VALUE ADDED PRODUCTS UTILIZING ACID WHEY: DEVELOPMENT OF A FRUIT YOGURT BEVERAGE AND A SPORTS DRINK

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ABSTRACT

Disposal of acid whey, a by product of Greek yogurt production, remains a significant problem. Scientists and industry are seeking feasible alternatives to utilize yogurt acid whey (YAW), including value-added beverages. A yogurt acid whey-based (AWB) fruit yogurt beverage was developed; addition of stabilizer and homogenization are recommended to improve beverage's refrigerated stability to over 1 month. Sensory studies showed consumers (n=120) liked samples containing YAW equally to ones containing water, finding beverages with 35-45% YAW acceptable. In addition, a yogurt AWB, clean label, sports drink was developed incorporating 60% YAW (natural source of electrolytes). Lime juice, mint and ginger were selected as ingredients to mask the distinctive flavor of YAW. The final formulation matched the nutrition profile of commercial sports drinks and consumers found the drink acceptable in sensory studies (n=119). The product was rated favorably but too tart, thus further research is needed to optimize the %YAW while maintaining the nutritional profile.

BIOGRAPHICAL SKETCH

Marcela Villarreal was born in Santa Rosa, La Pampa located in the center of Argentina. She grew up in Santa Rosa living with her parents and her two brothers. After finishing High School she moved to Buenos Aires city to begin her undergraduate studies. Marcela studied Food Science and Technology at Universidad de Buenos Aires (UBA), a 5.5-year degree. While studying, Marcela worked in two different laboratories as a laboratory analyst and collaborator. From 2011-2014 Marcela worked as a Teacher Assistant (TA) in the department of Food Science, UBA, assisting with different courses such as Food Chemistry, Food Technology and Food Analysis. In addition, in 2011 Marcela started working as an intern in the National Institute of Industrial Technology (INTI). After graduating in September of 2011 she was hired as permanent staff in INTI and continued working in the Toxicology and Nutritional lab as a professional laboratory analyst. In 2014, Marcela won a Fellowship to perform a Master Degree Program in Science and Technology in the U.S. sponsored by the Argentinean President´s cabinet and the Buenos Aires Embassy of the United States, administered by the Fulbright Commission-Argentina with support from LASPAU.

Marcela joined Cornell in 2014 as a graduate student in Dr. Olga Padilla-Zakour's group. In 2015, Marcela was the TA for FDSC 4190 Food Chemistry Laboratory. She also became the captain of the Cornell Ocean Spray product development team that developed a Cranberry beef jerky for the national competition.

Marcela received the Outstanding TA Award (2016) and the Western New York IFT Award (2015-2016) for excellence in food science.

After completing her Master’s degree, Marcela will return to Argentina where she plans to continue working at INTI while pursuing her PhD program in Food Science at UBA.

DEDICATION

Dedicated to the best family I could have ever asked for, Estela, Diego, Alejandro and David

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I feel that even though these pages were the last ones to be written in paper, they were written in my mind and heart since the very beginning.

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LIST OF ABBREVIATIONS

AWB: Acid whey-based

BOD: Biological Oxygen Demand

COD: Chemical Oxygen Demand

FDA: Food and Drug Administration

FYB: Fruit yogurt beverage

FYBs: Fruit yogurt beverages

GSY: Greek style yogurt

LAB: Lactic acid bacteria

NF: Nanofiltration

TA: Titratable acidity

WHC: Water holding capacity

YAW: Yogurt acid whey

CHAPTER 1

INTRODUCTION

Greek style yogurt (GSY) has become popular in the US in the past few years. By 2015 it captured 50% of the yogurt market share which is slated to grow 5% each year before reaching $4bn in 2019 (Hal Conick 2015). GSY is seen by consumers as higher in protein, more fulfilling, low fat and natural in comparison with regular yogurt (Kilara and Chandan 2013).

Greek yogurt is manufactured as stirred-style yogurt but involves a strain step in which the yogurt is allowed to drain in cloth bags (small scale production) by gravity or by centrifugation and membrane filtration (commercial production) until the desired total solids in the yogurt is obtained (Kilara and Chandan 2013). The water removed in this step is known as acid whey and accounts for about two thirds of the volume of milk that goes into the manufacture of GSY (Kyle and Amamcharla 2016).

For most of the 20^{th} century, whey had been disposed into the ocean, municipal sewage treatment facilities and/or into fields (Smithers 2015). Nowadays, untreated whey disposal is prohibited through strict environmental regulations (Smithers 2008; Smithers 2015) mainly because of its high organic matter content exhibiting high Biological Oxygen Demand (BOD > 35,000 ppm) and Chemical Oxygen Demand (COD > 60,000 ppm) (Siso 1996; Smithers 2015; Mawson 1994). Land disposal affects the soil's physical and chemical composition resulting in decreased crop yield. Dumping the whey into water streams reduces the aquatic life by depleting the dissolved oxygen (Kosseva et al. 2009). High lactose and protein content in whey is the main reason for its high BOD (Smithers 2015; Mawson 1994; Siso 1996) although in the case of yogurt acid whey (YAW) the protein content is very low to zero (Smith, Smith, and Drake 2016b).

Whey types and composition

Whey is a greenish and translucent liquid (Jelen 2011) that can be defined as the serum or watery part of the milk that remains after separation of the curd due to coagulation of milk proteins by acid or proteolitic enzymes (Panesar et al. 2007). Type and composition of whey depends on processing procedures utilized for casein removal and any other technological practices performed to pretreat the milk before processing (Panesar et al. 2007). The most commonly known type of whey originates from cheese manufacturing or from industrial casein production where the casein is coagulated by rennet, usually with chymosin or any other casein-coagulating enzyme (Jelen 2011). This type of whey is known as sweet whey since the rennet induced coagulation of casein and subsequent whey drainage occurs at a approximate pH of 6.5-6.0 (Jelen 2011). The second basic whey type is known as acid whey (pH < 5.0), resulting from processes in which casein is coagulated by fermentation or addition of organic or mineral acids, as in the processing of fresh, acid coagulated cheeses (e.g. cottage cheese or quark) (Jelen 2011) or strained yogurt (e.g. Greek style yogurt).

Table 1.1 shows the main components for both types of wheys (Jelen 2011). Water constitutes approximately 93% of the whey, while the total solids fraction contain lactose (70-72%), minerals (12-15%) and whey proteins (8-10%).

Table 1.1. Typical composition of sweet and acid whey

Component	Sweet whey (g/L)	Acid Whey (g/L)
Total Solids	63.0-70.0	63.0-70.0
Lactose	46.0-52.0	44.0-46.0
Protein	6.0-10.0	6.0-8.0
Calcium	0.4-0.6	1.2-1.6
Phosphate	1.0-3.0	2.0-4.5
Lactate	2	6.4
Chloride	1.1	1.1

Source:(Jelen 2011)

The main difference between both wheys are mineral content, the acidity and the composition of the whey protein fraction (Jelen 2011). The notably higher acidity in acid whey (final pH approximately 4.5), necessary for casein precipitation, results from acid coagulation in which some of the lactose converts to lactic acid by lactic acid bacteria (LAB) and/or addition of acidulants and various acids (e.g. glucono-δ-lactone, sulfuric, phosphoric, hydrochloric, citric or lactic acid) (Jelen 2011; Keller 2015). Acid whey can also have a lower concentration of lactose compared to sweet whey as a fraction of it is converted to lactic acid in the yogurt fermentation process (Keller 2015). In addition, acid whey can have high levels of galactose due to enzymatic breakdown of lactose to glucose and galactose (Keller 2015). Acid whey has higher calcium content as, at this low pH, the colloidal calcium contained in the casein micelles in normal milk solubilised and partitioned into the whey (Jelen 2011). Composition of whey protein fraction is different as sweet whey contains glycomacropeptide, a fragment of the κ-casein molecule produced by rennet clotting, constituting 20% of the whey protein fraction of sweet, rennet-based wheys (Jelen 2011).

