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Egenskaper och smak på en probiotisk havrebaserad dryck

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Physico-Chemical Properties, Probiotic Survival and Sensory Acceptability of a Liquid Oats-Based Beverage

Degree Project in Food Engineering KLTM01

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Abstract

In the present thesis were studied oat based real fruit puree products. The aim was to create a new synbiotic beverage by combining the health benefits of the probiotic bacterial strain with the prebiotic ones derived from the β-glucans in oats. This new synbiotic oat-based real fruit puree beverage called Nordic Berries Skaka Smaka consisted of 48% whole-grain oat substrate and 52% Nordic berries fruit puree, and was inoculated with the bacterial strain of *Prevotella copri* DSM18205.

Factors such as physic-chemical properties and the stability of the products within their life-span were analyzed. The new synbiotic product, was examined in different aspects (eg. pH, viability of strain, organoleptic characteristics) for 20 days under different storage conditions such as temperature and acidity level. It was found that the addition of the specific strain into the oat-based real fruit puree product was not affected by the used different storage conditions. Two sensory tests were performed, one hedonic and one in focus group for the Nordic Berries Skaka Smaka product. In both of them, panelists presented a willing to buy the new Nordic berries Skaka Smaka product, but only if the beneficial health effects are targeted in its package.

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Background

1.1 The need of symbiotic food products and the concept of *Prevotella copri*

Continuous development of new functional food products is the challenge of science and industry to the increased consumer awareness regarding the role of food in the human health and wellbeing (Bland & Medcalf, 1994). Nowadays, one of the most increasing trends when it comes to new functional food products are the ones based on oats. Globally, most of probiotic food products are milk based, but lately there are made a few attempts for development of probiotic foods based on non-dairy substrates (eg.cereals). Northern parts of the planet, like Canada, Scandinavian countries, Russia and Scotland have a long tradition of cultivating high quality oats and apply it in a variety of foods. Oats (*Avena sativa L.*) are an important source of animal feed as they are a good source of protein, fibre and minerals. Despite that, oats are also used in production of many food products and industrial applications. In the late 80s, many scientific studies have proven that the functional ingredients oats have beneficial effects on human health (Webster and Wood, 2011).

As a result of many researches, cereal based products have been recognized for their numerous health effects due to their nutritional value. As an example, oats and barley have the functional component of β-glucans (fibres belonging to carbohydrate complex) as they are reach in them (Mathey *et al.,* 1999). Oats have a high content of β-glucans. According to both European Food Safety Authorities (E.F.S.A.) and Food and Drugs Administration (FDA) in U.S.A. (F.D.A.), β-glucans have been officially acknowledged as a functional ingredient in food products, since they can possibly have a positive effect in the prevention of non-communicable diseases such as obesity, type II diabetes (T2D), metabolic syndrome and cardiovascular disease. When β-glucans are consumed, fibres are partially digested in the small intestine and as soon as they reach the large intestine fermented by the gut microbiota. This fermentation has as the consequence to release of very important low-molecular fatty acids (eg. butyric and propionic acid) which return back to the body and regulates the blood glucose response (Wrick, 1993). In addition, β-glucans are known as prebiotic, stimulating the growth of some beneficial residential colon microorganisms (Wood & Beer, 1998).

Thus, there is a need to increase the number of beneficial bacteria in the gut micro flora. This can be achieved by supplying viable bacteria through blood, or through food products in sufficient amounts of substrates (prebiotic and probiotic substrates) (Björk *et al*., 2014).

In accordance to resent research findings, bacterial strains isolated from human faeces belonging to the family of *Prevotellaceae* (such as *P.copri*), proved to have positive health effects in both the glucose metabolism and the weight regulation. As a conclusion, there is a public need to develop food products that could improve the blood glucose metabolism and reduce the risk of metabolic syndrome and other related diseases (Björk *et al*., 2014).

A balanced diet monitored by professional individuals containing a variety of energy-dense foods, more fruits, vegetables and grains, foods containing adequate amounts of calcium and vitamin D should be suitable for all the groups of people (Gariballa, 1998; Gariballa, 2004). In the market nowadays, the need of energy-dense products is increasing since this category

seems essential for some target groups. Since the health issues and the eating disorders are increasing rapidly in all the age groups of population, there is need of good eating habits establishments. Synbiotic products, seem to be able to prevent several problems, when they are being consumed in a healthy diet for the most groups of people.

1.2 Design of new functional products

To design new innovative products is the result of an accurate and precise management of knowledge sources all over its life cycle, such as technology, market, competitors and suppliers. Nowadays, it has been reached a high level of maturity in terms of product development. New products are released to the market every day, ready to solve existing problems, or create new needs in customers' demands. As a result, food companies are involved in a continuous product design and development competition where every single decision could have an effect on the final result perceived by the costumer. Every product that is already out in the market or is new, has a continuously changing life, which is affected mostly by the market's needs and the customers' requirements. The goal every time, is to ensure the satisfaction of the customers by full-filing all of their expectations (Vila & Albiñana, 2015).

1.3 Requirements for probiotic products

During the last 10 years the need and interest for probiotic products in the market has increased. Although the majority of the probiotic products are in the dairy field, but the number of the non-dairy ones is in rise. While developing probiotic products, many parameters have to be considered. First and foremost, since probiotics are viable bacteria they must be delivered to the gut microbiota alive in order to express their beneficial health effects (Swidan, 2009). A variety of health benefits have been attributed to ingesting probiotics, including enhanced immune health and improved digestion. Each probiotic strain and dosage results in different health benefits. The products formulation affects the viability of the probiotic strain. Parameters as water activity, pH, and storage conditions affect the probiotic strain's survival (Vinderola, Bailo & Reinheimer, 2000; Swidan, 2009).

More specifically, the pH tolerance is depended upon the product's composition and strain's tolerance and it is a very important factor as it works as an indicator for the strain's survivability into the food product. Another important factor is the temperature including both storage temperature and heat treatments during the production process (eg. pasteurization). It is recommended that most of the probiotic strains must be added into the drink after the thermal process since the majority of the bacteria are mesophilic microorganisms and they have no heat tolerance.

Conditions during storage and distribution can play an important role in the probiotic viability, primarily as they relate to temperature control and temperature fluctuation. In general storage in lower temperatures (cooling temperatures) improve the probiotic stability (Swidan, 2009).

In order to develop a new synbiotic food product, several steps need to be taken into consideration. Bacteria in the transition between the exponential phase and the stationary

phase are more susceptible to the stress of storage conditions than cells in the stationary phase (Heller, 2001).

1.4 Commercially used probiotic strains in food products

Probiotic microorganisms are isolated from human gastrointestinal system. *Lactobacillus* and *Bifidobacterium* species are widely known. *E. faecium, E. faecalis, S. thermophilus, Lc. lactis subsp. lactis, Leuconostoc mesenteroides, Propionibacterium freudenreichii, Pediococcus acidilactici, Sporolactobacillus inulinus, E. coli*, bacteria such as some *Bacillus species*, other lactic acid bacteria species, yeast such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* are commonly used as probiotics. (Foulquié Moreno *et al*., 2006; Granato *et al.,* 2010; Nagpal *et al*., 2012).

Interest for probiotics has arisen in recent years especially in relation to the *addition* of *Bifidobacterium*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, Lactobacillus *reuteri* to the fermented dairy products such as yoghurt (Guldas & Irkin, 2010).

The bacterial strain of *P.copri* DSM18205, was used in this current project, was isolated from human faeces according to the DSMZ Laboratory in Germany.

1.5 Characterization of the food product by means of chemical and nutritional analyses before launching the market

It is important to characterize the physicochemical properties of a newly developed product before launching the market. Food products, with different consistencies require different kind of analysis. In this particular study case, products to be analysed were in liquid form, so the nutritional aspects that were characterized were the followings: fat, fibre, protein, carbohydrate analysis and the measurement of the β-glucan content. All used methods were approved by AOAC International.

Moving to the structural analysis, the structure of the different components in the oat base can affect both colloidal and rheological properties of the oat drink. The oat base is a colloidal system, a suspension of oat in water, meaning the oats are dispersed in the water, not dissolved. The whole Skaka Smaka product which contains apart from the oat base, oils and other proteins and starch sources, can be characterized as a dispersion, where, the oat drink also is an oil in water emulsion, with oils dispersed in water. The salt, which is into the solution - water, is fully dissolved in it. Some sedimentation of particles can occur in the oat drink, due to the tendency of the insoluble dietary fibres to stay crystalline (Lorén, 2013).

An emulsion can be destabilized in several ways, such creaming, flocculation, coalescence, Ostwald ripening, and phase inversion (Hidalgo-Álvarez, 2010). The two phenomena assumed most probable to occur is creaming and coalescence. Creaming means that there will be a separation of different sized particles and coalescence that particles merge. Therefore, it is important to take the structure of additives in consideration. One way in order to stabilize an emulsion is the use of emulsifiers which is very common. Emulsifiers work by reducing the interfacial tension between the phases. In the oat drink the polar lipids work as emulsifiers (Lorén, 2013).

Since the product is a beverage made from two phases, it is an emulsion. Food products that characterized as emulsion systems show typical colloidal instabilities (creaming, sedimentation, flocculation, coalescence). It is important to test its stability in long time storage.

The method was used based upon to test the products' stability was the Turbiscan Stability Index method. The Turbiscan is a complete technique that can be used during all the development of a product from the formulation in the lab through the production of the products. It enables to measure stability of various types of beverage emulsions and to identify what sort of instability is taking place, even when several phenomena occur simultaneously or when nothing is visible to the eye. Moreover, the emulsion droplet size distributions was analysed using laser light scattering technique in a Mastersizer S long bench apparatus (Malvern Instruments, Ltd. UK) and laser light scattering technique in a Mastersizer S long bench apparatus (Malvern Instruments, Ltd. UK).

2. Aim

The goal of this thesis was consisted of several parts, which are described as following:

- Firstly, the general concept behind the development of the Skaka Smaka oat based energy-densed drink products, is to prevent the status of malnutrition among elderly patients.
- Secondly, the Skaka-Smaka products were analyzed as for their physicochemical characteristics.
- Thirdly, a new synbiotic oat-based real fruit puree product was developed. A highly promising bacterial strain from the genus *Prevotella spp.* was inoculated into the new product and several factors were tested.
- Last but not least, since it is very important the product to be market oriented, two sensorial tests-one hedonic and one in focus group, were conducted so as to realize the potential customers' preferences.

3. Material and Method

3.1 Product development pre-trials

Before deciding the final ingredients' composition of the Nordic berries Skaka Smaka beverage, several trials (120 trials) were performed. In these pre-trials, different concentrations and combinations of the ingredients were evaluated according to their organoleptic characteristics. In the pre-trials the following berries were investigated; raspberry, blue berry, black berry in different ratios. Also different ratios oat base and fruit purée were tested.

