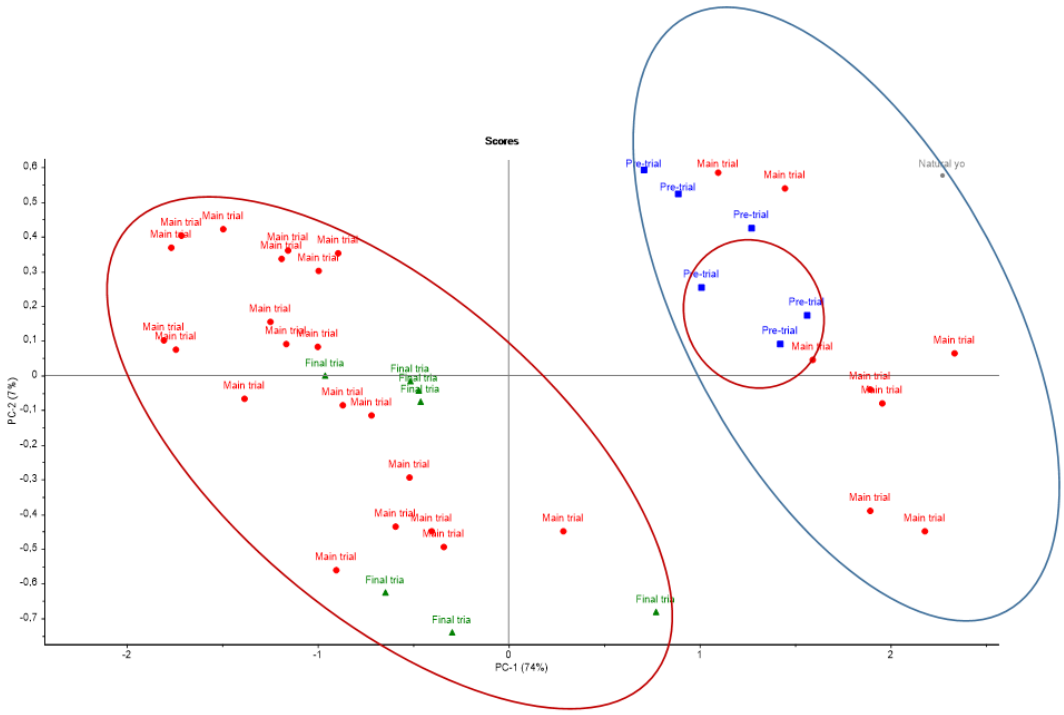


Figure 39. PCA score plot of all NIR spectra analysed in the project. The red ellipses indicate yoghurts made from support milk, while the blue ellipse indicates yoghurts processed from regular milk.

4.4.5 NMR

Unfortunately, incorrect settings were used for the NMR analysis during the final trial, making them incomparable to the results from the earlier trials. Repetition of this analysis is therefore recommended if further product development of the products will be performed.

4.4.6 Follow-up experiment summary

Both products had an appropriate nutrition value for infants. However, only the support yoghurt with fibres and starch had a yoghurt-like consistency. The yoghurt with increased protein content was very thin-flowing. The follow-up experiment confirmed the results from the main experiment, i.e. that the maize starch is the most convenient stabiliser, of the stabilisers tested in this study, to achieve a desirable consistency in a yoghurt processed from Support milk. Although both products fulfilled the nutritional requirements of a yoghurt product for infants and were both stable towards the formation of mould and yeast, and had an excellent survival rate of lactic acid bacteria, the consistency of the support yoghurt with fibres and starch would be the decisive factor when choosing a product for further research or marketing.

5 Conclusion

The consumer survey provided some information concerning the baby food market in Iceland. Overall, the participants in the survey were interested in a new dairy product for infants and small children. The majority of the participants in the survey had given their children some kind of a fermented milk product before they reached one year of age. Yoghurt is convenient as a quick and easy made meal, but it is not recommended for infants under 12 months of age. Thus, there seems to be a room on the baby food market for a new yoghurt product for the youngest age group.

Four different types of yoghurts made from Support milk were measured and compared to both regular yoghurt and vitamin enriched regular yoghurt. All types of support yoghurts met the EFSA requirements for nutritional value in infant and follow-on formula. Furthermore, the products were stable towards the formation of mould and yeast, and had a good survival rate of lactic acid bacteria over the storage time, which was four weeks. However, only one sample, the support yoghurt with fibres and maize starch, had a consistency in resemblance to regular yoghurt, and had also the best ability to retain water compared to the other support yoghurts.

These results indicate that Support milk is fermentable but there is a need for using fibres and stabilisers to obtain the most desirable consistency. However, choosing the appropriate stabiliser can be challenging, whereas gelatine, for example, does not suit all social/religious groups, as it is usually made from pig bones. Furthermore, some may find the usage of maize starch undesirable because they may think that it is genetically modified. However, maize starch is something that most infants have already consumed between 6-12 months and therefore a convenient thickening agent for the product. Maize starch is also not a common allergy cause.

Then there is the matter of whether making a probiotic yoghurt for infants is appropriate, whereas EFSA considers that there are not enough evidence for the beneficial effects of giving infants probiotics to advise the addition of probiotics to IF and FOF, and the long term effects of doing so are not well known. However, yoghurt does not fall in to the same category as IF and FOF regarding nutrition for infants, so the EFSA regulation on IF and FOF does not fully apply, but it can be used as a guideline for the nutritional value in the support yoghurt.

It would have been better define the age groups for the children in the consumer survey more accurately. It could even have been better to focus only on the target group, i.e. parents of children aged seven months to two years of age. There was a lack of defining the target group from the beginning, which may have led to the survey being put out for parents of children of larger age range than intended, and therefore interpreted in a broad sense by the participants.

6 Future perspectives

The next step for the support yoghurt with fibres and maize starch would be a sensory evaluation. It could also be interesting to make a new consumer survey designed for the support yoghurt with fibres and maize starch and lay it out for the target group, i.e. parents of children from seven months to two years of age. A more detailed consumer survey with more participants could give more accurate information about consumers' attitude on the new product, for example on whether or not maize starch is an undesirable additive in a product for infants and young children.

It could be interesting to try different composition of fibres and stabilisers to achieve a product that suits more social groups. Then there is the possibility of using different types of lactic acid bacteria with probiotic effects, such as *Lactobacillus acidophilus* or *Bifidobacterium bifidum.* It could also be interesting to try adding flavours without the addition of sugar or sweeteners.