Challenges of drying acid whey

Traditionally, products from sweet whey as whey powder, lactose, whey protein concentrate and demineralised whey powder are usually obtained by spray drying the sweet whey (Chen et al. 2016). Conversely, drying acid whey by conventional equipment is not a feasible task as the dry powder will stick to the walls of the dryer and cyclone (Modler and Emmons 1978). In particular, high concentration of lactate in acid whey, either as lactic acid or dissociated into lactate ions, reduces the possibility of obtaining acid whey spray dried powders as lactate increases powder stickiness causing operational problems in the dryer (Chandrapala et al. 2016) due to the hygroscopic nature of lactate ions. The formation of powder agglomerates and sticky deposits in the dryer depends of the proportion of crystalline lactose rather than amorphous state which in fact depends upon the powder glass transition temperature, relative to the dryer operating temperature (Chen et al. 2016). A reduction in this ratio will allow acid whey to be converted into high value powders using conventional spray drying units (Chandrapala et al. 2016; Chen et al. 2016). Different approaches have been studied to remove lactate content from acid whey including the use of electrodyalisis and nanofiltration (NF), both of them well proven demineralization technologies (Chen et al. 2016; Kentish and Rice 2015b). Chen et al. 2016, utilized electrodyalisis to remove lactate ions from acid whey. In order to achieve the same ratio of lactic acid to lactose as the one found in sweet whey, they removed 80% of the lactic acid in acid whey, and simultaneously 90% of minerals. After removing lactic acid, and diminishing the ratio of lactic acid to lactose, the glass transition temperature of the powder increased. This physical change in the dried acid whey should reduce stickiness problems in the spray drying operations system allowing the effective recovery of acid whey components (Chen et al. 2016).

Nanofiltration (NF) is a membrane filtration operation that separates and concentrates molecules with weight between 100 and 500 Dalton (Roman et al. 2009). The principal function of NF in dairy is to remove water and salts from dairy fluids (Kentish and Rice 2015a). It is common to nanofiltrate the permeate from the ultrafiltration process which allows the use of the retained concentrated lactose solution (Kentish and Rice 2015a). After this process, a permeate containing lactic acid, galactose and monovalent minerals, and a retentate containing peptides, lactose and calcium are obtained (Mike Molitor, 2016). NF has lower investment cost and simpler installation compared to electrodialysis, and generates reduced volume of effluent with lower BOD (Gernigon, Schuck, and Jeantet 2011). Nevertheless, permeates from membrane filtration cannot be discharged in the environment as still have high potential for contamination (Prazeres, Carvalho, and Rivas 2012). NF is an ideal technology to separate lactate from acid whey as there is no need for chemicals and the acid whey solution is demineralized and concentrated simultaneously (Chandrapala et al. 2016). Previous researchers successfully separated lactate from lactose in acid whey. At the natural acid whey pH, only 30% of lactate ions were able to pass through the membrane but when the pH was lowered to 3, the percentage increased to 50%. The results obtained suggest that NF may be used to produce a retentate that can be spray dried with great efficiency once lactate ions are removed (Chandrapala et al. 2016).

Smith and others, 2014, published a patent under the tittle: “Food products with yogurt whey” in which acid whey from GSY is pasteurized, concentrated and neutralized. Neutralization is done by adding a base such as sodium, potassium, calcium or magnesium hydroxide among others, to achieve acid whey pH greater than 5.0-6.2. Acid whey is concentrated by removing water to any useful solids or moisture content using methods such as filtration, evaporation or membrane separation. Once the acid whey is concentrated and neutralized it can be dried by any drying method. Even spray drying is possible because with a pH of 6 or greater, the spray dried neutralized whey forms a free flowing powder. The final product can be utilized as food product bulking or sweetening agent, nutrient fortificant, bakery product, beverage product, etc.

Challenges of working with liquid acid whey

Acid whey has a large potential to be used as the main component of beverages because of its nutritional composition. The utilization of liquid acid whey offers an interesting approach as there is no need for using complex technology other than pasteurization.

Acid whey can be sourced as zero or full fat, depending on the fat content of the GSY made. Even though full fat acid whey can be desirable in products such as a beverages because of better palatability, it will be prone to more rancidity issues than when working with zero fat acid whey. Liaw et al. 2011, reported that lipid oxidation products were more pronounced in whey without fat separation in comparison with whey with fat separation. In addition, Baccouche et al. 2013, reported the need to eliminate fat content in whey as it deteriorates organoleptic quality of drinks. Even if pasteurized, storage time of liquid acid whey should be as short as possible as it has been determined that lipid oxidation compounds and off-flavors increased with storage time (Roman et al. 2009; Smith, Smith, and Drake 2016)

Smith et al. 2016, described acid whey as having higher aroma compared to other types of wheys. They determined sour aromatic and distinct potato/brothy flavors in acid whey and reported that cardboard flavor and lipid oxidation aldehydes increased with storage time.

Whey is not a standardized product. YAW whey collected from different dairy companies as well as different yogurt lines will vary in composition (total sugar, protein, fat and titratable acidity, etc), affecting odor, flavor, aftertaste and nutritional composition of the final product (table 1.2).

Table 1.2. Low and high composition values of yogurt acid whey samples[1]

Acid whey components	Lower value	Higher value
Total Sugars (mg/g)	33.6	41.1
Total Protein (mg/g)	1.71	4.4
Total Fat (mg/g)	0.0	15.0
Calcium (mg/g)	1.2	1.28
Sodium (mg/g)	0.38	0.42
Phosphorus (mg/g)	0.67	0.69
Potassium (mg/g)	1.56	1.69
Magnesium (mg/g)	0.10	0.11
Chloride (mg/g)	0.78	1.08
TA (Lactic Acid)	4.24	5.42
pH	4.21	4.48

[1]Data collected by Pedro Menchik, PhD student at Cornell University from 7 yogurt acid whey samples from NY state dairies collected during 2015-16

Moreover, a flaw during GSY production might be reflected on the remaining YAW. As an example, YAW should normally contain no casein but if the separator does not work properly during GSY production, substantial amounts will remain in the whey. Visual inspection might not be enough to detect it, but once pasteurized, the casein will precipitate, resulting in quality problems to the finished product.

Utilization of acid whey could be increased if the objectionable flavor of whey could be reduced or eliminated (Mcgugan, Larmond, and Emmons 1972). A challenge of working with acid whey resides in its sour dairy flavor and aroma, which is not easy to mask. Its natural tartness is difficult to balance with many flavors as the dairy aroma might still be strongly perceived. In some cases the dairy aroma is strong, described as expired milk. Furthermore, acid whey is astringent and its lingering acidity dries the tongue, a concern when developing whey beverages such as sports drinks where consumers expect a thirst quenching attribute.

Acid whey applications toward natural products

The development of natural products is an area of active research. Stadnik et al, 2016, used sea salt in combination with acid whey to study the production of dry-cured organic pork loins without the addition of nitrite. The investigators reported that the use of sea salt with acid whey positively affected the physicochemical parameters of the dry cured organic loins. Samples with acid whey had similar overall sensory quality compared to controls made with curing salts. The combination of sea salt and acid whey successfully reduced the browning reaction that produces dark dry-cured loins and yielded similar polyunsaturated fatty acids content, suggesting that acid whey has a comparable effect to nitrite against oxidation. Wojciak et al., 2014, reported that acid whey and mustard seed can be used to replace nitrites during cooked sausage production, as they found beneficial effects on physicochemical and sensory qualities of no-nitrite sausage. Dolatowski et al., 2015, used acid whey and probiotic strains to obtain safe and microbiologically stable fermented sausages. They stated obtaining extended shelf life and excellent overall quality, comparable to the fermented sausage made with curing salt.

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CHAPTER 2

Development of an Acid Whey Based Fruit Yogurt Beverage

INTRODUCTION

Yogurt drinks have seen strong growth in adoption and sales in recent years (increasing dollar sales by 62% and volume sales by 73% from 2011-16) (Yogurt and Yogurt Drinks US, Mintel database, Aug. 2016).