Of each trial, key parameters such as organoleptic acceptance, pH, viscosity, dry matter and total solid content were analysed. The recipe was the one had the best organoleptic characteristics according to the panellists, was chosen as the final one (See Table 3 Results section). All the experiments regarding the product's development were performed at the

Aventure's AB laboratory, while most of the nutritional analysis was performed at the Food Engineering department of the Lund's University Chemical centre.

3.2 Nutritional analysis methods

Before performing any kind of nutritional analysis, it was crucial all of the three liquid beverages to be used in a powder form for more convenience and accuracy in the obtaining result values.

3.2.1 Preparation of samples

In order to prepare the beverages at the laboratory level, the following steps were taken: Firstly, all the raw materials, apart from the whey protein, were weighted and mixed till the mixture becomes homogenous. Once the mixture consistency was achieved, the mixture was passing through a sieve (approximately 0.5μm size) so as to get rid of the solid particles. Later, the mixture was heated up while stirring continuously to approximately 72°C. After that step, the mixture was stored under cooling conditions, in a cool water bath (4°C) till it reaches the room temperature (approximately 22°C). Once it was in room temperature, the whey protein was added slowly under continuously mixing. The product mixture was then transferred in a plastic store bottle and was stored at 4°C for further use. In case of the symbiotic samples, the addition of the bacterial strain *P.copri* is well explained bellow. It is well known that there are some physicochemical factors that might influence the survival of probiotic bacteria into products. The most important factors might be affected in this case are: acidity, temperature, oxygen concentration and storage conditions, which were examined (Vinderola, Bailo & Reinheimer, 2000).

These prepared beverages were freeze-dried into powder form. The dried powders were stored at room temperature for further use. Samples were analysed for their chemical composition (total solids, protein, fat, carbohydrate, ash and β-glucan as a dietary fiber indicator) using standard method procedures. The two reference products (Skaka Smaka berries flavour and Skaka Smaka strawberry flavour) were also analysed in liquid form within 1 month stored at room temperature for their stability index.

3.2.2 Analysis of Protein, Fat, Moisture, Total solids, Carbohydrates and β-glucans' content

To start with the analysis of the protein content into the beverages, it can be determined by different methods. The most commonly used ones are the Kjelldahl method and the nitrogen combustion -Dumas method, which are based on nitrogen determination. The method was used in order to estimate the protein content in the current thesis was the Dumas one. The results are given from that method, are as % or mg nitrogen, which are lately being converted into protein by using conversion factors. In order to measure the amount of protein in the freeze dried powder form products, 25 replicates from each powder product in room temperature were analysed and their mean value and standard deviation were calculated. As

standard component in this method was used Ascorbic Acid (Sigma Aldrich, St Luis, USA). Results are shown in the Table 4 Results section.

In order to estimate the fat content of a food, one needs to measure the lipid content. The term 'lipid' refers to compounds that are sparingly soluble in water and somehow soluble in organic solvents (eg. petroleum ether CAS No.8032-32-4). In this current case, it was determined by extraction with one solvent–petroleum ether (Soxhlet method). For this experiment, the results were coming out after three replicates of each sample and are shown in the Table 4 Results section. Additionally, in the methods, the total calorie content, was measured by using the Bomb Calorimeter Parr6200 by determining the enthalpy of combustion.

Also, moisture and total solid measurements were evaluated in the dried powder samples. Total solids and consequent moisture, were determined by using oven drying and gravimetric method.

The fibres were analysed in the liquid drink products (4°C) by estimating the β-glucan content by using the Megazyme Assay kit (Megazyme Inc., Chicago, Illinois 60603,USA). According to the method, samples were suspended and hydrated in a buffer solution of pH 6.5 and then incubated with purified lichenase enzyme and at the end filtered. An aliquot of the filtrate was then hydrolysed to completion with purified β-glucosidase. The D-glucose produced was assayed using a glucose oxidase/peroxidase reagent. Results were obtained by using the Megazymes' calculator and are shown in the Table 4 Results Section.

3.2.3 Turbiscan Stability Index

The method was used based upon to test the products' stability was the Turbiscan Stability Index method. The Turbiscan is a complete technique that can be used during all the development of a product from the formulation in the lab through the production of the products. It enables to measure stability of various types of beverage emulsions and to identify what sort of instability is taking place, even when several phenomena occur simultaneously or when nothing is visible to the eye. In this experiment, both reference products, Skaka Smaka strawberry and berries flavour were analysed at room temperature storage conditions, for their stability index. It deserves to be mentioned that the new flavor Nordic Berries Skaka Smaka was not analysed due to the lack of time. The results are shown in the Images 1 and 2 at Results section 4.4.

3.2.4 Microscope and particle size distribution

Emulsion droplet size distributions were also analysed using laser light scattering technique in a Mastersizer S long bench apparatus (Malvern Instruments, Ltd. UK). The refractive index of the double emulsions was taken as 1.462 (refractive index of rapeseed oil). Samples were first diluted with deionized water to prevent multiple scattering effects. Then, they were circulated through the measuring zone using a Hydro SM small volume sample dispersion unit, following the manufacturer's recommendations for this type of emulsion. Samples were also

diluted 10 and 100 times in deionized water, and pictures of their systems were taken by using the microscope. The pictures are illustrated in the Images 3 and 4 at the Results section.

3.3 Outline of the experiments with *Prevotella copri*

The main goal was to combine the prebiotic effect of the β-glucans coming from oats with the probiotic effect of the bacterium *P.copri* in order to develop a new functional synbiotic beverage, which will benefit the public health after consumption always in combination with a health dietary status.

In order to develop a new synbiotic food product, several steps need to be taken into consideration. It is well known that there are some physicochemical factors that might influence the survival of probiotic bacteria into both dairy and non-dairy products. The most important factors might be affected are: acidity, temperature, oxygen concentration and storage conditions (Vinderola, Bailo and Reinheimer, 2000).

In this current project, the twelve samples listed in the Table 1, were used for the experiments. Samples from the Nordic berries Skaka Smaka beverage were analysed using as control the oat based beverage. Samples were stored under both aerobic and anaerobic conditions so as to check the effect of the oxygen in the bacterial's survival. Samples were also stored at different temperatures so as to examine if the temperature has any effect in the bacterial survival (4°C ,22°C and 38°C).

Prevotella copri cells are Gram negative rods, anaerobic and non- spore forming. Upon the agar that the bacterium is being plated, colonies differ on the color. The optimal growth is at 37°C under 100%CO_2 (Hayashi *et al.,* 2007).

Table 1: Different storage conditions in samples that used during the current master thesis study

Sample	Storage conditions
1	Nordic berries drink, anaerobic 4°C
2	Nordic berries drink, aerobic 4°C
3	Nordic berries drink, anaerobic 22°C
4	Nordic berries drink, aerobic 22°C
5	Nordic berries drink, anaerobic 38°C
6	Nordic berries drink, aerobic 38°C
7	Oat based control sample drink, anaerobic 4°C
8	Oat based control sample drink, aerobic 4°C
9	Oat based control sample drink, anaerobic 22°C
10	Oat based control sample drink, aerobic 22°C
11	Oat based control sample drink, anaerobic 38°C
12	Oat based control sample drink, aerobic 38°C

The recipes for both the Nordic berries drink and the control sample are based on the product's final recipe and shown on the Table 2.

Table 2: Recipes used in order to make the samples for the current master thesis study

Ingredients	
Nordic berries drink	**Weight %**
Black currant berries	11,85
Blueberries	20,2
Banana puree	5
Oat base 10% (LWO*+enzymes)	48
Rapesseed oil	3,14
Omega 3	0,55
Sunflower oil	1,47
Whey protein	4,34
Maltodextrin	5,06
Apple juice concentrated	0,35
Salt	0,04
Oat based Control samples	**Weight %**
Oat base 10% (LWO+enzymes)	48
Rapesseed oil	3,14
Omega 3	0,55
Sunflower oil	1,47
Whey protein	4,34
Maltodextrin	5,06
Apple juice concentrated	0,35
Salt	0,04

**LWO: Referred to the oat flour was used in order to prepare the oat base (LWO:Liquid Whole Oats)*

3.3.1 Bacterial cell preparation

The strain *P.copri* DSM18205 was used in this study. The strain was provided from the DSMZ company form Germany and cultured in PYG $_{\text{VAS 100 KAN 100}}$ selective broth at 38°C for 5 days (optimum growth).

From the bacterial culture that was incubating at 38°C PYG $_{\text{KAN 100 VAS 100}}$ selective broth, 10mL were transferred into an 50mL sterile tube and were centrifuged at 4500rpm, 22°C for 20 min (Eppendorf Centrifuge 5804R). The supernatant was then withdrawed and the pellet cell was re-suspended with 9% of saline solution to its initial volume (10mL). In order to ensure that

the strain was alive before the inoculation into the beverages, the optical density (OD $_{620}$nm) was measured (0,4) using 9% saline solution as blank (OD Lambda Bio, Perkin Elmer).

3.3.2 Beverages inoculation and storage under different conditions

The 10mL of the re-suspended pellet cell were inoculated in the food samples in concentration of 1% v/v. Twelve samples were created (six from the Nordic berries drink and six from the oat based control sample) and stored under different conditions for 20 days (See Table 1). Observations and measurements for the colony forming units were carried out at 4°C, 22°C and 38°C for both aerobic and anaerobic conditions for t=0 days, t=5 days, t=10 days, t=15 days and t=20 days. After serial dilutions, the samples were plated in the specific PYG agar for *P.copri* cultures.

Measurements for pH (PHM 220 Lab pH meter MeterLab ^{TM} Radiometer, Copenhagen) and viable counts were taken for t=0 days, t=5 days, t=10 days, t=15 days and t=20 days. Also, the viscosity (Brookfiels DVE viscometer) was measured for t=0 days and t=20 days, as well as, the β-glucan content (Megazyme International Ireland Ltd., Co Wicklow, Ireland). Moreover, the organoleptic characteristics were evaluated by the odor/smell and color of the samples for for t=0 days, t=5 days, t=10 days, t=15 days and t=20 days.

Since the purpose was to examine the survival of the bacterium and the effect of different storage conditions, samples were stored in both aerobic and anaerobic conditions as well as under different temperature conditions (4°C –cooling temperature, 22°C-stable room temperature, 38°C- accelerated death of the product).

3.4 Sensory analysis (Hedonic test & Focus group)

In order to evaluate the acceptance of the Nordic berries product (always compared to the Skaka Smaka berries flavour as the reference one), sensory evaluations were performed, one Hedonic and one in Focus Group. Starting with the Hedonic sensorial analysis, it took place at the Gerdahallen gym (Lund Sweden) where 30 participants (age variant 15-85) were tasted both products and replied to questions shown at the Questionnaire according to the instructions (See Appendix_Section 1). On the other hand, about the Focus Group session, the aim was to let the participants have a discussion about their eating habits and their concept of the new product. Ten participants (age 21-27), moderate exercising were participated. The Questionnaire 2 (See Appendix_Section 2), is the one that used for the Focus Group. Results for both organoleptic sessions are shown in the Tables 7 and 8 and in the Graphs 6 and 7 in the Results section 4.