References

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences, 19*(2), 164-174.

Aldredge, D. L., Geronimo, M. R., Hua, S., Nwosu, C. C., Lebrilla, C. B., & Barile, D. (2013). Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. *Glycobiology, 23*(6), 664-676. doi:10.1093/glycob/cwt007

Andersen C. M., Frøst, M. B., Viereck N. (2010). Spectroscopic characterization of low- and non-fat cream cheeses. *International Dairy Journal 20*(1), 32-39. doi:10.1016/j.idairyj.2009.07.006

AOCS Ba 3-38. (1997). Method for analysing total fat in fish and fish meal.

Asakuma, S. et al. (2008). Variation of major neutral oligosaccharides levels in human colostrum. *European Journal of Clinical Nutrition, 62*(4), 488-494. doi:10.1038/sj.ejcn.1602738

Atladottir, H., & Thorsdottir, I. (2000). Energy intake and growth of infants in Iceland-a population with high frequency of breast-feeding and high birth weight. *European Journal of Clinical Nutrition, 54*(9), 695-701.

Barłowska, J., Szwajkowska, M., Litwińczuk, Z., & Król, J. (2011). Nutritional Value and Technological Suitability of Milk from Various Animal Species Used for Dairy Production. *Comprehensive Reviews in Food Science and Food Safety, 10*(6), 291-302. doi:10.1111/j.1541-4337.2011.00163.x

Bertram, H. C., Wiking, L., Nielsen, J. H., & Adersen, H. J. (2005). Direct measurement of phase transitions in milk fat during cooling of cream - a low-field NMR approach. *International Dairy Journal, 15*(10), 1056-1063. doi:10.1016/j.idairyj.2004.10.005

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology., 37*(8), 911-917. doi:10.1139/o59-099

Braegger, C. et al. (2011). Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. *Journal of Pediatric Gastroenterology & Nutrition, 52*(2), 238-250. doi:10.1097/MPG.0b013e3181fb9e80

Carbone, E. T., & Zoellner, J. M. (2012). Nutrition and Health Literacy: A Systematic Review to Inform Nutrition Research and Practice. *Journal of the Academy of Nutrition and Dietetics, 112*(2), 254-265. doi:10.1016/j.jada.2011.08.042

Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *American Journal of Physiology, 94*(3), 630-638. doi:10.1103/PhysRev.94.630

Chassard, C., de Wouters, T., & Lacroix, C. (2014). Probiotics tailored to the infant: a window of opportunity. *Current Opinion in Biotechnology, 26*, 141-147. doi:10.1016/j.copbio.2013.12.012

Chuang, C. K., Lin, S. P., Lee, H. C., Wang, T. J., Shih, Y. S., Huang, F. Y., & Yeung, C. Y. (2005). Free amino acids in full-term and pre-term human milk and infant formula. *Journal of Pediatric Gastroenterology & Nutrition, 40*(4), 496-500. doi:10.1097/01.MPG.0000150407.30058.47

Claeys, W. L. et al. (2014). Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control, 42*, 188-201. doi:10.1016/j.foodcont.2014.01.045

Cohen, Y. (2009). Phosphorus dissolution from ash of incinerated sewage sludge and animal carcasses using sulphuric acid. *Environmental technology, 30*(11), 1215-12126. doi:10.1080/09593330903213879

Corrieu, G., & Béal, C. (2016). Yogurt: The Product and its Manufacture A2 - Caballero, Benjamin. In P. M. Finglas & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (pp. 617-624). Oxford: Academic Press.

Damodaran, S., Parkin, K. L., & Fennema, O. R. (2008). *Fennema's Food Chemistry* (4th edition ed.). Boca Raton: Taylor & Francis Group.

Di Mauro, A., Neu, J., Riezzo, G., Raimondi, F., Martinelli, D., Francavilla, R., & Indrio, F. (2013). Gastrointestinal function development and microbiota. *Italian Journal of Pediatrics, 39*(1). doi:10.1186/1824-7288-39-15

DiGiulio, D. B. (2012). Diversity of microbes in amniotic fluid. *Seminars in Fetal and Neonatal Medicine, 17*(1), 2-11. doi:10.1016/j.siny.2011.10.001

DiGiulio, D. B. et al. (2008). Microbial Prevalence, Diversity and Abundance in Amniotic Fluid During Preterm Labor: A Molecular and Culture-Based Investigation. *PLoS ONE, 3*(8), e3056. doi:10.1371/journal.pone.0003056

Directorate of Health. (2013). Recommended Daily Intake of vitamins and minerals.