Drinkable yogurt is consumed as a refreshing drink and is characterized as a stirred yogurt with low viscosity (Tamime and Robinson 2007). Differently from set yogurt in which the coagulum is handled very carefully, in drinkable yogurt production, centrifugal pumps are used to transfer the yogurt from the incubation tank to the cooler. To break the coagulum after fermentation, higher agitation speeds are used or the cold yogurt can be passed through a homogenizer without the application of pressure (Tamime and Robinson 2007). Drinkable yogurt can also be made from diluting the yogurt to the appropriate consistence with e.g. water, milk, whey. In the production of Ayran, a traditional Turkish yogurt drink, the fermented yogurt is mixed with about 35% water (Tamime and Robinson 2007). A similar product to Ayran is the Lebanon, in which the fermented milk is diluted with water or whey from Labneh making. In Iran, people consume a fermented and diluted yogurt drink called dough, similar to Ayran (Tamime and Robinson 2007). Additionally, in Brazil yogurt is diluted with cheese whey.

Over the years, researchers have been exploring how to transform large volumes of whey into different products suitable for consumption (Djurić et al. 2004). Belloso-Morales et al. 2003 developed a fermented beverage using fresh sweet whey (from panela cheese), fresh acid whey (from Guayanes cheese) and reconstituted sweet whey with a kombucha culture as inoculum.

Carbon dioxide production was minimal as fermented beverages obtained were not sparkling. Even though the culture was able to grow well in the three kinds of whey, researches indicated the best results were achieved with reconstituted sweet whey. Authors reported the beverage to be too sour and salty, stating that demineralising the whey previous to be used and adding fruit flavor to the finish product could improve the sensory characteristics of the fermented beverage.

The objective of this project was to develop an acid whey-based fruit yogurt beverage utilizing yogurt acid whey (YAW), determining the acceptability of the product in a formal sensory study. In addition, physicochemical measurements over time were performed, allowing optimization of formulation and processing for a refrigerated beverage.

MATERIALS AND METHODS

PHYSICOCHEMICAL EVALUATION

Materials and Ingredients

Yogurt: Freshly made low fat, mango flavour, commercial yogurt was purchased from the Cornell Dairy Plant (Ithaca, NY) and stored at 4°C for up to 3 days before using. (Ingredients: cultured pasteurized grade A reduced fat milk, skim milk, sugar, mangos, corn starch, agar, pectin, natural flavors, fruit and vegetable concentrate for color). Mango yogurt was selected because it is the most popular flavour from the Cornell Dairy Plant.

YAW: Acid Whey from Greek style yogurt (GSY) processing was provided by Byrne Hollow Farm (Cortland, NY). Acid Whey was pasteurized at 72°C for 15 seconds in compliance with FDA Grade “A” Milk Ordinance provided and stored at 4° C until use for up to 3 days.

Stabilizer: Dairy Blend Acidified Beverage 120 stabilizer (cellulose gum, pectin), was provided by TIC GUMS (White Marsh, MD).

Chemicals

Chemical reagents were purchased from VWR (VWR analytical, Radnor, PA, USA) including NaOH 0.1N standard solution, buffer reference standards at pH 7.00 ± 0.01 and at pH 4.00 ± 0.01

Yogurt Beverage Preparation

To assess the effect of homogenization, YAW addition and stabilizer concentration, different treatment combinations were studied (see Table 2.1), based on preliminary testing to reduce the number of variables. To prepare the fruit yogurt beverage (FYB) samples, a stock solution of stabilizer mixed with YAW was prepared in advance in order to ensure total hydration of the stabilizer. The stock solution was prepared in an induction heated mixer (Kenwood Cooking Chef with heat induction technology, NJ, USA) at 60°C to facilitate hydration. After weighing the amount of yogurt, YAW and stock solution for each different sample formulation (see Table 1 for formulation and respective treatment), the ingredients were mixed together in an induction heated mixer at low speed to avoid air incorporation. Once the mixtures were ready, some samples were heated to 55°C and then passed through a homogenizer (FT9 Armfield, Ringwood, Hampshire, UK) at 1000 psi. All samples were packaged in 300 g plastic containers with lids and stored at 4°C. The control sample was prepared following the same procedure except that water was added instead of YAW. For physicochemical evaluation, samples were analyzed every 10 days for up to 40 days.

Table 2.1. Experimental design to assess the effect of homogenization, yogurt acid whey addition and stabilizer concentration on the quality of refrigerated, mango yogurt beverage.

Treatment Description	Abbreviation	Ingredient (%)			
		Mango Yogurt (%)	Acid Whey (%)	Water (%)	Stabilizer (%)
Non-Homogenized yogurt beverage with 35% acid whey and 0.2% stabilizer	35% NH 0.2	64.8	35	0	0.2
Non-Homogenized yogurt beverage with 35% acid whey and 0.4% stabilizer	35% NH 0.4	64.6	35	0	0.4
Homogenized yogurt beverage with 35% acid whey and no stabilizer	35% H + NS	65	35	0	0
Non-Homogenized yogurt beverage with 35% acid whey and no stabilizer	35% NH +NS	65	35	0	0
Homogenized yogurt beverage with 35% acid whey and 0.2% stabilizer	35% H 0.2	64.8	35	0	0.2
Homogenized yogurt beverage with 35% acid whey and 0.4% stabilizer	35% H 0.4	64.6	35	0	0.4
Homogenized yogurt beverage with 40% acid whey and 0.2% stabilizer	40% H 0.2	59.8	40	0	0.2
Homogenized yogurt beverage with 40% acid whey and 0.4% stabilizer	40% H 0.4	59.6	40	0	0.4
Homogenized yogurt beverage with 45% acid whey and 0.2% stabilizer	45% H 0.2	54.8	45	0	0.2
Homogenized yogurt beverage with 45% acid whey and 0.4% stabilizer	45% H 0.4	54.6	45	0	0.4
Homogenized yogurt beverage with 45% water and 0.2% stabilizer	Control 0.2	54.8	0	45	0.2
Homogenized yogurt beverage with 45% water and 0.4% stabilizer	Control 0.4	54.6	0	45	0.4

Experimental design

Two experiments were conducted to assess the effect of homogenization and stabilizer, and to determine the optimal concentration of YAW addition to produce a stable yogurt beverage. Samples with 35% YAW and 0, 0.2 and 0.4% stabilizer were prepared with and without homogenization. Homogenized samples with 0.2 and 0.4% stabilizer were prepared with 35, 40 and 45% added YAW and with 45% added water as a control. Samples were prepared in triplicate, kept refrigerated at 4°C and analyzed every 10 days.

Analytical procedures

pH

pH was measured at using a Thermo Scientific Orion 2 Star pH meter (Thermo Fisher Scientific, Beverly, MA) calibrated using commercial pH 4.00 and 7.00 buffers (VWR analytical, Radnor, PA, USA)

Water Holding Capacity

The water holding capacity (WHC) of all the samples was measured following a modified version of the procedure stated by Sodini et al (2003). Fifteen grams of sample was centrifuged at 2500 rpm for 10 minutes in a refrigerated high speed centrifuge (Beckman Avanti J-25 Centrifuge), at 4 °C. The amount of whey expelled during centrifugation was removed and weighed. Being W the amount of whey expelled, WHC was calculated as:

$$WHC = (15-W)/15 * 100\ (\%) \qquad \text{(Eq 1.0)}$$

Titratable Acidity

Titratable acidity (TA) was determined using a G20 compact titrator (Mettler Toledo, Schwerzenbach, Switzerland). TA was expressed as g of lactic acid/100 g of sample and was obtained by titrating 10 g of sample diluted with 50 ml of deionised water with 0.1N NaOH.

Viscosity

Viscosity was measured in a Brookfield DV – III ULTRA (Brookfield Engineering Lab Inc., Stoughton, MA) with a VL-1 spindle at 114 rpm at 10 °C. A shear rate of 25 s^{-1} was selected, in the range 10 s^{-1} to 37 s^{-1} reported for foods of different viscosities by Sharma and Sherman (1973).

Before each measurement, samples were agitated for 5 s to follow consumers' mixing instructions before drinking a yogurt beverage. The data was acquired using the Rheocalc software (Brookfield Engineering Lab Inc., Stoughton, MA). The viscosity measurements were continuous over 30 s and the viscosity value was recorded after 10 seconds.

Colorimetric analysis

Yogurt samples (approximately 30 mL) were transferred to a 2-cm path length quartz cell (Hunter Labs, Reston, Va., U.S.A.) and read for CIE *L*, *a*, *b*, chroma, and hue angle values using a Hunter ColorQuest XE (Hunter Labs). Samples were read using reflectance specular included with D65 illuminant, and 10° observer angle.