4. Results

4.1 Final recipe and production process in a laboratory scale

As it previously mentioned in the Method section, the recipe was chosen to be the final one for the Nordic Berries drink is presented in the Table 3 below:

Table 3: Composition (%) of the new product called Nordic Berries Skaka Smaka

Ingredients	Weight % (g)
Black currant	11,85
Blueberries	20,20
Banana puree	5,00
Oat base 10% (LWO+enzymes)	48,00
Rapesseed oil	3,14
Omega 3	0,55
Sunflower oil	1,47
Whey protein	4,34
Maltodextrin	5,06
Apple juice concentrated	0,35
Salt	0,04

In the final product's recipe, the total solids (expressed as Brix), pH and viscosity were measured. The pH value found to be 4,13, the Brix value 18,1 (at 20°C), and the viscosity 761 cp (s62,10min, 4°C). As for the organoleptic characterization, the taste was astringent due to the high in berries concentration. Also, the color was dark purple due to the high content of flavonoids-anthocyanins in the berries, and the texture was semi-liquid. The texture of the final product is expected to be more viscous when the product will be produced in a large scale.

4.2 Physicochemical analyses

4.2.1 Nutritional analyses

All the results of the physic-chemical analyses were performed are shown in the Table 4 below. From the results, it can be concluded that the reference products have similar nutritional values, while the Nordic berries one shows slightly different values. Since the oat base level is 48% in the new created beverage, the total calorie (%) and β-glucan content (%) are higher compared to the reference products. As it can be seen, in Table 4, standard deviations are not

presented, because they were less than 0,02 so they are not making any significance difference.

Table 4: Results of the nutritional analyses in the Skaka Smaka products

Nutritional analyses (%)			
	Skaka Smaka - berries	**Skaka Smaka- strawberry**	**Nordic berries Skaka Smaka**
Energy (kcal) in the drink	137,7	131,1	151,1
Fat content in the drink	4,1	3,7	3,6
Carbohydrate content in the drink	21,1	20,1	26,1
Protein content in the drink	4,3	4,3	3,6
β-glucan content in drink	2,1	2,1	3,6
Moisture in freeze dried powder	0,9	1,7	0,6
Dry matter in freeze dried powder	99,1	98,3	99,4

4.2.2 Turbiscan Stability Index (TSI) analyses

The stability of the reference Skaka Smaka products was evaluated. The following images 1and present the stability results of the products within a period of 1 month. The results are shown as a calculation of the Δbackescatering light into the emulsions. It can be concluded that both products show no coallesence, no sedimentation and no creaming effects.

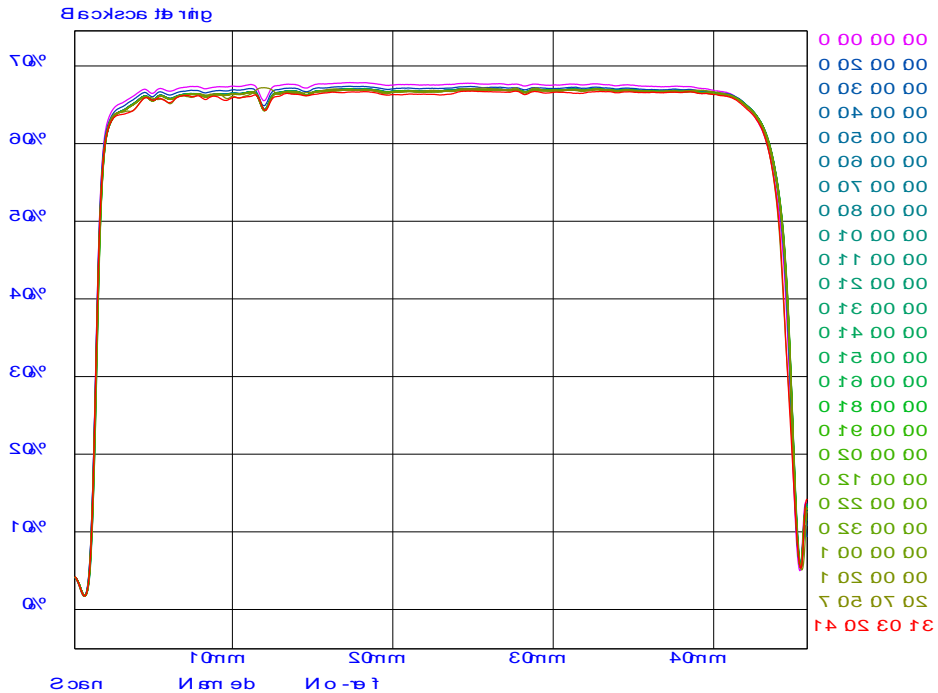


Image 1: Results of the TSI for the reference product Skaka Smaka berries flavor

TSI was estimated for the Skaka Smaka products by taking measurements -once per week for each product for 1 month (room temperature). Each graph line in the image illustrates the stability of the product per measurement.

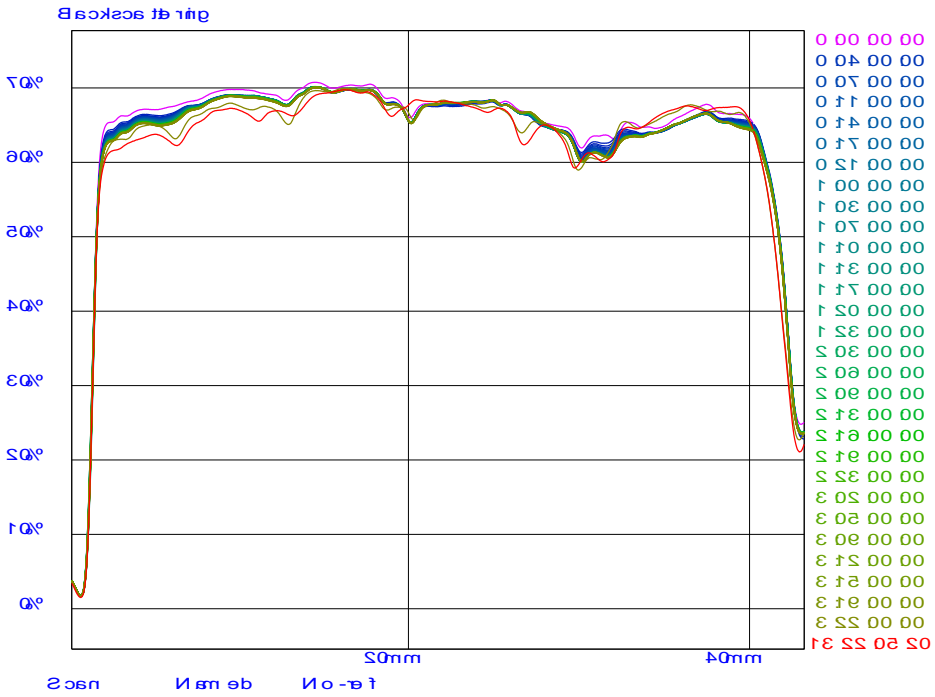
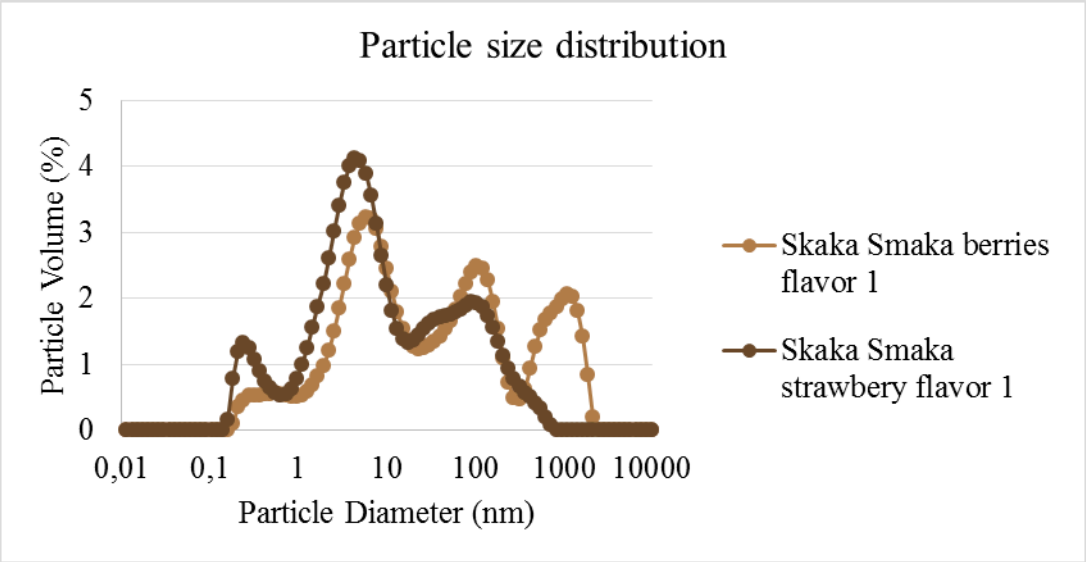


Image 2: Results of the TSI for the reference product Skaka Smaka strawberry flavor

TSI was estimated for the Skaka Smaka products by taking measurements-once per week for each product for 1 month (room temperature). Each graph line in the image illustrates the stability of the product per measurement.

4.2.3 Results of the Particle size distribution and the Microscope

Once the results obtained, the mean values of the volume distribution D [4,3] and the surface D [3,2] were used in order to calculate the volume (%). As it can be seen from the Graph 1 bellow, both Skaka Smaka reference samples shown multiple peaks, which means that the food model is multimodal since it is consisted from different ingredients.



Graph 1: Particle size distribution in reference products
**Particle size distribution results illustrate the mean values of two replicates per sample.*

The images were obtained from the microscope using the 50 times focus lenses in both of the cases, shows that the Skaka Smaka products are indeed multiplex systems. In both of the Images 3 and 4 bellow, it can be seen that the matrix of the products is complex. Very spherical big particles are representing the oil droplets into the system. Bigger particles, with no specific size are probably the whey protein aggregates. Other very dark either smaller or bigger particles, are probably coming from the berries external cell.