Directorate of Health & Healthcare Service of the Capital area. (2009). Nutrition for infants.
Domellöf, M., Thorsdottir, I., & Thorstensen, K. (2013). Health effects of different dietary iron intakes: a systematic literature review for the 5th Nordic Nutrition Recommendations. *Food & nutrition research., 57*. doi:10.3402/fnr.v57i0.21667
Ella's Kitchen. (2014). Ella's Kitchen. Retrieved from http://www.ellaskitchen.com/our-products/ on the 27th august 2015
Elmadfa, I., & Meyer, A. L. (2012). Vitamins for the first 1000 days: preparing for life. *International Journal for vitamin and nutrition research, 82*(5), 342-347. doi:10.1024/0300-9831/a000129
European Food Safety Authority. (2011). Scientific Opinion o n the substantiation of health claims related to Lactobacillus rhamnosus ATCC 53103 (LGG) and "gastro-intestinal health" (ID 906) and maintenance of tooth mineralisation (ID 3018) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *The EFSA journal, 9*(6), 2233. doi: 10.2903/j.efsa.2011.2233
European Food Safety Authority. (2014). Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. *The EFSA journal, 11*(10), 1-103. doi:10.2903/j.efsa.2013.3408
Foodstuffs. (2007). Determination of trace elements. Determination of iodine by inductively coupled plasma mass spectrometry. (BS EN 15111: 2007).
Foodstuffs. (2006). Determination of Vitamin B_2 by high performance liquid chromatography. (ONORM EN 14152: 2006).
Foodstuffs. (2009). Determination of Vitamin D by high performance liquid chromatography. Measurement of cholecalciferol (D_3) or ergocalciferol (D_2). (BS EN 12821: 2009).
Fox, M. J., Ahuja, K. D., Robertson, I. K., Ball, M. J., & Eri, R. D. (2015). Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. *BMJ Open, 5*(1), e006474. doi:10.1136/bmjopen-2014-006474
Fox, M. K., Pac, S., Devaney, B., & Jankowski, L. (2004). Feeding infants and toddlers study: what foods are infants and toddlers eating? *Journal of the American Dietetic Association, 104*(1), 22-30. doi:http://dx.doi.org/10.1016/j.jada.2003.10.026
Fox, P. F., & Kelly, A. L. (2006). Indigenous enzymes in milk: Overview and historical aspects—Part 1. *International Dairy Journal, 16*(6), 500-516. doi:http://dx.doi.org/10.1016/j.idairyj.2005.09.013
Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., & O'Mahony, J. A. (2015). Water in Milk and Dairy Products. In P. F. Fox, T. Uniacke-Lowe, P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Dairy Chemistry and Biochemistry* (pp. 299-320). Cham: Springer International Publishing.
German, J. B., & Dillard, C. J. (2006). Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. *Critical reviews in food science and nutrition, 46*(1), 57-92. doi:10.1080/10408690590957098
Granato, D., Branco, G. F., Cruz, A. G., de Assis Fonseca Faria, J., & Shah, N. P. (2010). Probiotic Dairy Products as Functional Foods. *Comprehensive Reviews in Food Science and Food Safety., 9*(5), 455-470. doi:10.1111/j.1541-4337.2010.00120.x
Gritz, E. C., & Bhandari, V. (2015). The Human Neonatal Gut Microbiome: A Brief Review. *Frontiers in Pediatrics, 3*. doi:10.3389/fped.2015.00017
Guandalini, S. et al. (2000). Lactobacillus GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *Journal of Pediatric Gastroenterology & Nutrition, 30*(1), 54-60.
Guarner, F., Perdigon, G., Corthier, G., Salminen, S., Koletzko, B., & Morelli, L. (2005). Should yoghurt culture be considered probiotic? *British Journal of Nutrition, 93*(6), 783-786. doi:10.1079/BJN20051428
Guerin-Danan, C. et al. (1998). Milk fermented with yogurt cultures and Lactobacillus casei compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants. *The American Journal of Clinical Nutrition, 67*(1), 111-117.
Gunnarsdottir, I., Helgadottir, H., Thorisdottir, B., & Thorsdottir, I. (2013). Diet of six-year-old Icelandic children - National dietary survey 2011-2012. *The Icelandic medical journal, 99*(1), 17-23.
Gunnarsson, B. S., Thorsdottir, I., Palsson, G., & Gretarsson, S. J. (2007). Iron status at 1 and 6 years versus developmental scores at 6 years in a well-nourished affluent population. *Acta Paediatrica, 96*(3), 391-395. doi:10.1111/j.1651-2227.2007.00086.x
Hassiotou, F. E. et al. (2013). Breastmilk Cell and Fat Contents Respond Similarly to Removal of Breastmilk by the Infant. *PLoS ONE, 8*(11), e78232. doi:10.1371/journal.pone.0078232
Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition – a review. *Lipids in Health and Disease, 6*. doi:10.1186/1476-511X-6-25

Hinrichs, R., Götz, J., & Weisser, H. (2003). Water-holding capacity and structure of hydrocolloid-gels, WPC-gels and yoghurts characterised by means of NMR. *Food Chemistry, 82*(1), 155–160. doi:10.1016/S0308-8146(02)00539-3

Hill, C., & Ross, R. P. (1998). Starter cultures for the dairy industry. In S. Roller & S. Harlander (Eds.), *Genetic Modification in the Food Industry: A Strategy for Food Quality Improvement* (pp. 174-192). Boston, MA: Springer US.

Hipp organic. (2016). Hipp organic. Retrieved from http://www.hipp.co.uk/products/our-baby-food-and-drinks on the 27th august 2015

Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *The Journal of Clinical Investigation, 116*(8), 2062-2072. doi: 10.1172/JCI29449

Hoppe, C., Molgaard, C., Juul, A., & Michaelsen, K. F. (2004). High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. *European Journal of Clinical Nutrition, 58*(9), 1211-1216. doi:10.1038/sj.ejcn.1601948

Hornell, A., Lagstrom, H., Lande, B., & Thorsdottir, I. (2013). Protein intake from 0 to 18 years of age and its relation to health: a systematic literature review for the 5th Nordic nutrition recommendations. *Food & nutrition research., 57*. doi:10.3402/fnr.v57i0.21083

Hurrell, R, & Egli, I. (2010). Iron bioavailability and dietery reference values. *The American Journal of Clinical Nutrition, 91(5),* 1461-1467. doi: 10.3945/ajcn.2010.28674F

Indrio, F., & Neu, J. (2011). The intestinal microbiome of infants and the use of probiotics. *Current opinion in pediatrics, 23*(2), 145-150. doi:10.1097/MOP.0b013e3283444ccb

Insel, P., Ross, D., McMahon, K., & Bernstein, M. (2016). *Nutrition* (6th edition ed.). Sudbury: Jones and Bartlett Publishers.

Institute of Medicine. (1998). *Dietary Reference Intakes for Thiamin, Riboflavin, Niacn, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotine, and Choline*. Washington (DC): National Academies Press (US).

ISO 5983-2. (2005). Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content - Part 2: Block digestion/steam distillation method (ISO 5983-2:2005).

ISO 5984. (2002). Animal feeding stuffs - Determination of crude ash (ISO 5984:2002).

ISO 6496. (1999). Animal feeding stuffs - Determination of moisture and other volatile matter content (ISO 6496: 1999).

ISO 11885. (2007). Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES). (ISO 11885: 2007).

Kattula, D. et al. (2014). The first 1000 days of life: prenatal and postnatal risk factors for morbidity and growth in a birth cohort in southern India. *BMJ Open, 4*(7), e005404. doi:10.1136/bmjopen-2014-005404

Kotowska, M., Albrecht, P., & Szajewska, H. (2005). Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. *Alimentary Pharmacology & Therapeutics, 21*(5), 583-590. doi:10.1111/j.1365-2036.2005.02356.x

Kunz, C., & Rudloff, S. (2006). Health promoting aspects of milk oligosaccharides. *International Dairy Journal, 16*(11), 1341-1346. doi:http://dx.doi.org/10.1016/j.idairyj.2006.06.020

Lankaputhra, W. E. V., & Shah, N. P. (1998). Antimutagenic properties of probiotic bacteria and of organic acids. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 397*(2), 169-182. doi:10.1016/S0027-5107(97)00208-X

Laurentin, A., & Edwards, C. A. (2005). *Encyclopedia of human nutrition, four-volume set* (2nd edition ed.). Oxford: Elsevier.