Soluble solids

The soluble solids content were determined as degree Brix with a Leica Auto Abbe refractometer model 10500-802 (Leica Inc., Buffalo, NY).

Particle size

Particle size distribution was determined using a laser diffraction particle size analyzer Mastersizer 2000 (Malvern instruments, Worcestershire, UK). Samples were added into a water-continuous dilution accessory (200 Hydro-S) filled with deionised water. The particle size distribution was calculated using the instrument software (Mastersizer 2000, version 5.40).

Statistical Analysis

Evaluation of significant effect on the physicochemical measurement of samples with different percentage of acid whey was performed by building a full factorial design with four levels for samples (35%, 40%, 45% and Control), two levels for percentage of stabilizer (0.2% and 0.4%), and 5 levels for day of measurement (day 0, 10, 20, 30 and 40) with the measured values of pH, viscosity, TA, WHC, soluble solids (°Brix), and color (hue, C*, a*, L*, b*) as the response. Means were further compared using student´s t. Statistical analyses were performed using JMP® version 12.0 (SAS Institute, Cary, USA). Differences were considered significant when $p < 0.01$.

Assessment of significant effect on the physicochemical measurement of samples with 35% YAW and different treatment was performed by building a full factorial design with one level for samples (35%), two levels for percentage of stabilizer (0.2% and 0.4%), two levels for treatment (homogenized and none homogenized) and 5 levels for day of measurement (day 0, 10, 20, 30 and 40) with the measured values of pH, viscosity, TA, WHC, soluble solids °(Brix), and color (hue, C*, a*, L*, b*) as the response. Means were further compared using student´s t. Statistical analyses were performed using JMP® version 12.0 (SAS Institute, Cary, USA). Differences were considered significant when $p < 0.01$.

SENSORY EVALUATION

Sample preparation

Sensory evaluation was conducted following the guidelines and policies of the Cornell Institutional Review Board for Human Participants.

Based on preliminary results, a subset of samples was selected for the sensory evaluation. Samples were prepared as detailed above and described in Table 2.2. A fresh batch of the three FYB plus the control were prepared just for the consumer taste panels.

The samples were served in identical 60 ml plastic cups with lids. Water and unsalted crackers were provided for palate cleansing. Each sample was labeled using four-digit random numbers and presented to each participant in random order.

All samples were tested for total coliforms before serving, guaranteeing counts of <5 cfu/mL.

Table 2.2. Treatment description, fruit yogurt beverage abbreviation and ingredient % for sensory evaluation set of samples.

Treatment Description	Abbreviation	Ingredient (%)			
		Mango Yogurt (%)	Acid Whey (%)	Water (%)	Stabilizer (%)
Homogenized yogurt beverage with 25% acid whey	25%	74.8	25	0	0.2
Homogenized yogurt beverage with 35% acid whey	35%	64.8	35	0	0.2
Homogenized yogurt beverage with 45% acid whey	45%	54.8	45	0	0.2
Homogenized yogurt beverage with 45 % water	Control	54.8	0	45	0.2

Consumers

Consumers were recruited from the Cornell Sensory testing database to participate in a FYB test. Participants were scheduled using doodle poll with a maximum of 8 participants each 15 min section. Walk-ins were also allowed in this study as there was no particular requirement to participate except that participant must like dairy and dairy-based products and have no allergies or intolerances to milk or other dairy products/ingredients.

Sensory evaluation was conducted at the Cornell Sensory testing facility with 120 participants (85 females and 35 males) between the ages of 18-60.

Test design and Evaluation

RedJade software (RedJade ®, Redwood Shores, CA, USA) was used to design the test, questionnaires and to collect data.

At the beginning of the test, each participant had to accept the participant study agreement in order to continue with the study. In this agreement, participants had a description of the research, risk and benefits of performing the study, confidentiality protection and contact emails in case of any concern.

The study consisted of two parts, commencing with a preference test where each participant had to evaluate two yogurt beverages: one with 45% of YAW and the control (45% of water instead of YAW) on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) for one attribute, overall liking. Then the participant had to choose the preferred sample among both samples. Sample order was randomized to avoid placement bias.

In the second part of the Sensory study, each participant rated 3 different yogurt beverages with 25, 35 and 45% of YAW on a 9-point hedonic scale for five liking attributes (overall liking, appearance, texture, mouth feel and flavor) and on a 5-point Just About Right (JAR) scale (1 = much too low, 2 = a little too low, 3 = just about right, 4 = a little too much, and 5 = much too much) for five attributes (consistency, texture, tartness, fruity flavor and serving temperature). In addition, participants were asked how they would consume each of the samples (as a standalone beverage, in a bowl mixed with cereal, as a dressing for fruit salad or other). Participants were also asked about acceptability of aftertaste of samples. Sample order was randomized to appear in each of the three possible positions so that not every participant will receive the samples in the same order.

After completion of these two parts of the test, participants answered demographics questions and whether they would purchase this product or not knowing that the beverage had YAW coming from Greek yogurt processing as part of the formulation.

Participants received a reward of $5 for participating in the study.

Statistical Analysis

RedJade software (RedJade ®, Redwood Shores, CA, USA) was used to analyse data. Means were compared using Tukey's test. Differences were considered significant when $p < 0.05$.

Focus group

A focus group session was scheduled to obtain consumer feedback on the FYB samples containing 45% YAW or 45% water (control) after 40 days of refrigerated storage. Focus group was conducted in one discussion session of 45 min. Eight people (6 female, 2 male) participated in the focus group. Participants were a mix of students, staff and faculty of the Food Science department at Cornell University which consume dairy products regularly (including yogurt, Greek-style yogurt with fruit and other additions, drinkable yogurt, kefir, etc.). Two randomly coded samples were tested: the control first and the 45% YAW second. Twenty ml of test product per participant were served in 5 oz cups, covered by matching lids to create a head space for aroma elements to develop. Products were served chilled on ice. Water was used in between the samples' tasting as a palate cleanser. Focus group was moderated by the Manager of the Cornell Sensory facility Alina Stelick and it took place in Stocking Hall at Cornell University.

RESULTS AND DISCUSSION

Preference test

After performing a preference test for samples with 45% of YAW vs. control, 52.5% of the consumers (n=120) preferred the sample containing YAW compared to 47.5% for the control. Both the preference test and the overall liking scores (Table 2.3) were not significantly different ($p < 0.05$) indicating that the addition of YAW, even at 45%, does not influence the acceptability of the freshly made beverage. After choosing the preferred sample, participants were asked to explain their answer. Participants that chose sample with 45% of YAW over the control stated that the control was too watery and that 45% sample had a better flavor profile, better balance of sweetness, was more refreshing and the taste was more memorable.

Table 2.3. Mean score for overall liking preference test on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) for fruit yogurt beverage samples containing mango yogurt, stabilizer and 45% yogurt acid whey or 45% water (control).

Sample	Overall liking mean value
45%	6.06_{A}
Control	5.95_{A}

(n=120)-Means not connected by the same letter are significantly different ($p<0.05$) following Tukey´s HSD

Consumer acceptability test

In the second part of the study, each participant rated 3 different yogurt beverages prepared with 25, 35 and 45% YAW on a 9-point hedonic scale for different liking attributes (1=dislike extremely to 9= like extremely). Results shown in table 2.4 indicate that samples with 25-35% YAW were rated significantly higher than the 45% for overall liking, appearance, flavor and taste. For texture and mouthfeel, the 35% sample was rated significantly higher than the 45% one, and similarly to the 25% sample.

Table 2.4. Mean score for five liking attributes for the fruit yogurt beverage on a 9-point hedonic scale (1=dislike extremely to 9=dislike extremely).