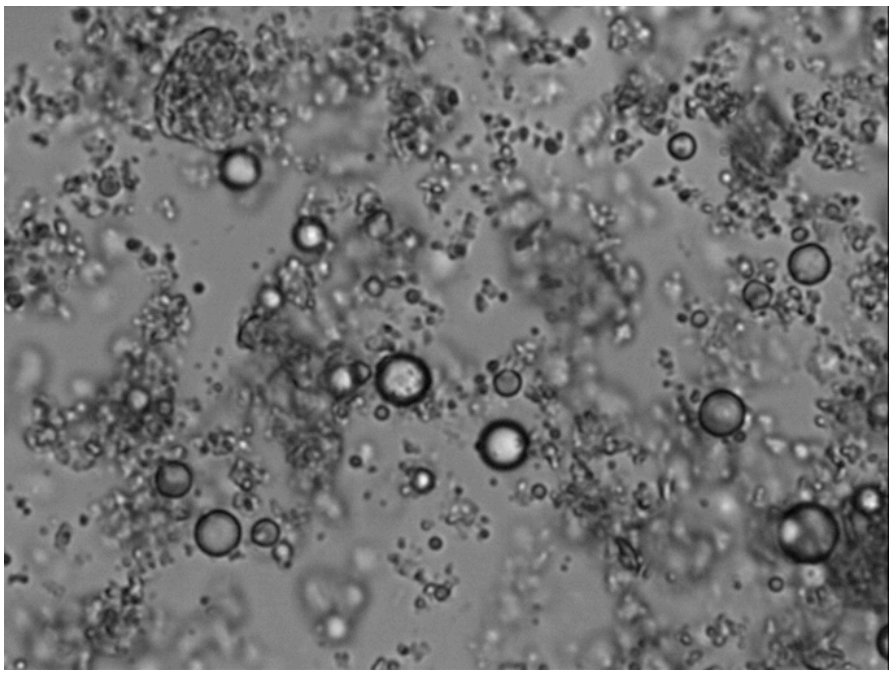


*Image 3: Skaka Smaka berries flavour product's complex system under the microscope (50*focus lenses). Spherical droplets illustrate the oil droplets into the system. The big aggregates illustrate the ones shaped due to the high protein content.*

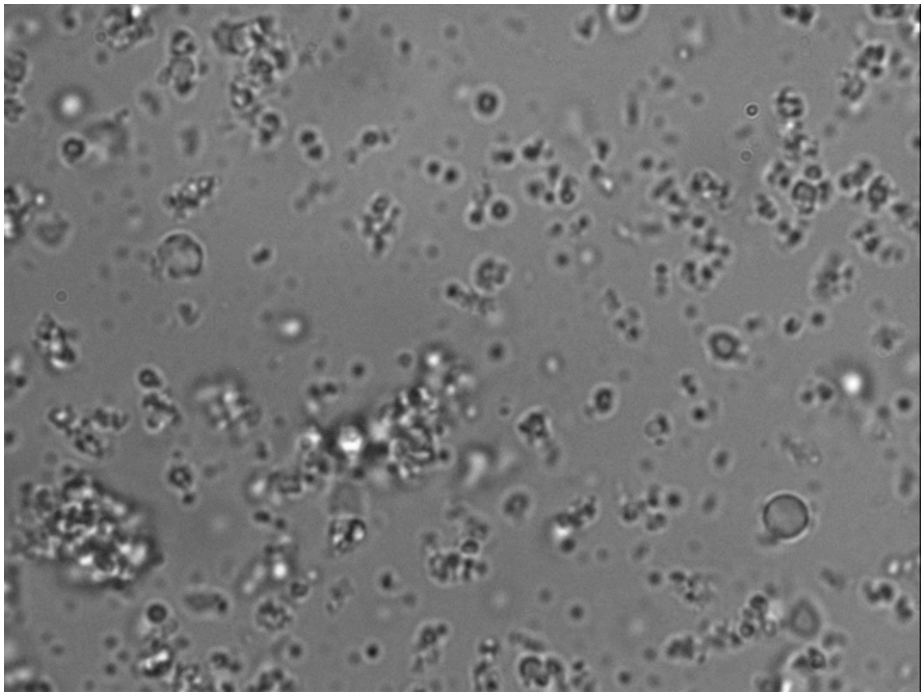
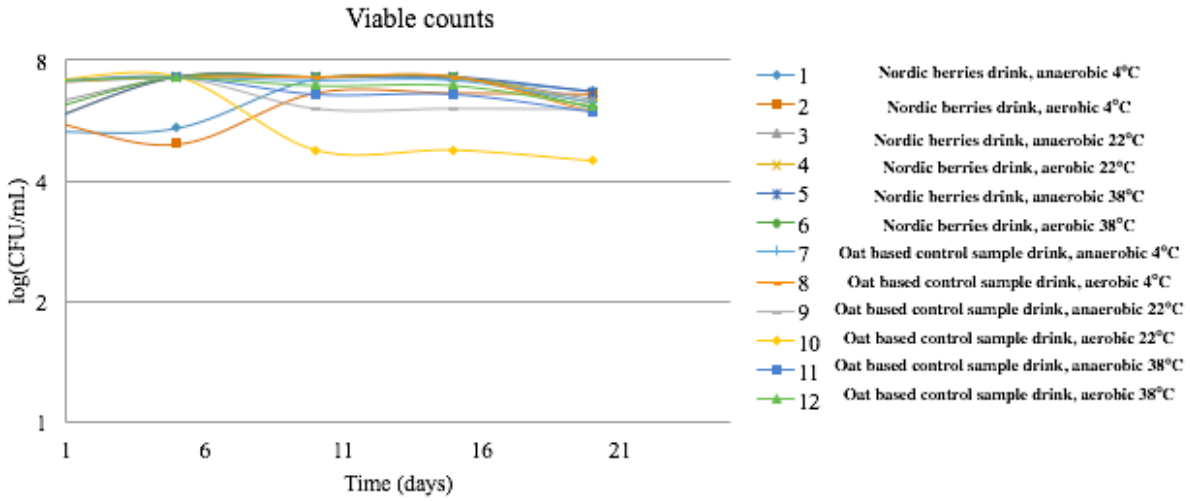


*Image 4: Skaka Smaka strawberry flavour product's complex system under the microscope (50*focus lenses). Spherical droplets illustrate the oil droplets into the system. The big aggregates illustrate the ones shaped due to the high protein content.*

4.3 Probiotic survival and how it affects the viscosity and the β-glucan concentration of the beverage tested samples

Enumeration of viable cells of *P.copri* was performed by estimation of colony forming unit number on PYG – agar plates after incubation at 38℃ for 5 days as described by Hayashi (2007).For each sample triplicates were made. Sterile saline solution (9%) was used for making dilutions of samples.

The results are shown in both Table 5 and Graph 2, illustrate the bacterial survival under different storage conditions. More specifically in the Graph 2, curves from 1 to 6 describing the results coming from the Nordic Berries drink and curves from 7 to 12 the ones coming from the Control sample (for samples were used see Table 1).



Graph 2: Viability of the bacterial strain into the food products.
**Numbers from 1 to 12 are representing the samples were tested and can be seen in the Table 5.*

Viable counts (cfu/mL) of the bacterial strain P.*copri* were measured for all the samples stored in different conditions for 1 month.

Table 5: Viability expressed as cfu/mL of the bacterial strain into food products

Viability of P.*copri* (cfu/mL)					
	0 days	**5 days**	**10 days**	**15 days**	**20 days**
1.Nordic berries drink, anaerobic 4°C	$5,32* 10^4$	$5,43*10^4$	$7,19*10^4$	$7,19 *10^4$	$6,71 *10^4$
2.Nordic berries drink, aerobic 4°C	$5,73 *10^4$	$4,95 *10^4$	$6,62 *10^4$	$6,62 *10^4$	$6,59 * 10^4$
3.Nordic berries drink, anaerobic 22°C	$6,17 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$6,42 *10^4$
4.Nordic berries drink, aerobic 22°C	$5,56 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$6,25 *10^4$
5.Nordic berries drink, anaerobic 38°C	$5,56 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$6,68 *10^4$
6.Nordic berries drink, aerobic 38°C	$5,94 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$6,08 *10^4$
7.Control sample drink, anaerobic 4°C	$7,06*10^4$	$7,26 *10^4$	$7,15 *10^4$	$7,15 *10^4$	$6,31*10^4$
8.Control sample drink, aerobic 4°C	$7,02 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$7,26 *10^4$	$5,95 *10^4$
9.Control sample drink, anaerobic 22°C	$7,07 *10^4$	$7,26 *10^4$	$6,05 *10^4$	$6,05 *10^4$	$6,03 *10^4$
10.Control sample drink, aerobic 22°C	$7,07 *10^4$	$7,26 *10^4$	$4,78 *10^4$	$4,78 *10^4$	$4,50 *10^4$
11.Control sample drink, anaerobic 38°C	$7,06 *10^4$	$7,26 *10^4$	$6,56 *10^4$	$6,56 *10^4$	$5,92 *10^4$
12.Control sample drink, aerobic 38°C	$7,10 *10^4$	$7,26 *10^4$	$6,93 *10^4$	$6,93 *10^4$	$6,18 *10^4$

**For each sample they were made truplicates. The standard deviation is not presented in the table as it was not more than 0,01cfu/mL.*

Generally, Nordic berries samples were more stable compared to the control samples, regarding the bacterial survival which is confirmed by the Graph 2. Samples 1 and 2 were stored at 4℃. From the curves obtained from both of them, it can be concluded that the bacterial strain survives in an oat based real fruit puree drink for 20 days without having any significance change in the concentration.

On the other hand, the oat based control samples were stored on 4℃ for 20 days, show a more stable pattern, without presenting any peak on their survival. This difference between the Nordic Berries drink and the oat based control sample can be explained by the acidity effect on the strain. *P.copri* as any other bacterium seems to be affected of an acidic environment, as it takes more days for it to be adjusted into the new environment. In the Nordic Berries drink, the bacterium survives for more time compared to the control sample, where for 20 days, in the Nordic Berries drink there were $6,71*10^4$cfu/mL (anaerobic conditions) and $6,59*10^4$cfu/mL (aerobic conditions), while for the oat based control sample were $6,31*10^4$cfu/mL (anaerobic conditions) and $5,95*10^4$cfu/mL (aerobic conditions).

For the samples were stored at 22℃, for both Nordic berries and oat based control sample drinks, there seems to be no difference on the survival curves. By that, it is obvious that the bacterium adjusts better to a new environment in temperatures closer to its optimal growth temperature compared to the lower ones. In the Nordic Berries drink, the bacterium survives for more time compared to the control sample, where for 20 days, in the Nordic Berries drink there were $6,42*10^4$cfu/mL (anaerobic conditions) and $6,25*10^4$cfu/mL (aerobic conditions), while for the oat based control sample were $6,03*10^4$cfu/mL (anaerobic conditions) and $4,50*10^4$cfu/mL (aerobic conditions).

For the samples were stored at 38℃, there is difference on the bacterial survival into the drink between the different samples. In the Nordic Berries drink, the bacterium survives for more time compared to the control sample, where for 20 days, in the Nordic Berries drink there were $6,68*10^4$cfu/mL (anaerobic conditions) and $6,08*10^4$cfu/mL (aerobic conditions) , while for the Control sample were $5,92*10^4$cfu/mL (anaerobic conditions) and $6,18*10^4$cfu/mL (aerobic conditions).