Le Botlan, D. J., & Helie, I. (1994). A novel approach to the analysis of fats by low-resolution NMR: application to milk and vegetable fats. *Analusis, 22*, 108-113.

Le Dean, A., Mariette, F., Lucas, T., & Marin, M. (2001). Assessment of the state of water in reconstituted milk protein dispersions by nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC). *Lebensmittel-Wissenshaft und –Technologie*, *34*(5), 299–305. doi:10.1006/fstl.2001.0765

Le Dean, A., Mariette, F., & Marin, M. (2004). 1H Nuclear Magnetic Resonance Relaxometry study of water state in milk protein mixtures. *Journal of Agricultural and Food Chemistry*, *52*(17), 5449–5455. doi:10.1021/jf030777m

Lee, H.-J., & Yun, Z.-S. (2015). Consumers' perceptions of organic food attributes and cognitive and affective attitudes as determinants of their purchase intentions toward organic food. *Food Quality and Preference, 39*, 259-267. doi:10.1016/j.foodqual.2014.06.002

Lourens-Hattingh, A., & Viljoen, B. C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal, 11*(1–2), 1-17. doi:http://dx.doi.org/10.1016/S0958-6946(01)00036-X

Lucey, J. A. (2004). Cultured dairy products: an overview of their gelation and texture properties. *International Journal of Dairy Technology, 57*(2-3), 77-84. doi:10.1111/j.1471-0307.2004.00142.x
Mahan, L. K., Escott-Stump, S., & Raymond, J. L. (2012). *Krause's Food and the Nutrition Care Process* (13th edition ed.). St. Louis: Elsevier.
Masulli, D. (2016). Measuring pH of yoghurt. *Food Quality and Safety.*
Månsson, H. L. (2008). Fatty acids in bovine milk fat. *Food Nutr Res, 52*. doi:10.3402/fnr.v52i0.1821
Marteau, P. R., de Vrese, M., Cellier, C. J., & Schrezenmeir, J. (2001). Protection from gastrointestinal diseases with the use of probiotics. *The American Journal of Clinical Nutrition, 73*(2), 430-436.
McFarland, L. V. (2006). Meta-Analysis of Probiotics for the Prevention of Antibiotic Associated Diarrhea and the Treatment of Clostridium difficile Disease. *The American Journal of Gastroenterology, 101*(4), 812-822. doi:10.1111/j.1572-0241.2006.00465.x
Mehra, R., & Kelly, P. (2006). Milk oligosaccharides: Structural and technological aspects. *International Dairy Journal, 16*(11), 1334-1340. doi:http://dx.doi.org/10.1016/j.idairyj.2006.06.008
Meiboom, S., & Gill, D. (1958). Modified spin-echo method for measuring nuclear times. *Review of Scientific Instruments, 29*(8), 688-691. doi:10.1063/1.1716296
Mikami, K., Kimura, M., & Takahashi, H. (2012). Influence of Maternal Bifidobacteria on the Development of Gut Bifidobacteria in Infants. *Pharmaceuticals, 5*(6), 629-642. doi:10.3390/ph5060629
Mjolkursamsalan. (2003). MS Stodmjolk - Barnsins stod og stytta. Retrieved from http://www.ms.is/heilsa/heilsuvorur/stodmjolk
Morelli, L. (2008). Postnatal development of intestinal microflora as influenced by infant nutrition. *The Journal of Nutrition, 138*(9), 1791-1795.
Morita, H. et al. (2002). Cytokine Production by the Murine Macrophage Cell Line J774.1 after Exposure to Lactobacilli. *Bioscience, Biotechnology, and Biochemistry, 66*(9), 1963-1966. doi:10.1271/bbb.66.1963
Muehlhoff, E., Bennett, A., & McMahon, D. (2013). *Milk and dairy products in human nutrition*: Food and Agriculture Organization of the United Nations (FAO).
Mugambi, M. N., Musekiwa, A., Lombard, M., Young, T., & Blaauw, R. (2012). Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutrition Journal, 11*. doi:10.1186/1475-2891-11-81
Nishimura, R. Y., de Castro, G. S. F., Jordão Junior, A. A., & Sartorelli, D. S. (2013). Breast milk fatty acid composition of women living far away from the coastal area in Brazil. *Jornal de Pediatria, 89*(3), 263-268. doi:10.1016/j.jped.2012.11.007
Nordic Council. (2012). *Nordic nutrition recommendations 2012 - integrating nutrition and physical activity* (5th edition ed.).
Ozdemir, N. (2015). Iron deficiency anemia from diagnosis to treatment in children. *Turk Pediatri Ars, 50*(1), 11-19. doi:10.5152/tpa.2015.2337
Panesar, P., & Shinde, C. (2012). Effect of Storage on Syneresis, pH, Lactobacillus acidophilus Count, Bifidobacerium bifidum count of Aloe vera Fortified Probiotic Yoghurt. *Current Research in Dairy Sciences, 4*, 17-23. doi:10.3923/crds.2012.17.23
Parker, M. W. et al. (2013). Rapid Adoption of Lactobacillus rhamnosus GG for Acute Gastroenteritis. *Pediatrics, 131*(1), 96-102. doi:10.1542/peds.2012-1427l
Pedersen, H. T., Bro, R., & Engelsen, S. B. (2002). Towards rapid and unique curve resolution of low-field NMR relaxation data: trilinear SLICING versus two dimensional curve fitting. *Journal of Magnetic Resonance, 157*(1), 141-155. doi:10.1006/jmre.2002.2570
Pizarro, F., Olivares, M., Maciero, E., Krasnoff, G., Cócaro, N., & Gaitan, D. (2015). Iron Absorption from Two Milk Formulas Fortified with Iron Sulfate Stabilized with Maltodextrin and Citric Acid. *Nutrients, 7*(11), 8952-8959. doi:10.3390/nu7115448
Rao, R., & Georgieff, M. K. (2007). Iron in fetal and neonatal nutrition. *Seminars in fetal & neonatal medicine, 12*(1), 54-63. doi:10.1016/j.siny.2006.10.007
Reglugerd um mjolkurvorur nr. 851/2012
Ren, Y. F., & Wang, L. L. (2010). Effects of probiotics on intestinal bacterial colonization in premature infants. *Chinese journal of contemporary pediatrics, 12*(3), 192-194.
Reykdal, O., Rabieh, S., Steingrimsdottir, L., & Gunnlaugsdottir, H. (2011). Minerals and trace elements in Icelandic dairy products and meat. *Journal of Food Composition and Analysis, 24*(7), 980-986. doi:http://dx.doi.org/10.1016/j.jfca.2011.03.002