	Attributes				
Sample	**Overall liking**	**Appearance**	**Texture**	**Mouthfeel**	**Flavor and Taste**
25%	6.59_{A}	6.73_{A}	6.53_{AB}	6.54_{AB}	7.08_{A}
35%	6.74_{A}	6.83_{A}	6.93_{A}	6.68_{A}	6.94_{A}
45%	5.16_{B}	6.24_{B}	6.39_{B}	6.13_{B}	6.10_{B}

(n=120) Means in the same attribute quadrant, not connected by the same letter are significantly different ($p<0.05$) following Tukey´s HSD

Food texture is particularly important for consumers, and is often used as a food quality indicator (Hildegarde and Heymann 2013). In addition, texture is a significant attribute in most dairy foods. Tournier et al. 2007, stated that consumers associate creaminess with texture and pleasantness. It has been stated that creaminess, smoothness and thickness are the texture characteristics that influence consumers the most in the liking attribute of dairy products such as yogurt (Bruzzone, Ares, and Giménez 2013). In this study, it was important to determine whether the texture was acceptable for a yogurt beverage and, as can be seen in Figure 2.1, most consumers liked (scores ≥ 6) the texture of the three different samples.

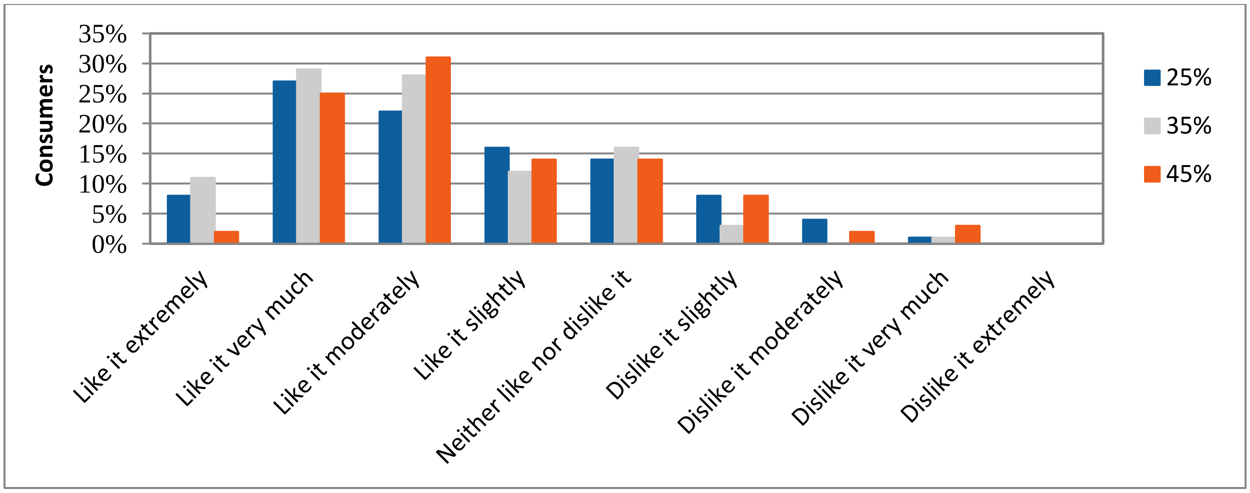

Figure 2.1. Consumer (n=120) response for Texture liking on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) of fruit yogurt beverage samples containing 25, 35 and 45% of YAW.

When analysing the mouthfeel of the samples, as seen in Figure 2.2, 58% of consumers liked the mouthfeel of sample with 25% YAW, 62% liked the sample with 35% YAW while only 40% of consumers liked the sample with 45% YAW. The lower percentage can be explained by penalty analysis below in this chapter as consumers thought the sample with 45% YAW was too thin.

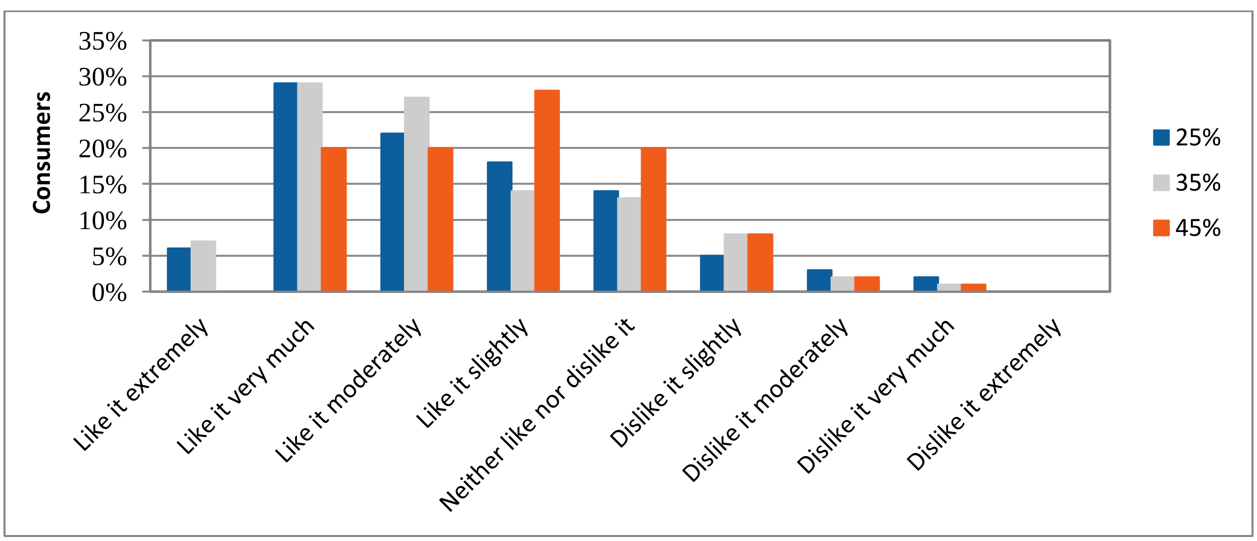

Figure 2.2. Consumer (n=120) response for mouthfeel liking on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) of fruit yogurt beverage samples containing mango yogurt, stabilizer and 25, 35 and 45% of YAW respectively.

Stabilizer was added to the samples to extend the product's shelf life, avoiding syneresis and milk solids sedimentation (Tamime and Robinson 2007). Acid whey has a considerable amount of salts and other solids (table 2.5) hence, it was important to determine consumers response to perceived smoothness and graininess of the samples. As seen in figure 2.3, more than 80% of consumers thought that smoothness for the three samples was just about right. The results are in agreement with the mouthfeel ratings of the samples as stated above.

Table 2.5. Typical composition of Acid whey[1].

Component	Acid Whey (g/L)
Total Solids	63.0-70.0
Lactose	44.0-46.0
Protein	6.0-8.0
Calcium	1.2-1.6
Phosphate	2.0-4.5
Lactate	6.4
Chloride	1.1

[1]Illustrative data compiled from various sources (Jelen 2011).

Penalty analysis

Penalty analysis or mean drop analysis is used by the food industry when developing new products, in order to optimize formulations (Narayanan et al. 2014). Penalty analysis combines just-about-right (JAR) and overall liking tests to correlate a decrease in consumer acceptance to attributes that are not at the JAR level and penalizes products in terms of penalty or deviation from JAR (Lawless and Heymann 2010).

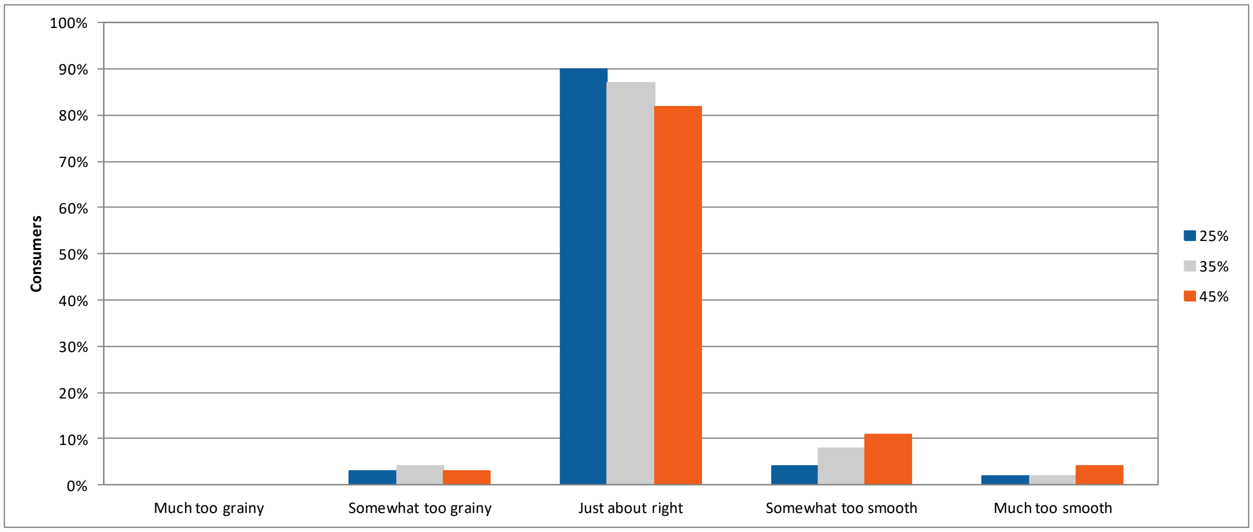

Figure 2.3. Consumer (n=120) response for smoothness liking on a Just About Right (JAR) scale of the fruit yogurt beverage samples containing mango yogurt, stabilizer and 25, 35 and 45% of YAW respectively.