B-glucan content was determined by the Megazyme's enzymatic kit (Megazyme International Ireland Ltd., Co Wicklow, Ireland). Absorbance was measured by spectrophotometer (OD Lambda Bio, Perkin Elmer) at λ=510nm.

It is crucial for the synbiotic drink to evaluate the β-glucans' content within the product's shelf life, since β-glucans must remain as the functional ingredient and not being fermented by the bacterial culture in the beverage (Angelov *et al.,* 2006).

Table 6 presents the β-glucan content (g/100g dwt) as it is obtained from the method for both Nordic Berries sample and oat based control sample.

Table 6: β-glucan content (g/100g dwt) changes during storage

Samples	β-glucan (g/100g dry wet basis)	
	t=0days	t=20days
1.Nordic berries drink, anaerobic 4°C	3,39	0
2.Nordic berries drink, aerobic 4°C	3,39	0
3.Nordic berries drink, anaerobic 22°C	3,39	0
4.Nordic berries drink, aerobic 22°C	3,39	0
5.Nordic berries drink, anaerobic 38°C	3,39	0
6.Nordic berries drink, aerobic 38°C	3,39	0
7.Control sample drink, anaerobic 4°C	1,04	0
8.Control sample drink, aerobic 4°C	1,04	0
9.Control sample drink, anaerobic 22°C	1,04	0
10.Control sample drink, aerobic 22°C	1,04	0
11.Control sample drink, anaerobic 38°C	1,04	0
12.Control sample drink, aerobic 38°C	1,04	0

Generally, the Nordic berries drink showed to had a significant higher β-glucan concentration (g/100g dwt) compared to the oat based control sample one. A possible explanation for that is that the high concentration of flavonoids into the berries might interfere with the method and the final results are not trustable. At the end of the experiment (t=20 days) the β-glucan content (g/100g dwt) was evaluated again in all of the samples and it found to be approximately equal to 0 g/100g dwt

Along with the β-glucan concentration, the viscosity of the samples was measured. The viscosity of the samples was measured by using the Brookfield viscometer for t=0 days and t=20 days. The aim was to examine whether the strain has any effect on the texture of the beverages within the 20 days of storage under different conditions. The Table 7, presents the viscosity (cp) results of all the samples from the beginning of the experiment t=0 days and at the end of the experiment t=20days.

Table 7: Viscosity changes during storage

Samples	Brookfield viscosity (CP 2spindle 60)	
	t= 0days	t=20days
1.Nordic berries drink, anaerobic 4°C	761	54,1
2.Nordic berries drink, aerobic 4°C	761	72,6
3.Nordic berries drink, anaerobic 22°C	761	39,6
4.Nordic berries drink, aerobic 22°C	761	45,6

5.Nordic berries drink, anaerobic 38°C	761	36,1
6.Nordic berries drink, aerobic 38°C	761	33,8
7.Control sample drink, anaerobic 4°C	39	25
8.Control sample drink, aerobic 4°C	39	25,4
9.Control sample drink, anaerobic 22°C	39	26
10.Control sample drink, aerobic 22°C	39	26,3
11.Control sample drink, anaerobic 38°C	39	22
12.Control sample drink, aerobic 38°C	39	22,2

From the obtained results for the viscosity changes, we can see differences in the initial viscosities of the products. The Nordic Berries drink showed to have higher initial viscosity compared to the oat based control sample, since it is more acid. At the end of the experiment all of the samples lost their viscosity as it was decreased.

As it has been mentioned above, the acidity was evaluated in terms of pH for t=0, 5, 10, 15 and 20 days, for the twelve different samples. Table 8 presents the pH effect within the 20 days of bacterial survival into the Nordic berries drink and the Control sample drink.

From the results were obtained, there is a difference between the Nordic Berries drink and the Control sample regarding the acidity. In the Nordic Berries drink, for 20 days the pH remained stable or decreased 0,2 degrees at approximately 4,29 without being affected by the aerobic – anaerobic conditions of storage. On the other hand, for the Control sample drink, the pH value was decreased from 6,12 (t=0 days) to lower values. For the Control samples were stored at 4°C, the decrease of the pH was slower (from 4,12 (anaerobic and aerobic storage) to 4,75 (anaerobic storage) and 4,72 (aerobic storage) compared to the samples were stored at 22°C and 38°C. For these samples, the pH was decreased immediately to 4,25 (anaerobic 22°C), 4,28 (aerobic 22°C) and 4,37 (anaerobic 38°C) and 4,38 (aerobic 38°C) for 20 days. By far, there is no difference in the bacterial survival between the samples that are stored under aerobic and anaerobic conditions.

Table 8: Changes in the pH during storage

pH					
Samples	**0 days**	**5 days**	**10 days**	**15 days**	**20 days**
1.Nordic berries drink, anaerobic 4°C	4,28	4,39	4,35	4,29	4,32
2.Nordic berries drink, aerobic 4°C	4,28	4,31	4,34	4,26	4,37
3.Nordic berries drink, anaerobic 22°C	4,28	4,34	4,18	4,18	4,22
4.Nordic berries drink, aerobic 22°C	4,28	4,33	4,21	4,27	4,28
5.Nordic berries drink, anaerobic 38°C	4,28	4,25	4,29	4,2	4,28
6.Nordic berries drink, aerobic 38°C	4,28	4,2	4,22	4,22	4,23
7.Control sample drink, anaerobic 4°C	6,12	6,1	5,15	4,75	4,57

8.Control sample drink, aerobic 4°C	6,12	6,11	5,35	4,72	4,46
9.Control sample drink, anaerobic 22°C	6,12	4,28	4,25	4,25	4,39
10.Control sample drink, aerobic 22°C	6,12	4,28	4,22	4,28	4,36
11.Control sample drink, anaerobic 38°C	6,12	4,37	4,37	4,37	4,39
12.Control sample drink, aerobic 38°C	6,12	4,38	4,39	4,38	4,38

The quality characteristics of the samples were evaluated within 20 days of storage under different conditions as for their odor/smell and color. Since the strain has not been recognized yet by the European Food Safety Authorities (EFSA) as safe for oral consumption (G.R.A.S.), taste trials were not performed.

To begin with the color, it was evaluated by optical observation. The color in the Nordic berries beverage seems to be purple (due to the high content in anthocyanins coming berries), for 15 days storage at 4℃ samples. The color of the samples of the Nordic Berries drink were stored at 22℃ did not changed at all within the 15 days of observation. The color of the Nordic berries samples were stored at 38℃, after 10days were changed to pale purple, since this temperature accelerates all the metabolic functions of the plant cells.

Similar results were observed for the Control sample beverages. Samples were stored at 4℃ and 22℃ preserved their color within 15 days observation, while samples stored at 38℃ after 10days changed their color to pale beige. Again, the aerobic-anaerobic conditions did not affect at all the color of both samples.

Moving to the odor results evaluation, Nordic berries samples were stored at 4℃ were stable for 15 days and no off-flavor was observed. For the ones stored at 22℃ and 38℃, after 10 days there was an off – flavor development which can be described as too much astringent (due to the low pH). Control samples were stored at 4℃, remained stable without any off- flavour for 15 days, while the ones stored at 22℃ and 38℃ after 10 days started developing an off-flour flavour which can be explained due to the fast decreasing of the pH.

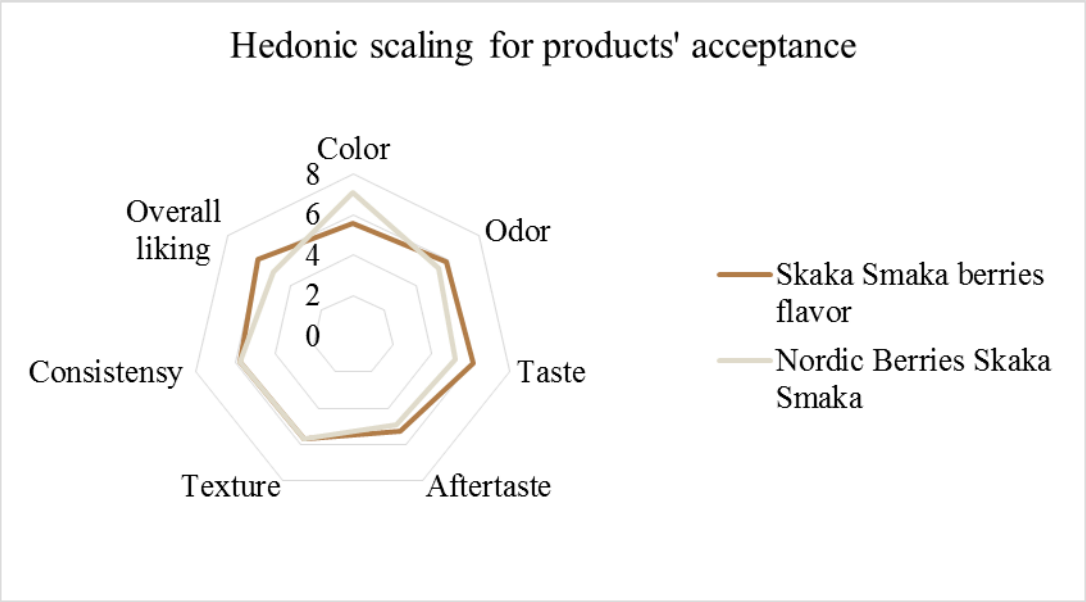
4.4 Sensory evaluation

4.4.1 Hedonic test

In order to evaluate the acceptance of the Nordic berries flavour product (always compared to the Skaka smaka berries flavour one), sensory evaluations were performed, one hedonic and one focus group. Starting with the hedonic sensorial analysis, it took place at the Gerdahallen gym (Lund Sweden) where 30 participants (age variant 15-85) tested the products and replied to the Questionnaire 1 (See Appendix) according to the instructions. The distribution for the people was 84% women and 16% men. 48,4% were in between 20-40 years old, 22,6% 41 to 60 year old, 22,6% above 60 year old and 3,2 bellow 20 year old. From them, 48,8% were students, 23% employees and 23% retired. As for their nationality, 80,6% were Swedish, 9,7% German and 3,2% Latin American and Finish.

The aim was to evaluate the acceptance of the Nordic berries new flavour, compared to the already existed Skaka smaka berries flavour.

The scale of the organoleptic evaluation was from 1-9 where 1=extremely dislike and 9=extremely like. Results were analysed using the statistic tool Prism 7.0 for Mac. Results are presented on the Graph 3 bellow.