Routray, W., & Mishra, H. N. (2011). Scientific and Technical Aspects of Yogurt Aroma and Taste: A Review. *Comprehensive Reviews in Food Science and Food Safety, 10*(4), 208-220. doi:10.1111/j.1541-4337.2011.00151.x

Saavedra, J. M., Bauman, N. A., Perman, J. A., Yolken, R. H., Saavedra, J. M., Bauman, N. A., & Oung, I. (1994). Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *The Lancet, 344*(8929), 1046-1049. doi:http://dx.doi.org/10.1016/S0140-6736(94)91708-6

Schiffrin, E. J., Rochat, F., Link-Amster, H., Aeschlimann, J. M., & Donnet-Hughes, A. (1995). Immunomodulation of Human Blood Cells Following the Ingestion of Lactic Acid Bacteria. *Journal of Dairy Science, 78*(3), 491-497. doi:http://dx.doi.org/10.3168/jds.S0022-0302(95)76659-0

Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal, 17*(11), 1262-1277. doi:10.1016/j.idairyj.2007.01.014

Sieuwerts, S., de Bok, F. A. M., Hugenholtz, J., & van Hylckama Vlieg, J. E. T. (2008). Unraveling Microbial Interactions in Food Fermentations: from Classical to Genomics Approaches. *Applied and Environmental Microbiology, 74*(16), 4997-5007. doi:10.1128/AEM.00113-08

Statistics Iceland. (2015a). Mannfjoldi eftir kyni, aldri og sveitarfelogum1998-2015. Retrieved from http://gamli.hagstofa.is/?PageID=2593&src=https://rannsokn.hagstofa.is/pxis/Dialog/varval.asp?ma=MAN02001%26ti=Mannfj%F6ldi+eftir+kyni%2C+aldri+og+sveitarf%E9l%F6gum+1998%2D2015+%2D+Sveitarf%E9lagaskipan+hvers+%E1rs%26path=../Database/mannfjoldi/sveitarfelog/%26lang=3%26units=Fj%F6ldi

Statistics Iceland. (2015b). Mannfjoldi eftir menntunarstodu samkvæmt ISCED 2011 menntunarflokkuninni 2003-2014. Retrieved from http://px.hagstofa.is/pxis/pxweb/is/Samfelag/Samfelag__skolamal__5_menntunarstada/SKO00001.px/table/tableViewLayout1/?rxid=66848e75-1ae8-457e-a78a-864c648895b9

Stephen, A. et al. (2012). The role and requirements of digestible dietary carbohydrates in infants and toddlers. *European Journal of Clinical Nutrition, 66*(7), 765-779. doi:10.1038/ejcn.2012.27

Thorgeirsdottir, H. et al. (2012). *Study on Icelanders' nutrition*. Retrieved from Reykjavík:

Thorisdottir, A. V., Gunnarsdottir, I., Palsson, G. I., Gretarsson, S. J., & Thorsdottir, I. (2013). Iron status and developmental scores in 6-year-olds highlights ongoing need to tackle iron deficiency in infants. *Acta Paediatrica, 102*(9), 914-919. doi:10.1111/apa.12316

Thorisdottir, A. V., Ramel, A., Palsson, G. I., Tomassson, H., & Thorsdottir, I. (2013). Iron status of one-year-olds and association with breast milk, cow's milk or formula in late infancy. *Eur J Nutr, 52*(6), 1661-1668. doi:10.1007/s00394-012-0472-8

Thorisdottir, A. V., Thorsdottir, I., & Palsson, G. I. (2011). Nutrition and Iron Status of 1-Year Olds following a Revision in Infant Dietary Recommendations. *Anemia, 2011*, 986303. doi:10.1155/2011/986303

Thorisdottir, B., Gunnarsdottir, I., Palsson, G. I., Halldorsson, T. I., & Thorsdottir, I. (2014). Animal protein intake at 12 months is associated with growth factors at the age of six. *Acta Paediatrica, 103*(5), 512-517. doi:10.1111/apa.12576

Thorisdottir, B., Gunnarsdottir, I., Steingrimsdottir, L., Palsson, G. I., Birgisdottir, B. E., & Thorsdottir, I. (2016). Vitamin D Intake and Status in 6-Year-Old Icelandic Children Followed up from Infancy. *Nutrients, 8*(2), 75. doi:10.3390/nu8020075

Thorisdottir, B., Gunnarsdottir, I., Steingrimsdottir, L., Palsson, G. I., & Thorsdottir, I. (2014). Vitamin D intake and status in 12-month-old infants at 63-66 degrees N. *Nutrients, 6*(3), 1182-1193. doi:10.3390/nu6031182

Thorsdottir, I., & Gunnarsson, B. S. (2006). Dietary quality and adequacy of micronutrient intakes in children. *Proc Nutr Soc, 65*(4), 366-375. doi:10.1079/PNS2006512

Thorsdottir, I., Gunnarsson, B. S., Atladottir, H., Michaelsen, K. F., & Palsson, G. (2003). Iron status at 12 months of age -- effects of body size, growth and diet in a population with high birth weight. *Eur J Clin Nutr, 57*(4), 505-513. doi:10.1038/sj.ejcn.1601594

Thorsdottir, I., & Thorisdottir, A. V. (2011). Whole cow's milk in early life. *Nestle Nutrition Workshop Series. Pediatric Programme, 67*, 29-40. doi:10.1159/000325573