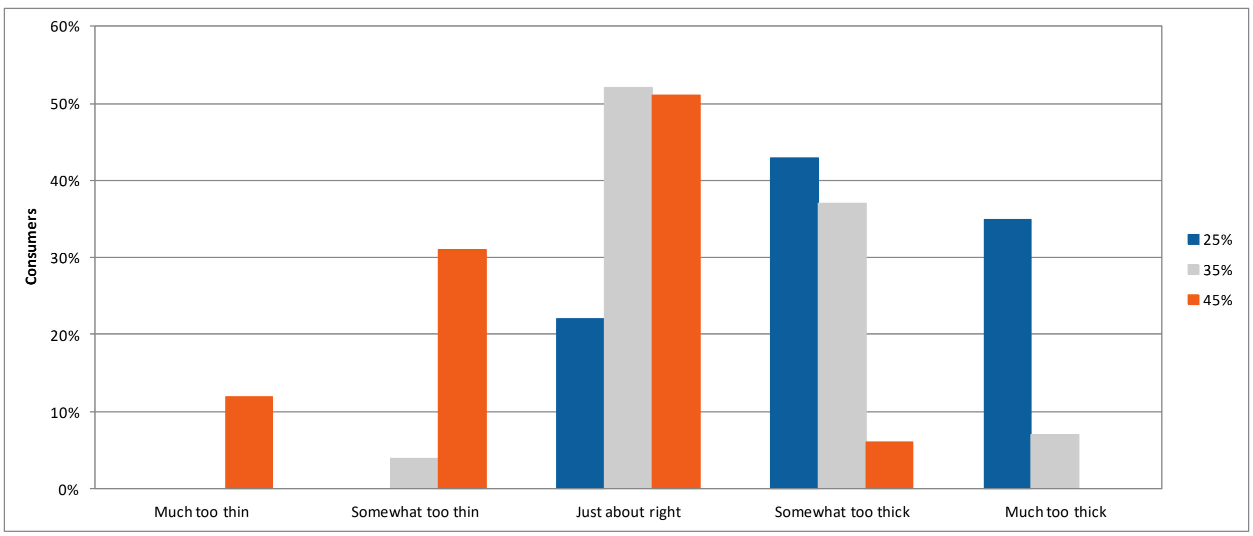

Figure 2.4. Consumer (n=120) response for texture liking on a Just About Right (JAR) scale of the fruit yogurt beverage samples containing mango yogurt, stabilizer and 25, 35 and 45% of yogurt acid whey respectively.

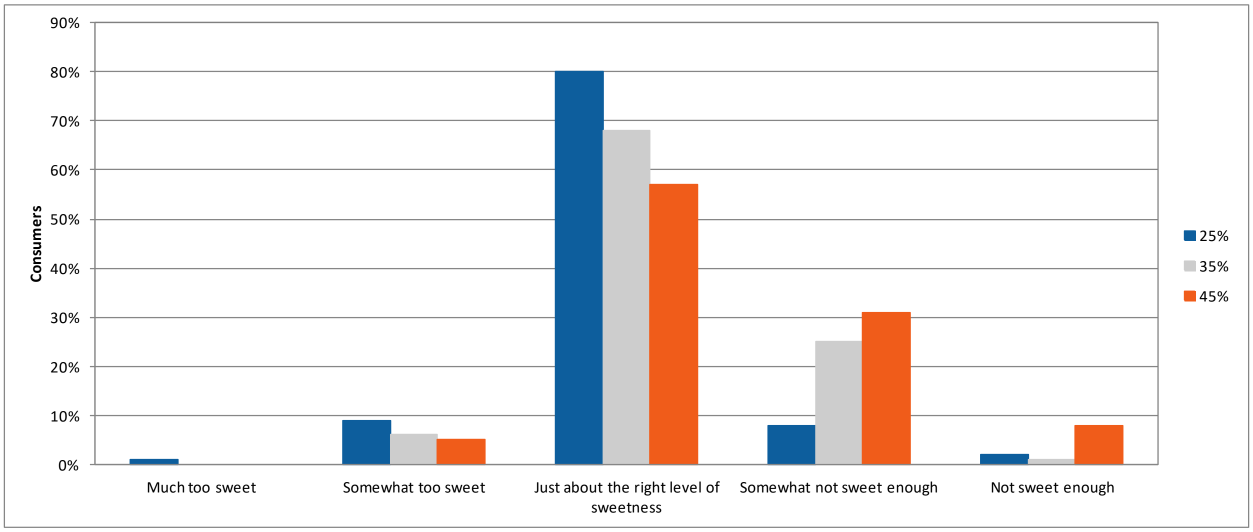

Figure 2.5. Consumer (n=120) response for sweetness liking on a Just About Right (JAR) scale of the fruit yogurt beverage samples containing mango yogurt, stabilizer and 25, 35 and 45% of yogurt acid whey respectively.

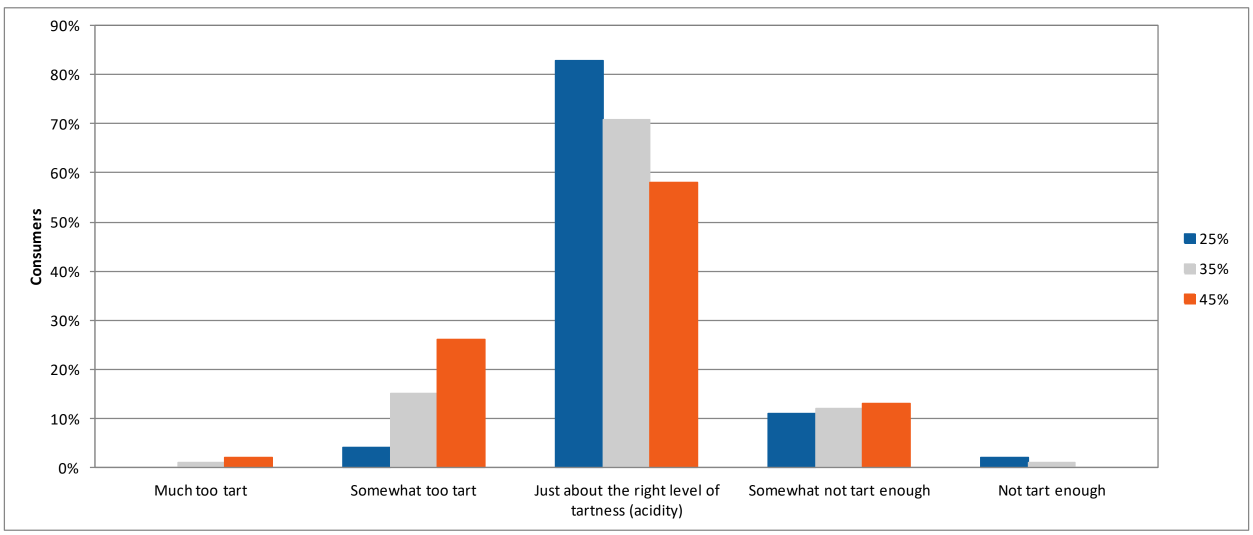

Figure 2.6. Consumer (n=120) response for tartness liking on a Just About Right (JAR) scale of the fruit yogurt beverage samples containing mango yogurt, stabilizer and 25, 35 and 45% of yogurt acid whey respectively.