Graph 3: Hedonic scaling for Skaka Smaka products acceptance (30 panelists, Gerdahallen Lund)

As it can be concluded from the results, there is a significant difference for the color of the two products. The color of the new Nordic Berries flavour drink is more appreciated by the people (Mean value 7,13±1,38) compared to the already excisted Skaka Smaka one (Mean value 5,62±1,72). Regarding the second attribute- odor, there is no significant difference between the two samples. As for the taste, there was a significant difference, where the Skaka Smaka berries flavor found to be more appreciated compared to the Nordic Berries one (Mean values of Skaka Smaka berries flavor 6,10±1,74, and Nordic Berries flavor drink 5,62±1,65). This can be explained from the more neutral taste of the Skaka Smaka berries drink, since it contains more banana puree and less oat base according to its recipe. For the aftertaste, texture and consistency no significant differences were found in between the two samples. The overall liking found to be significantly different where the Skaka Smaka berries flavor was slightly more acceptable (Mean value 6,16±1,59) compared to the Nordic berries one (Mean value 5,19±1,74). Last but not least, the majority of the potential customers, were confused whether they will buy the product (58%) and they stated that they will buy it only if there is a clear statement on it, about its health beneficial aspects. For 250mL on the go packages, they have the willing to pay around 15-20sek, and for the 1L packages 25-30sek.

On the other hand, for the new Nordic Berries flavour the majority of the potential customers, were confused whether they will buy the product (58%)and they stated that they will buy it only if there is a clear statement on it, about its health beneficial aspects. For 250mL on the go packages, they seem to have the willing to pay around 15sek, and for the 1L packages 20sek.

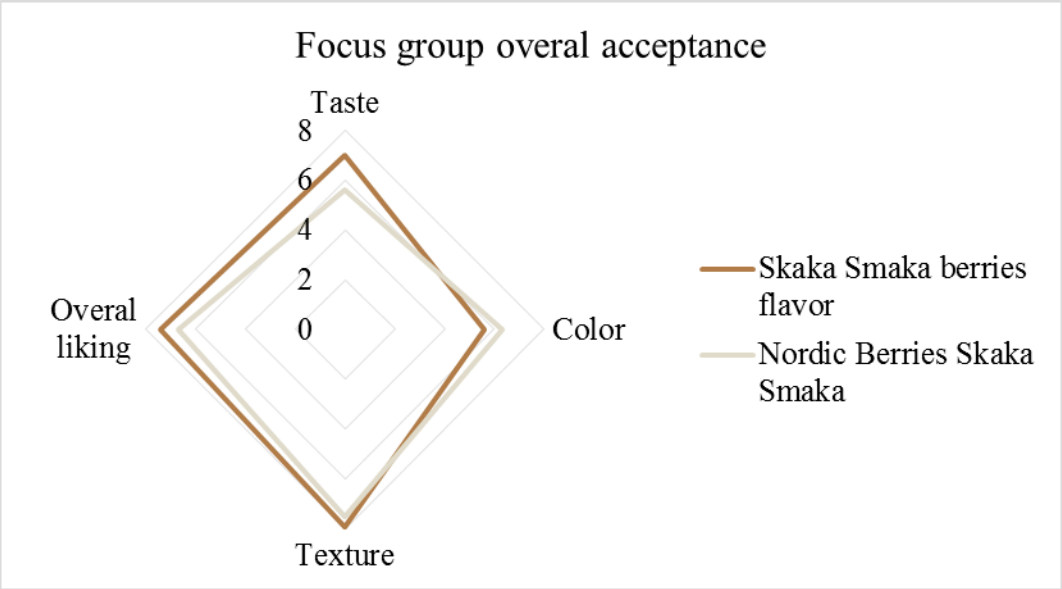
4.4.2 Focus group

The aim of the focus group questionnaire was to let the participants have a discussion about their eating habits and their opinions about the product. Ten participants (age 21-27), with moderate exercising were participated. After analysing the results of the focus group, it can be seen that most of the participants prefer to consume high in energy fruits or coffee/tea for in between breakfast and lunch meals. The reason is because these products are neutral, easy to find and consume and they do not affect their appetite desire for lunch. When they got asked about what they think it is missing from the food market nowadays, they all replied that a healthy –well stated product is missing. They suggested a salty type of cereal bar, an oat based porridge on the go, soft bread products and sliced fruits. They also suggested that vegan options of smoothies and drinks high in nutrients that will work potentially as meal replacements are missing.

The same panel group when it was asked if there are some products that will never buy, they replied based on their personal preferences. Products that are too much processed like complex ones, they are not preferable for consuming. In the question what they think is important for them for an in between meals product they all replied that they want a balanced taste, healthy- proper labelled product, high in nutrients and especially fibres without too much industrial processing. Also, the product must be easily found like from a distribution machine (eg. smoothie machine). They want this product not to have too many calories and to be healthy with a good taste at the same time. As for its consistency, the replies were according to their preferences. Some of them they prefer a product that gives a smooth mouthfeeling, while others prefer a crunchy one.

When they asked if they had the chance to buy a new semi-beverage product, in what amount they will buy it 50% said in 300-500mL of packing bottles, 45% in 200-250mL of packing bottles and 5% in 1L of packing bottles.

Most of them consider first the taste then price and then the calories before buying a product. Some believed to be marketing victims since they buy whichever product is in a good packing. The Graph 4 below, illustrate the results of the organoleptic evaluation statistical analysis performed by using Prism 7.0 for Mac.



Graph 4: Focus group overall acceptance of Skaka Smaka products (10 participants, Aventure AB. Lund)

As it can be seen from the results, there are differences in the responses of the panellists for all the questions. To be more precise with the results, panellists preferred the taste of the Skaka Smaka berries flavour (Mean value 7±1,3) compared to the Nordic berries one (Mean value: 5,6±1,4), because the taste of the first one is more neutral and sweet. As for its color, they preferred the Nordic berries one (Mean value 6,3±1,8) compared to the Skaka Smaka berries one (Mean value 5,6±0,8). For the texture they preferred the Skaka Smaka berries one (Mean value 7,9±0,8) rather than the Nordic Berries (Mean value: 7,9±0,8). This is due to the fact that the Nordic berries one developed in a lab scale, so it was impossible to reach the same viscosity levels compared with the Skaka Smaka berries one, which was produced in a industrial scale and had the appropriate viscosity. The viscosity of the Nordic Berries product is expected to be increased when the product will be produced in a large industrial scale. The overall liking, the Skaka Smaka berries flavour was mostly preferred by the panellists (Mean value 7,4±0,8) compared to the Nordic Berries one (Mean value6,7±1,0).

5. Discussion

In the current Master thesis project oat based beverages were studied as for their several parameters. The already existing Skaka Smaka (berries and strawberry flavour) were analysed for their physicochemical characteristics and their stability as emulsions within 1 month of storage. These products, later were used as reference ones. Moreover, at the same time, a new flavour beverage belonging to the Skaka Smaka category called Nordic Berries, was developed. This product had 12% more oat base compared (48%) compared to the preference ones. In this newly developed product, physicochemical characteristics were measured. From the results once can be seen, that there are small differences in between the nutritional profile of the products.

One of the most important methods to check whether a product will be successfully launched in the marker, is the consumer organoleptic test trials. For this reason, the acceptance and the overall liking of the newly developed Nordic Berries Skaka Smaka were evaluated by both the consumer hedonic test at the Gerdahallen gym where a high variety in range of people is going and by focus group analysis at Aventure AB. The product compared using as reference the Skaka Smaka Nordic Berries flavour one. From the results it can be inferred that people prefer to consume products that have a sweeter taste compared to the very acidic ones. In terms of color and texture the Nordic Berries drink was more preferable, since the color was highly purple due to the high concentration of flavonoid compounds (anthocyanins) and the texture can be described as liquid. Participants at Gerdahallen mostly at ages between 18-35 were positive to buy the new Nordic Berries Skaka Smaka beverage, since they are more aware about their nutritional habits and they prefer to consume less sweet products. On the other hand, seniors, showed higher preference to the Skaka Smaka berries flavour, because since it contained more banana puree the taste is sweeter and the texture smoothier. Seniors prefer to consume smoothier products.

As for the focus group results, participants were discussing freely, without any oppositions about their food preferences and what they think is mostly missing from the market. All of them had different opinions, but they came into an agreement that a synbiotic oat based real fruit puree product is needed. Once they tasted the products, they evaluated both of them and they seemed to be positive in buying the new Nordic Berries drink. As a conclusion of the organoleptic evaluations, it can be said that the new flavor of Nordic Berries has a great potential in the Swedish food market in the functional beverages category, as long as its special characteristics will be referred on its packing.

The new Nordic Berries beverage was used as a substrate in which the bacterial strain of *P.copri* was inoculated (1%) *in vitro* and several factors were examined in a duration of 20 days. As a reference was used the oat based beverage. Samples were stored under different oxygen and temperature conditions. From the results, it seemed that oxygen presence is not affecting at all the bacterial survival into both of the drinks. As for temperature, it does not play an important role in the bacterial survival for 20 days regarding the viability and other physical properties, but it affects the organoleptic characteristics of the products. The higher the temperature is, the faster the metabolic reactions are, so flavonoids are getting degraded (loss of color) and organic acids are being produced ("off" flavour). Though, it seemed that at cooling temperatures, the bacterium survives without affecting so much the organoleptic characteristics of the products. The suggested self-life of the beverage is 10 days at 4° C. Also, it seems that the bacterium might affect the degradation of the β-glucans into simpler sugars and thus, the viscosity is decreasing. Since the products were made in a laboratory scale and not being pasteurized in a proper way and total plate count was not measured, it can be that other bacteria that already excised can degrade the β-glucans. It is not known whether the degradation of β-glucans or other kind of components such as the starch or protein happened because of the addition of P.*copri* or other bacteria that already exist in the oats. One good suggestion will be in the future, each one of these components to be analysed individual in how they affect the texture of the product. Moreover, regarding the β-glucans, during the measurement the Nordic Berries drink seemed to have higher amount of this component compared to the oat based control sample (t=0 days). This results seems not very

trustworthy, since flavonoids coming form the berries' anthocyanins can interfere with peroxides and give higher results than the real ones. Last but not least, the bacterium seems very promising once it is inoculated into food products.

Additionaly, a future suggestion would be to inoculate the strain of *P.copri* DSM18205 to other food prebiotic selective mediums that work as substrates (eg. barley, or oat flour mixed with barley) in order to check the bacterial behavior (and fibres content) and to develop a beverage 'model' as the perfect substrate for the bacterial strain.

6. Conclusion

The tendency of the market and consumer needs, lead the industry to develop new functional food products. Synbiotic products, have been proven to have beneficial results in the human gut microbiota as they combine the prebiotic and probiotic effects, so, there is a crucial need in the market. Also, consumers nowadays tend to buy products with les or no sugar content because of health issues that might arise. There is more than a need for the Nordic Berries Skaka Smaka product to be launched in the market. *P. copri* is very promising to be a good probiotic inoculum to non-dairy, oat based products. By the current research's results, all the goals were covered. Important part to be examined was the survival of the bacterial strain. The bacterial strain survives without being affected from different storage conditions for 20 days. Generally, the low pH of the Nordic Berries beverage helps to preserve better the organoleptic characteristics of the product and the strain survival compared to the oat based control sample one. For the strain it takes more time to adjust to an acidic environment (Nordic berries beverage) compared to the neutral one (oat based control sample). *P. copri* seems to behave as a facultative anaerobe, according to the results of this report, since the aerobe/anaerobe conditions proved to have no effect at the strain's survival on the beverages at all. Skaka smaka products are a very good source of nutrients as it has been seen by the results, and they remain stable at room temperatures for a very long time. The new Nordic Berries beverage, can be clearly characterized as a meal replacement and it can be consumed by all the groups of people as long as its nutritional benefits are stated on its package.