Thorsdottir, I., Thorisdottir, A. V., & Palsson, G. (2008). *The diet of Icelandic infants - Results from a research on infants' diet, growth and iron status 2005-2007*. Retrieved from http://www.landlaeknir.is/servlet/file/store93/item11592/Ungbarnaskyrsla.pdf

Twigger, A. et al. (2015). Gene expression in breastmilk cells is associated with maternal and infant characteristics. *Scientific Reports, 5*, 12933. doi:10.1038/srep12933

Underwood, M. A., German, J. B., Lebrilla, C. B., & Mills, D. A. (2015). Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. *Pediatric research, 77*(0), 229-235. doi:10.1038/pr.2014.156

Valsdottir, T., Saevarsdottir, R. E., Einarsdottir, G., Thorkelsson, G., Olafsdottir, A., & Sveinsdottir, K. (2011). *Islenskur barnamatur - markadur og opinberar krofur*. Retrieved from http://www.matis.is/media/matis/utgafa/07-11-Islenskur-barnamatur-Samantekt.pdf

Wall, R., Ross, R. P., Ryan, C. A., Hussey, S., Murphy, B., Fitzgerald, G. F., & Stanton, C. (2009). Role of Gut Microbiota in Early Infant Development. *Clinical Medicine. Pediatrics, 3*, 45-54.

Watford, M. (2015). Glutamine and glutamate: Nonessential or essential amino acids? *Animal Nutrition, 1*(3), 119-122. doi:10.1016/j.aninu.2015.08.008

Weng, M., & Walker, W. A. (2013). The role of gut microbiota in programming the immune phenotype. *Journal of developmental origins of health and disease, 4*(3), 203-214. doi:10.1017/S2040174412000712

Westerbeek, E. A., van der Berg, A., Lafeber, H. N., Knol, J., Fetter, W. P., & van Elburg, R. M. (2006). The intestinal bacterial colonisation in preterm infants: a review of the literature. *Clinical Nutrition, 25*(3), 361-368. doi:10.1016/j.clnu.2006.03.002

World Health Organization. (2006). *Guidelines on Food Fortification with Micronutrients.* Geneva.

World Health Organization. (2009). *Infant and Young Child Feeding: Model Chapter for Textbooks for Medical Students and Allied Health Professionals.* (Vol. SESSION 1, The importance of infant and young child feeding and recommended practices.). Geneva.

Zhang, Z., Adelman, A. S., Rai, D., Boettcher, J., & Lőnnerdal, B. (2013). Amino Acid Profiles in Term and Preterm Human Milk through Lactation: A Systematic Review. *Nutrients, 5*(12), 4800-4821. doi:10.3390/nu5124800

Zivkovic, A. M., & Barile, D. (2011). Bovine milk as a source of functional oligosaccharides for improving human health. *Advances in Nutrition, 2*(3), 284-289. doi:10.3945/an.111.000455

Appendix I

The consumer survey participants answered.

Þróun mjólkurafurðar fyrir börn

Ágæti þátttakandi

Ég heiti Elva Björk og er meistaranemi í matvælafræði við Matvæla- og næringarfræðideild Háskóla Íslands. Meðfylgjandi könnun er hluti af meistaraverkefni mínu og er ætluð foreldrum/forráðamönnum barna undir 6 ára aldri. Í meistaraverkefni mínu er ég að kanna möguleikana á að þróa mjólkurafurð fyrir börn/ungbörn og er tilgangur könnunarinnar að skoða áhuga foreldra/forráðamanna á slíkri afurð. Könnunin er nafnlaus og ekki er hægt að rekja svör til einstakra þátttakenda. Það tekur um það bil 5-10 mínútur að svara könnuninni. Ef þú hefur einhverjar spurningar getur þú haft samband við mig.

Með fyrirfram þökk, Elva Björk ebt5@hi.is

1. Hvert er kyn þitt?

- ○ Karl
- ○ Kona

2. Hver er aldur þinn?

- ○ Yngri en 20 ára
- ○ 21-25 ára
- ○ 26-30 ára
- ○ 31-35 ára
- ○ 36-40 ára
- ○ Eldri en 40 ára

3. Hver er búseta þín?

- ○ Höfuðborgarsvæðið
- ○ Suðurnes
- ○ Suðurland
- ○ Austurland
- ○ Norðurland
- ○ Vesturland
- ○ Vestfirðir

4. Hvert er hæsta menntunarstig sem þú hefur lokið?

- Grunnskólapróf
- Stúdentspróf
- Iðnnám
- Grunnnám í háskóla
- Framhaldsnám í háskóla

5. Hversu mörg börn átt þú og á hvaða aldri eru þau?

	0	1	2	3	Fleiri en 3
0-6 mánaða					
7 mánaða - tveggja ára					
3-6 ára					
Eldri en 6 ára					

6. Hvert er viðhorf þitt til tilbúins barnamatar?

- Mjög jákvætt
- Frekar jákvætt
- Hlutlaus
- Frekar neikvætt
- Mjög neikvætt

7. Hversu oft gefur þú barninu þínu tilbúinn barnamat?

- 3-4 máltíðir á dag
- 1-2 máltíðir á dag
- Einu sinni á dag
- 5-6 sinnum í viku
- 3-4 sinnum í viku
- 1-2 sinnum í viku
- Sjaldnar en 1 sinni í viku
- Aldrei

8. Hefur þú gefið barni/börnum þínum stoðmjólk?

- Já
- Nei

9. Hversu gott eða slæmt finnst þér úrval barnamatar á íslenskum markaði?

- Mjög gott

- Frekar gott
- Hlutlaus
- Frekar slæmt
- Mjög slæmt

10. Hvenær hóf barn þitt neyslu á mjólkurvörum á borð við skyr, jógúrt, ab-mjólk o.þ.h.?

- Yngri en 6 mánaða
- 6-8 mánaða
- 9-10 mánaða
- 11-12 mánaða
- Eldri en 12 mánaða
- Aldrei
- Man það ekki

11. Hversu mikinn eða lítinn áhuga hefðir þú á að kaupa jógúrt sem væri sérstaklega sniðið að þörfum barna undir tveggja ára aldri m.t.t. næringargildis?