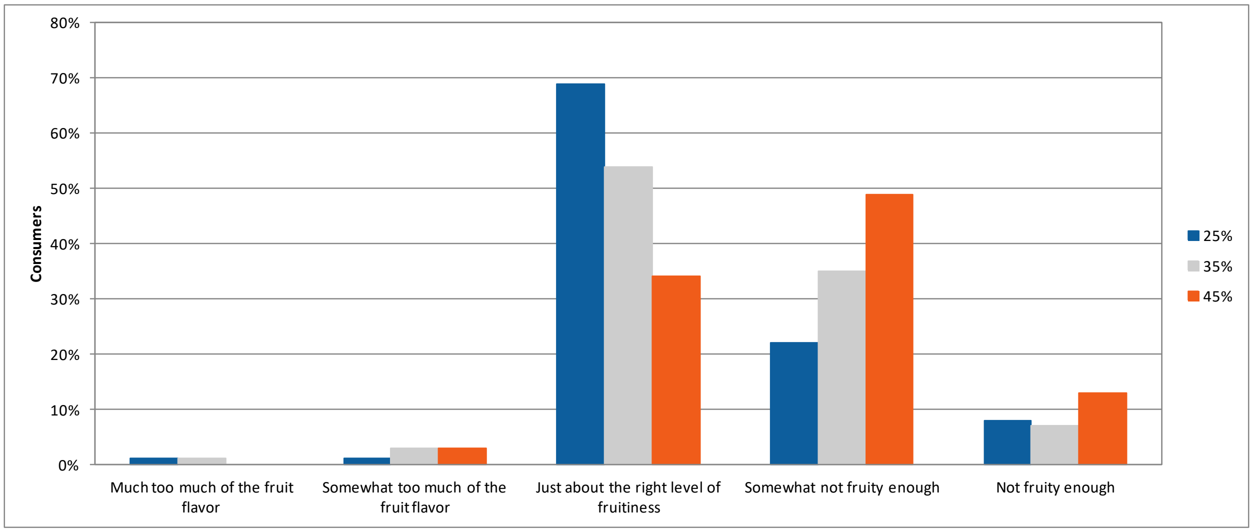

Figure 2.7. Consumer (n=120) response for fruity flavor liking on a Just About Right (JAR) scale of the fruit yogurt beverage samples containing mango yogurt, stabilizer and 25, 35 and 45% of yogurt acid whey respectively.

Penalty analysis showed that consumers perceived the three samples (25%, 35% and 45%) to be JAR in, smoothness, sweetness and tartness (Figures 2.3, 2.5 and 2.6) while texture and fruit flavour showed variations based on YAW concentration (Figures 2.4 and 2.7). Consumers found sample with 25% YAW as too thick (Figure 2.4) and when asked how they would improve the product, most of the answers mentioned the sample should be thinner and more drinkable. The 45% sample was perceived as not fruity enough by most consumers.

The results are better visualized in a penalty report plot by sample. Penalty report graphs the mean decrease in overall liking (assessed penalty) versus percentage of not-JAR evaluations (percentage of consumers that stated an attribute as not-JAR) and the preferred attributes are located at the lower left section of the plot (Narayanan et al. 2014). Attributes that received at least 20% responses in any of the not-JAR categories (“much too low” and “somewhat too low” responses) need to be taken into consideration for optimization (Narayanan et al. 2014).

Moreover, a penalty of 2.0 or higher is considered a challenge that needs to be reviewed in the formulation.

Figure 2.8 shows the penalty report for the sample with 25% YAW, indicating that 80% of consumers rated this sample as being too thick, scoring this attribute with a penalty of 0.9. The second penalty was for not fruity enough assessed by 29% of consumers with a 0.6 point decrease in acceptability.

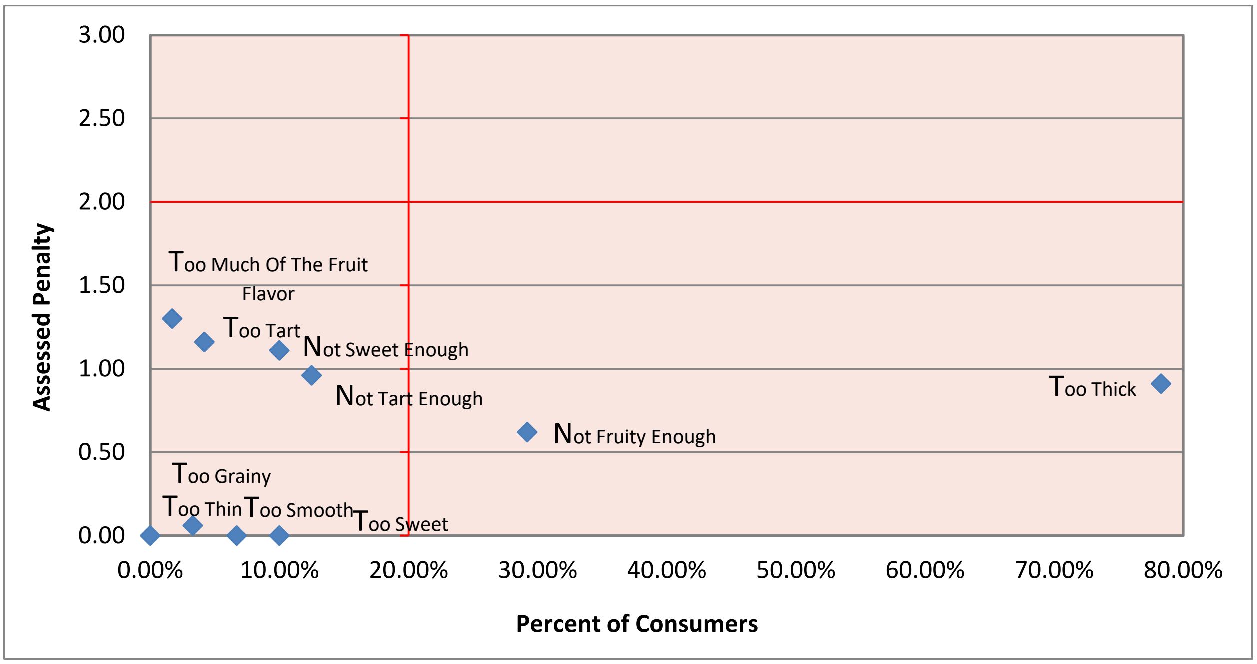

Figure 2.8. Assessed penalty report for homogenized fruit yogurt beverage sample containing yogurt, stabilizer and 25% YAW.

When asking consumers what usage they would give to this sample, 34% said they would use it as an ingredient in a smoothie or milk shake; 22% stated they would consume it in a bowl mixed with cereal in replacement of milk, 23% as a fruit salad dressing and 17% as a stand alone beverage.

The sample with 35% YAW had no attribute penalty above 1.0 (Figure 9), but more than 40% of consumers rated the sample to be too thick and not fruity enough while 25% rated it not sweet enough. These results can also be visualised in figures 2.4, 2.5 and 2.7. Comments for this sample are mostly about being too thick to be considered a beverage and about lack of fruity flavor. When asking about the possible usage for this samples, 33% of consumers said that they would use it as an ingredient in a smoothie or milk shake; 22% stated they would consume it in a bowl mixed with cereal in replacement of milk, 19% as a fruit salad dressing and 25% as a stand alone beverage.

Results for samples with 25% and 35% YAW indicate that even though these two sample might be too thick to consume as a drinkable yogurt beverage, both of them are still acceptable by panelist to be consumed in many other ways. Reformulation to increase the fruit component by adding juice might be beneficial to add more fruitiness and to decrease the viscosity.