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Appendix

1. HEDONIC TEST QUESTIONAIRE

New product development

In this current sensory evaluation, you will be given 2 food samples and tap water. These food samples are semi-beverages high energy oat based & real fruit puree products with no added sugars or any artificial aromas.

Before you start the taste trial, you are kindly requested to read the description that is given on each page.

The whole procedure takes 5-8min. You will be asked some questions which are divided into three sections. In the first one, some general information about yourself and your current status will be asked. In the second one, you have to reply in an organoleptic evaluation questionnaire about the products you tasted. There is a ranking scale from 1 to 9 (1= extremely dislike to 9=extremely like). The meaning of the scale will be provided in each section.

For any questions do not hesitate to ask me!!!!

OBS: *The product might contain milk and soy protein.*

We start on the next page

Section 1

1. Please specify your gender.

Male
Female

2. Please specify your age

below 20
20-40
41-60
Above 61

3. Please specify your occupation

Student
Employee
Housewife
Unemployed
Retired
Other, please specify

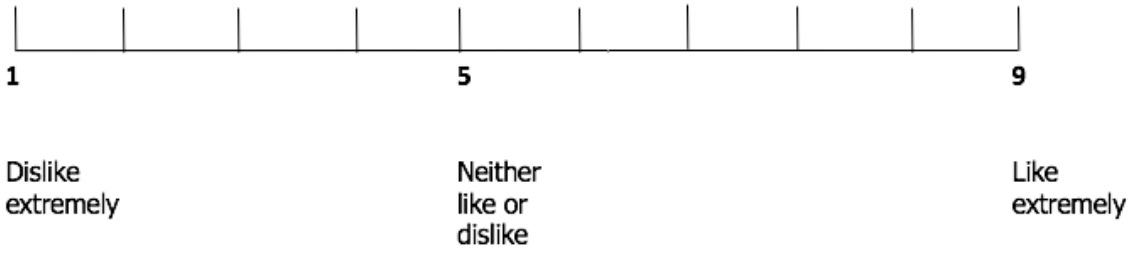
4. Please specify your nationality

Section 2

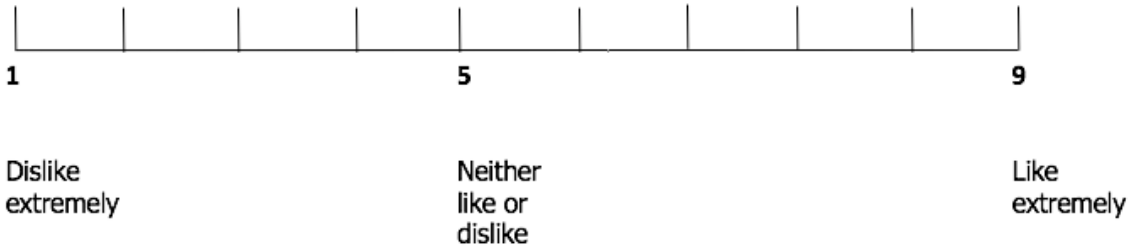
Now it's time for the product trial!!!

1. **Rinse your mouth with some water**
2. **Try now, sample nr 1**
3. **Reply on the following questions *(Scale definition: nr1= extremely dislike, nr5=neither like or dislike, nr9=extremely like)***

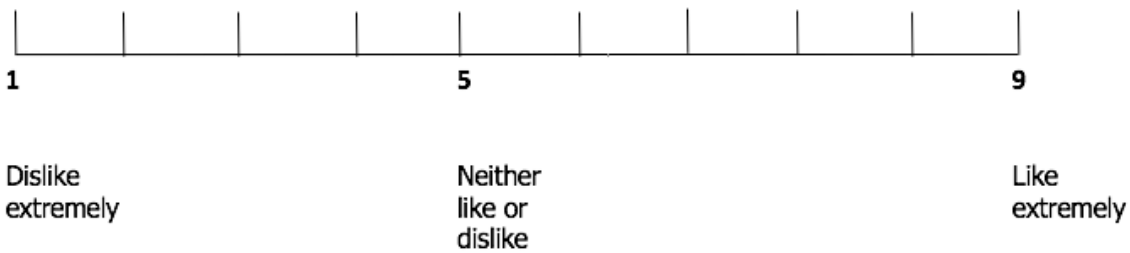
1.Do you like the color of the current product?



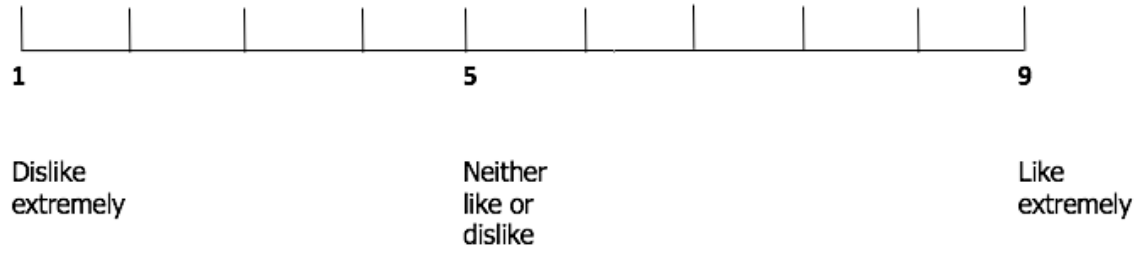
2. Do you like the odor/smell of the current product?



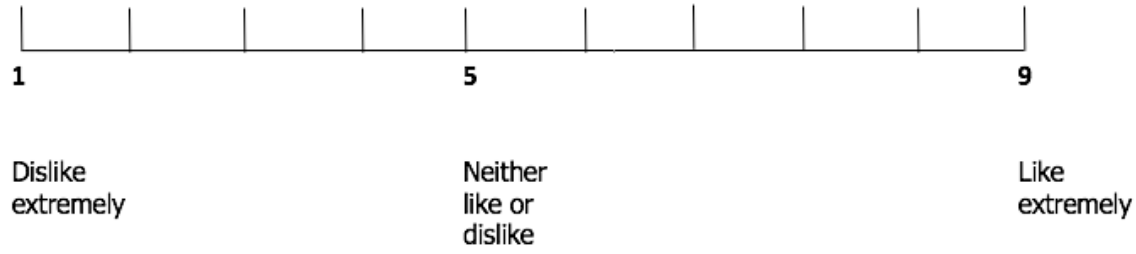
3.Do you like the taste of the current product?



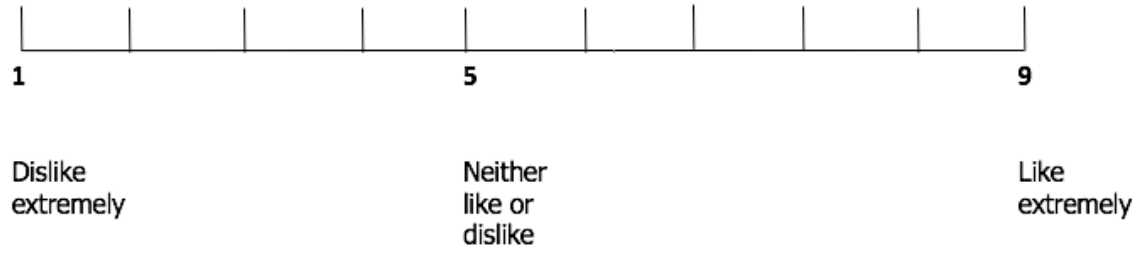
4.Do you like the aftertaste that the product leaves on your mouth (like mouthfeeling after having consumed the product)?



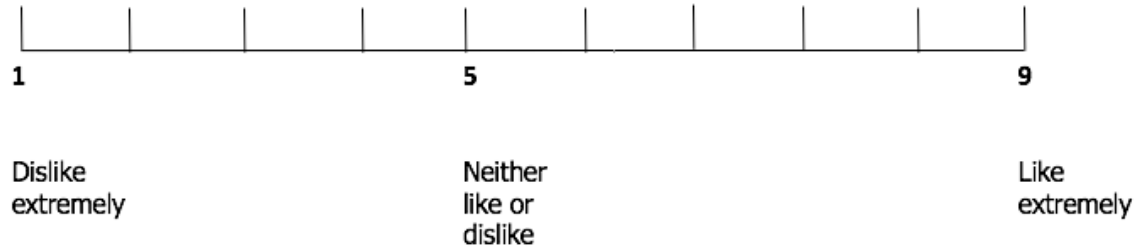
5.Do you like the texture of the current product?



6.Do you like the consistency of the current product?



7. Do you like the product in general?



8. Will you ever buy it?

YES
MAYBE
NO

9. If this product will be on the market in a 250mL of packing bottles, how much are you willing to pay?

Less than 10sek
15-20sek
25-30sek
More than 30sek

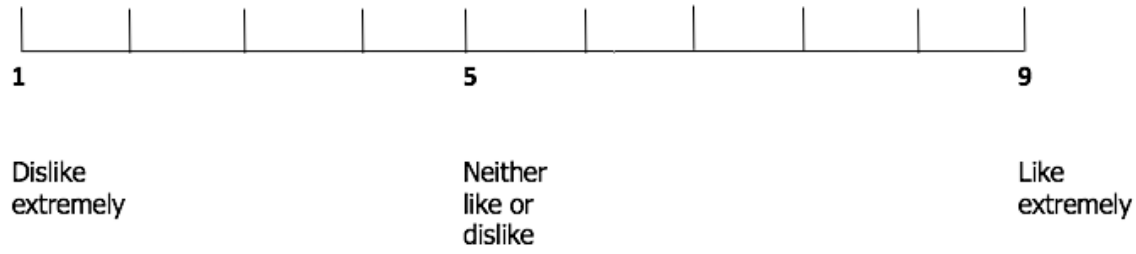
10.If this product will be on the market in 1L of packing bottles, how much are you willing to pay?