- Mjög mikinn
- Frekar mikinn
- Hlutlaus
- Frekar lítinn
- Mjög lítinn

12. Hversu sammála eða ósammála ert þú eftirfarandi fullyrðingu: Ég tel að slík vara sem fjallað er um í sp. 11 eigi að vera bragðbætt

- Mjög sammála
- Frekar sammála
- Hlutlaus
- Frekar ósammála
- Mjög ósammála

13. Hversu sammála eða ósammála ert þú eftirfarandi fullyrðingu: Ég tel að slík vara sem fjallað er um í sp. 11 eigi að vera vítamínbætt

- Mjög sammála
- Frekar sammála
- Hlutlaus
- Frekar ósammála
- Mjög ósammála

14. Hvaða eiginleika finnst þér mikilvægt að slík vara sem fjallað er um í sp. 11 hafi? Hér getur þú valið fleiri en einn möguleika.

- Bragðgóð

- ☐ Lífræn
- ☐ Ódýr
- ☐ Bragðbætt
- ☐ Næringarrík
- ☐ Íslensk framleiðsla
- ☐ Other: ☐

15. Hvað finnst þér sanngjarnt verð fyrir 120 g af slíkri vöru sem fjallað er um í sp. 11?

- ○ 85-94 kr.
- ○ 95-104 kr.
- ○ 105-114 kr.
- ○ 115-124 kr.
- ○ 125-134 kr.
- ○ 135-144 kr.
- ○ 145-149 kr.

16. Hversu líklegt eða ólíklegt er að þú borgir meira fyrir 120 g af mjólkurafurð sem er sérstaklega þróuð og framleidd fyrir ungbörn en aðrar sambærilegar mjólkurvörur á markaðinum?

- ○ Mjög líklegt
- ○ Frekar líklegt
- ○ Hlutlaus
- ○ Frekar ólíklegt
- ○ Mjög ólíklegt

17. Ef þú hefur athugasemdir varðandi einstakar spurningar, vinsamlegast skrifaðu þær hér.

Appendix II

Below are comments from participants in the net survey.

Er ekki hrifin af mjólkurvörum yfir höfuð
Barnið mitt fær ekki barnamat í dag þar sem það borðar allan venjulegan mat.
Kúamjólk er fyrir kálfa.
Margar spurningar eiga ekki við þar sem ég er ekki með ung börn.
Hef gefið stoðmjólk og er mjög á móti henni !
varðandi spurningu 14 mætti vera möguleikinn að hún væri sykurlaus eða Verulega sykurlítil - finnst það skipta mjög miklu máli í ungbarnavörum
Barnið mitt er 9 manaða og fær skyr, ab mjólk og svoleiðis þegar hann verðir aðeins eldri en eg skráði "aldrei" því möguleikinn "ekki ennþá" var ekki fyrir hendi
Mér finnst að mannabörn ættu ekki að neyta mjókurafurða frá kúm sem er gerð fyrir kálfa
spurning 7, spurningin er í nútíð en væri kannski gott að hafa hana í þátíð, "hversu oft gefurðu eða gafstu barninu tilbúinn barnamat". Ég er semsagt ekki að gefa tilbúinn mat í dag en gerði það þegar börnin voru yngri.
myndi bara kaupa ef sykurlaust
Frekar neikvætt viðhorf almennt til tilbúins barnamatar, en ánægð með nýlegu lífrænu skvísurnar sem eru ekki með viðbættum efnum, frá Ella's Kitchen aðallega
Yrði varan með hærri % af fitu og d vítamíni?
Það vantar aldursbil á milli 24 man og 3 ára :)
mjólkurlaust jógúrt með gerlum væri betri kostur
Spurning 10
Vantar svarmöguleika fyrir börn á aldrinum 2-3 ára
Barnið mitt er með mjólkurofnæmi svo ég myndi sjálf ekki kaupa vöruna en það sárvantar íslenska vöru fyrir börn með mjólkurofnæmi.
eg a barn með mjolkur ofnæmi og mer finnst vanta meira urval af mjolkyrlausum mat fyrir börn. og þegar maður finnur soja jogurt eða eitthvað alika þa ætti þap ekki að kosta halfan handlegg .
mætti fjalla meira um sykurmagn sem er allt of mikið í mjólkurvörum og söfum stílaðar inná börn
Vantar valmöguleikann að minnka sykurmagn

Appendix III

Results from all the analyses performed in the pre-trial, main experiment and follow-up experiment. The tables show the average of the measurements and the standard deviations are given within brackets.

Analysis	Date	Products in the pre-trial Yoghurt made from Support milk	Regular yoghurt enriched with the Support milk vitamins	Regular yoghurt (pure)
Protein (%)	23/5/16	1.8 (0.1)	3 (0.1)	
Fat (%)	23/5/16	5.1 (0.4)	4.1 (0.3)	
Carbohydrates (%)	23/5/16	6.4 (0.0)	4 (1.0)	
Ash (%)	23/5/16	0.8 (0.0)	0.7 (0.0)	
Water (%)	23/5/16	85.9 (3.4)	88.2 (3.5)	
WHC (%)	23/5/16	0 (0.0)	82.7 (1.5)	
pH	23/5/16	5.1 (0.0)	4.5 (0.0)	4.1 (0.0)
Colour (L-value)	23/5/16	90 (1.1)	93.5 (0.2)	91.3 (0.2)
Colour (a-value)	23/5/16	-2.7 (0.2)	-2.3 (0.1)	-2.8 (0.1)
Colour (b-value)	23/5/16	9.4 (0.8)	6.5 (0.1)	8.4 (0.1)
Lactic acid bacteria (Log)	23/5/16	3.3 (0.0)	5.1 (5.0)	
Yeast	23/5/16	<20	<20	
Mould	23/5/16	<20	<20	
Viscosity	23/5/16	116.3 (6.0)	958.3 (1.0)	

Analysis	Date	Products in the main experiment Support yoghurt with fibres and gelatine	Support yoghurt with fibres	Support yoghurt with fibres and starch	Vitamin enriched yoghurt with fibres
Protein (%)	5/1/17	2.1 (0.1)	1.7 (0.1)	1.7 (0.1)	3.1 (0.1)
Fat (%)	5/1/17	3.7 (0.3)	3.4 (0.3)	3.4 (0.3)	3.7 (0.3)
Carbohydrates (%)	5/1/17	8.9 (0.1)	8.5 (0.1)	10.7 (0.1)	5.4 (0.1)
Ash (%)	5/1/17	0.8 (0.0)	0.8 (0.0)	0.8 (0.0)	0.7 (0.0)
Water (%)	5/1/17	84.7 (3.4)	85.6 (3.4)	83.5 (3.3)	87.1 (3.5)
	12/1/17	84.9 (3.4)	85.9 (3.4)	83.6 (3.3)	87 (3.5)
	18/1/17	84.4 (3.4)	85.5 (3.4)	83.5 (3.3)	87.2 (3.5)
	23/1/17	84.5 (3.4)	85.9 (3.4)	83.6 (3.3)	88 (3.5)
WHC (%)	5/1/17	45.1 (5.5)	51.2	90.5 (0.2)	76 (0.7)
	12/1/17	37 (0.5)	0 (0.0)	38.1 (0.0)	34.1 (0.1)
	18/1/17		0 (0.0)	50.2 (1.3)	46.3 (3.6)
	23/1/17	39.3 (2.2)	0 (0.0)	45.2 (0.4)	41.6 (4.3)
pH	5/1/17	4.1 (0.0)	4.2 (0.0)	4.1 (0.0)	4.4 (0.0)
	12/1/17	4.2 (0.0)	4.1 (0.0)	4.1 (0.0)	4.4 (0.0)
	18/1/17	4 (0.0)	4.1 (0.1)	4.1 (0.0)	4.4 (0.0)
	23/1/17	4.1 (0.0)	4.1 (0.0)	4.1 (0.0)	4.3 (0.0)
Colour (L-value)	5/1/17	88.4 (0.2)	88.5 (0.1)	88.3 (0.2)	90.8 (0.1)
	12/1/17	88 (0.2)	87.8 (0.1)	87.2 (0.3)	89.6 (0.5)
	18/1/17	88.1 (0.5)	88.1 (0.3)	87.8 (0.6)	90.8 (0.2)
	23/1/17	88.4 (0.4)	88.3 (0.1)	88.3 (0.1)	90.5 (0.3)
Colour (a-value)	5/1/17	-2.9 (0.0)	-2.7 (0.1)	-2.9 (0.2)	-2.8 (0.1)
	12/1/17	-3.2 (0.0)	-3.2 (0.1)	-3.4 (0.1)	-3.3 (0.1)
	18/1/17	-2.8 (0.0)	-2.8 (0.1)	-2.9 (0.1)	-2.7 (0.0)
	23/1/17	-2.6 (0.1)	-2.7 (0.2)	-2.7 (0.1)	-2.6 (0.1)
Colour (b-value)	5/1/17	9.6 (0.0)	9.5 (0.1)	9.8 (0.5)	8.5 (0.2)
	12/1/17	10.1 (0.1)	10.4 (0.1)	9.8 (0.1)	9.1 (0.2)
	18/1/17	9.9 (0.2)	9.6 (0.1)	10 (0.2)	8.8 (0.1)
	23/1/17	9.9 (0.8)	9.4 (0.2)	9.2 (0.1)	8.4 (0.1)
Lactic acid bacteria	5/1/17	8.9 (8.4)	8.4 (6.9)	8.6 (8.0)	8.3 (6.9)
	12/1/17	8.8 (7.8)	8.3 (7.5)	8.7 (7.6)	7.8 (6.2)
	18/1/17	8.7 (8.0)	8.4 (8.0)	8.5 (7.3)	6.8 (5.2)
	23/1/17	8.8 (8.0)	8.4 (7.2)	9.15 (9.2)	7 (5.6)
Mould	5/1/17	<20	<20	<20	<20
	12/1/17	<20	<20	<20	<20
	18/1/17	<20	<20	<20	<20
	23/1/17	<20	<20	<20	<20
Yeast	5/1/17	<20	<20	<20	<20
	12/1/17	<20	<20	<20	<20
	18/1/17	<20	<20	<20	<20
	23/1/17	<20	<20	<20	<20

Analysis	Date	Products in the follow-up experiment Support yoghurt with fibres and starch (follow-up experiment)	Support yoghurt with fibres and protein
Protein (%)	9/3/17	1.9 (0.1)	2.2 (0.1)
Fat (%)	9/3/17	3.5 (0.3)	3.6 (0.3)
Carbohydrates (%)	9/3/17	10.6 (0.1)	8 (0.1)
Ash (%)	9/3/17	0.8 (0.0)	0.8 (0.0)
Vitamin-B2 (mg/100 g)	23/3/17	0.13 (0.0)	0.13 (0.0)
Vitamin-D (µg/100 g)	23/3/17	1.3 (0.0)	1.3 (0.1)
Iron (mg/kg)	23/3/17	6.3 (0.2)	5.9 (0.2)
Iodine (µg/kg)	23/3/17	40 (1.0)	40 (1.0)
Water (%)	9/3/17	83.3 (3.3)	85.5 (3.4)
	21/3/17	83.3 (3.3)	85.5 (3.4)
	4/4/17	83.4 (3.3)	85.5 (3.4)
WHC (%)	9/3/17	67.1 (0.3)	63 (6.4)
	21/3/17	72.4 (6.1)	0
	4/4/17	49.6 (0.2)	0
pH	9/3/17	4.3 (0.0)	4.4 (0.0)
	21/3/17	4.3 (0.1)	4.5 (0.0)
	4/4/17	4.2 (0.0)	4.2 (0.0)
Colour (L-value)	9/3/17	88.5 (0.7)	89 (0.1)
	21/3/17	88.8 (0.4)	89.5 (0.1)
	4/4/17	89.4 (0.1)	90.1 (0.2)
Colour (a-value)	9/3/17	-3.6 (0.0)	-3.6 (0.0)
	21/3/17	-3.1 (0.1)	-3.2 (0.1)
	4/4/17	-3.1 (0.0)	-3.1 (0.0)
Colour (b-value)	9/3/17	10 (0.4)	9.5 (0.1)
	21/3/17	9.8 (0.2)	9.5 (0.2)
	4/4/17	9.1 (0.1)	9.2 (0.2)
Lactic acid bacteria	9/3/17	8.6 (7.3)	8.7 (7.8)
	21/3/17	8.5 (7.7)	8.6 (7.5)
	4/4/17	8.4 (7.4)	8.6 (7.6)
Mould	9/3/17	<20	<20
	21/3/17	<20	<20
	4/4/17	<20	<20
Yeast	9/3/17	<20	<20
	21/3/17	<20	<20
	4/4/17	<20	<20