Less than 20sek
25-30sek
More than 35sek

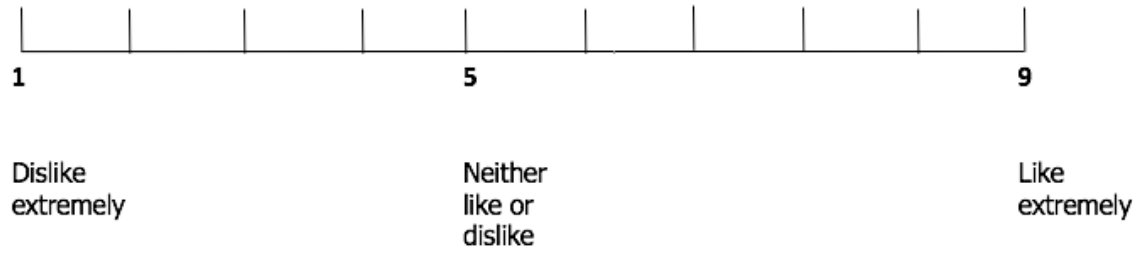
We are done with sample nr 1. Let's go to sample nr 2.

1. **Rinse your mouth with water (be sure you have no taste left from the previous sample)**
2. **Try now, sample nr2**
4. **Reply on the following questions *(Scale definition: nr1= extremely dislike, nr5=neither like or dislike, nr9=extremely like)***

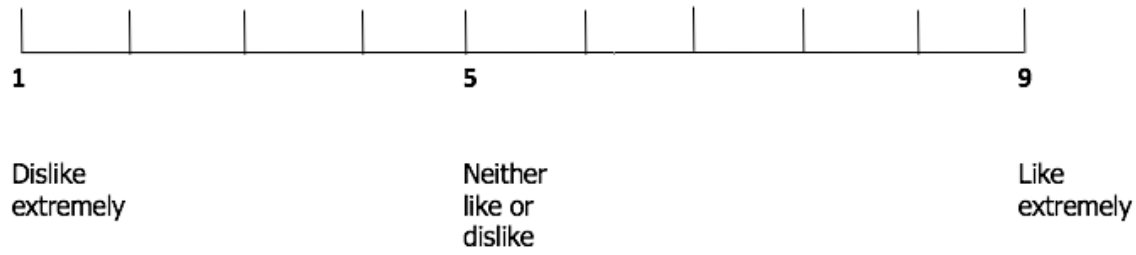
1.Do you like the color of the current product?



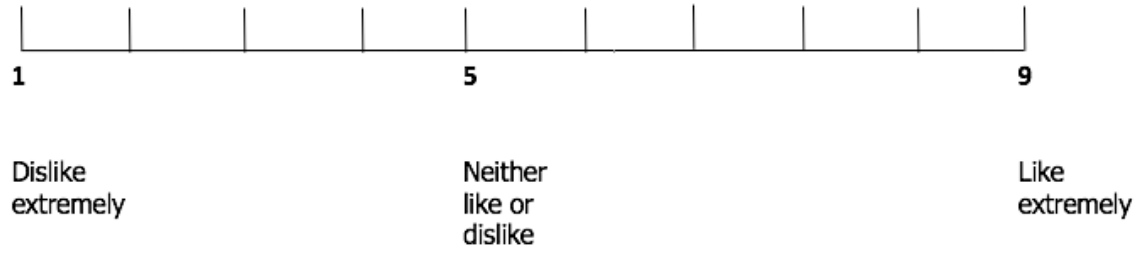
2. Do you like the odor/smell of the current product?



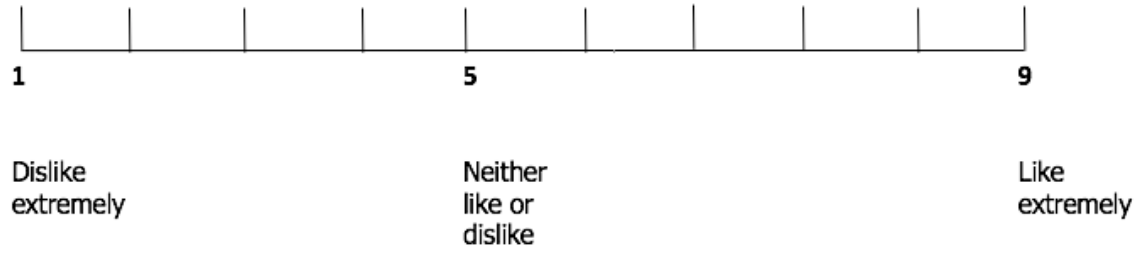
3.Do you like the taste of the current product?



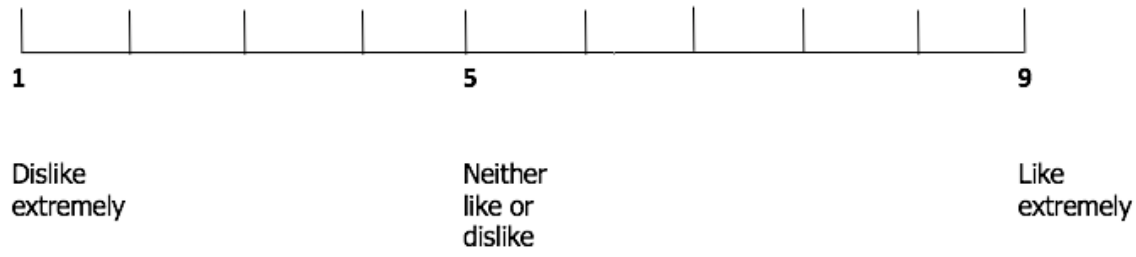
4.Do you like the aftertaste that the product leaves on your mouth (like mouthfeeling after having consumed the product)?



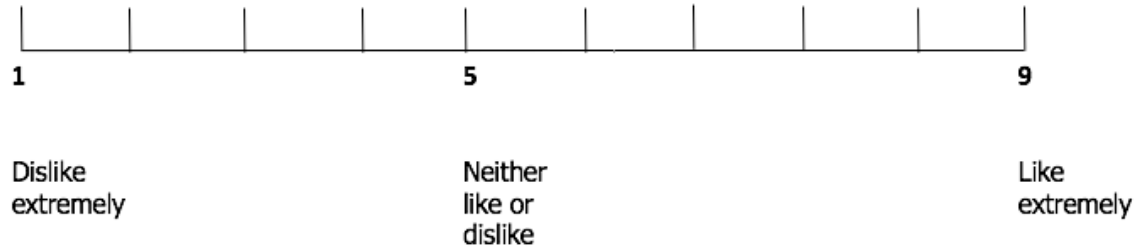
5.Do you like the texture of the current product?



6.Do you like the consistency of the current product?



7. Do you like the product in general?



8. Will you ever buy it?

YES
MAYBE
NO

9. If this product will be on the market in a 250mL of packing bottles, how much are you willing to pay?

Less than 10sek
15-20sek
25-30sek
More than 30sek

10.If this product will be on the market in 1L of packing bottles, how much are you willing to pay?

Less than 20sek
25-30sek
More than 35sek

Section 3

Now, you need to compare the two samples you tried.

1.Which sample do you like most according to its color?

Sample nr.1
Sample nr.2

2. Which sample do you like most according to its odor/smell?

Sample nr.1

Sample nr.2

3.Which sample do you like most according to its taste?

Sample nr.1
Sample nr.2

4.Which sample do you like most according to its aftertaste?

Sample nr.1
Sample nr.2

5.Which sample do you like most according to its consistency?

Sample nr.1
Sample nr.2

6.Which sample do you like most according to its texture?

Sample nr.1
Sample nr.2

7.Which one is your favourite sample in total?

Sample nr.1
Sample nr.2

2.FOCUS GROUP QUESTIONAIRE

Section 1

1. What did you have for in between meals (after breakfast and before lunch)?

-Why did you chose this product?

-What is good or bad with your chosen product?

2.What do you think is mostly missing from the food market nowadays?

-Any suggestions?

3.Are there any products that you will never chose to buy for any reason?

-Why?

4.What is important for you in a between meal product?

-Do you have any minimum or maximum desires about that?

5.Are the consistency and the texture important for you when you consume any product that belongs in a between meals category?

-If so, do you think that an easy to be consumed-swallowed product (like beverage or smoothie) will be the best or you prefer one that can be chewed (like cereal bars, fruits)?

6.Imagine now that you have a new beverage for this category. Which packing size you thing will be suitable for a beverage that you will probably consume as an in between meals product?

-200-250mL
-300-500mL
-1L

7. When you choose the in between meals product, are you considering the nutritional recommendations for macronutrients and micronutrients? If so, why?

-Are you aware of them? If so, why?

8. How many times per week do you exercise? (please choose one option that suits you the best)

I don't exercise due to the lack of time/willing
1-2times/week
2-4times/week
4-7times/week

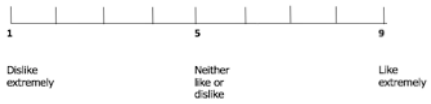
Now its time to taste the samples and response to the following questions. Please see next page →

Section 2

Taste

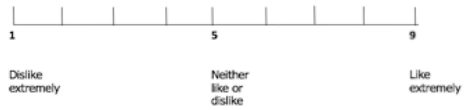
What do you think about the taste?

Please try **sample nr1**:



-Write if any comments:

Please try **sample nr2**:

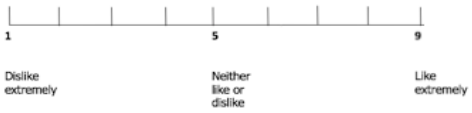


-Write if any comments:

Colour

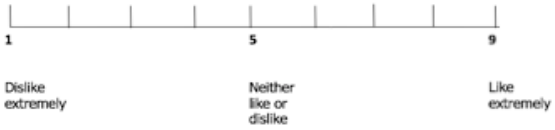
What do you think about the colour?

Please try the **sample nr1:**



-Write if any comments:

Please try the **sample nr2:**

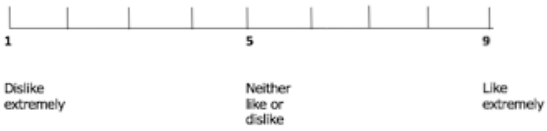


-Write if any comments:

Texture/consistency

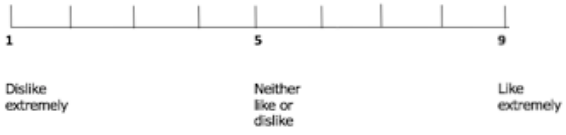
What do you think about the texture/consistency?

Please try the **sample nr1:**



-Write if any comments:

Please try the **sample nr2:**

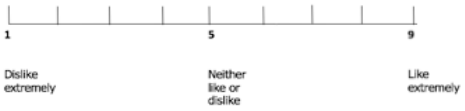


-Write if any comments

General opinion

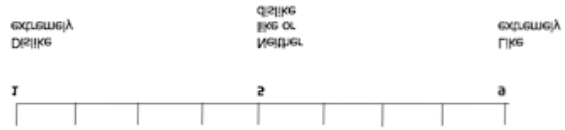
What do you think after in total after tasting all of the samples?

Sample **sample nr1:**



-Write if any comments:

Please try the **sample nr2:**



-Write if any comments:

-Which sample did you like the most?