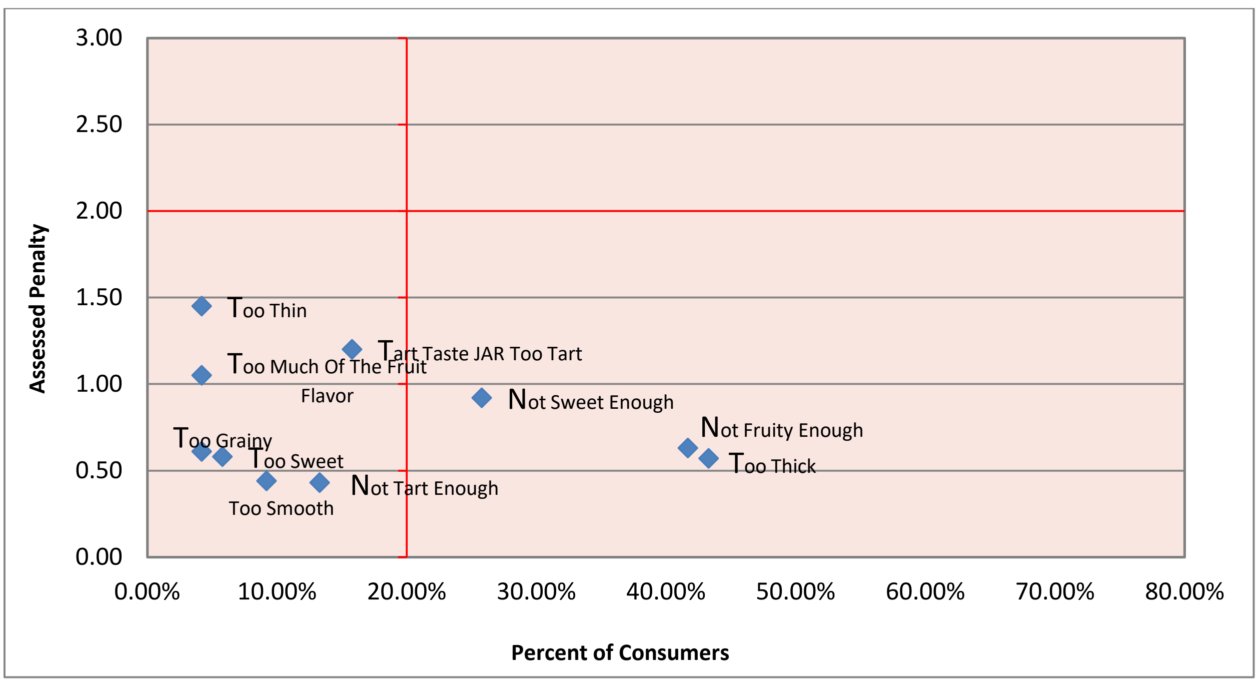

Figure 2.9. Assessed penalty report for homogenized fruit yogurt beverage sample containing yogurt, stabilizer and 35% yogurt acid whey.

For the sample containing 45% YAW, there were no attributes with a penalty over 2.0 (Figure 2.9) but 43% of consumers found the sample to be too thin (also seen in Figure 2.4), 62% not fruity enough (Figure 2.7), 38% not sweet enough and 28% too tart. When asked about the possible usage for this sample, 31% of consumers said they would use it as an ingredient in a smoothie or milk shake; 15% stated they would consume it in a bowl mixed with cereal in replacement of milk, 19% as a fruit salad dressing and 36% as a stand alone beverage. Interestingly enough, the 35% sample was considered too thick by 43% of consumers while the 45% sample was deemed too thin by the same consumer's percentage, indicating that thickness is a key attribute that changes significantly within the range studied, or that consumer's acceptability for a yogurt-based beverage shows high variability.

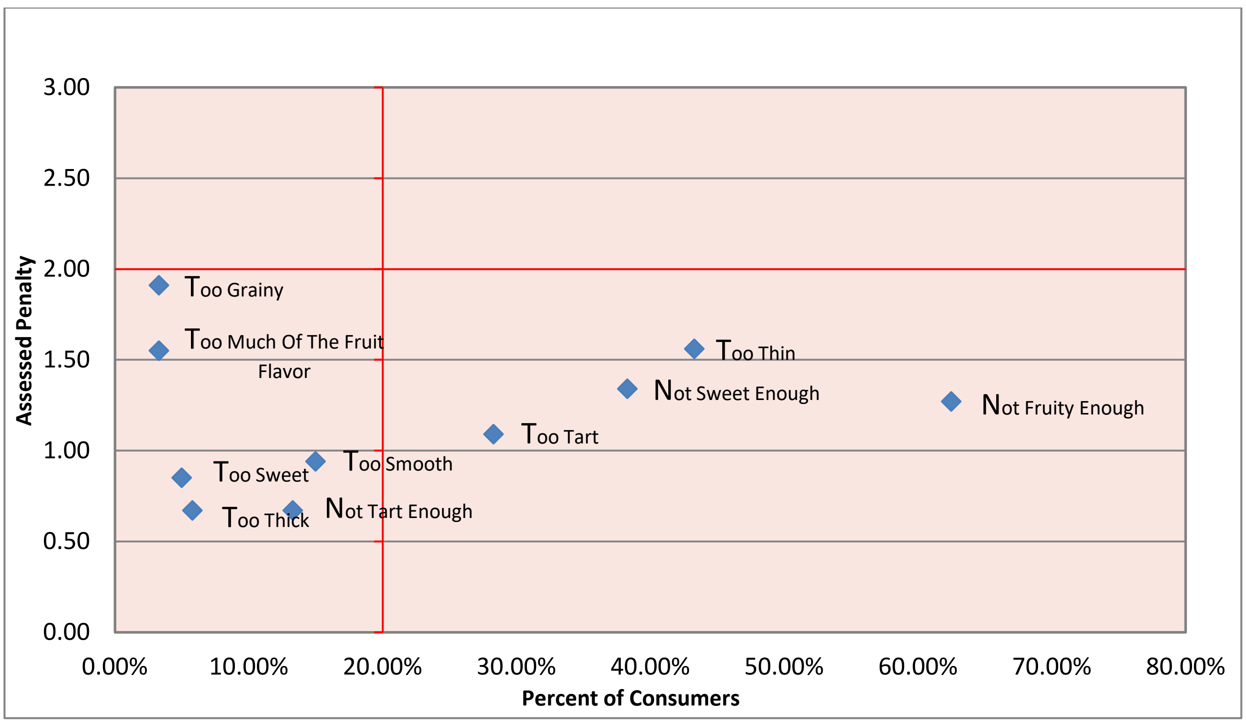

Figure 2.10. Assessed penalty report for homogenized fruit yogurt beverage sample containing yogurt, stabilizer and 45% yogurt acid whey.

Formulations for all of the samples for the sensory evaluation can be seen in Table 2.2. Since the fruit yogurt base was the same for all formulations, samples with higher percentage of YAW had proportionally less percentage of fruit yogurt, thus diluting the fruit flavor. For the sample with

45% YAW, consumers stated that it was not sweet enough and that the sample had not enough fruity flavor. These results are in agreement with the overall liking attribute where consumers preferred the 25% and 35% vs. 45% YAW. Reformulating to increase the fruity flavor by increasing the amount of mango pure will improve the acceptability of the 45% sample.

Samples with 25% and 35% YAW were thicker and not acceptable as a beverage for a large percentage of consumers. For these formulations, the thickness can be decreased by adding juice or water, or by mechanically thinning the beverage through high speed agitation or by homogenizing without applying pressure (Tamime and Robinson 2007). Consumers indicated that, a product similar to samples containing 25% and 35% YAW could be marketed as a multi use product as a beverage, to add to fruit salads, smoothies and milkshakes or for consumption in a bowl with cereal and/or fruit.

The taste panels conducted were important to determine the acceptability of the samples, and the percentage of YAW that was most appropriate to be used as part of the formulation of a drinkable yogurt. Based on the results obtained, samples with 35% to 45% YAW were selected for the FYB quality and shelf-life studies.

Fruit yogurt beverage aftertaste

Consumers were asked whether they noticed an aftertaste in samples with 25%, 35% and 45% YAW. There were no significant differences among samples, but consumers noticed an aftertaste after tasting all the samples. As aftertaste can be either a negative or positive connotation in a product sample, consumers were also asked if the aftertaste was acceptable to them. Of all consumers, 89, 95 and 86% of consumers stated that aftertaste of samples 25, 35 and 45% respectively was acceptable to them.

Purchase intent

An important part of this project was to assess if people would buy this product knowing that it contained acid whey in the formulation. In the demographics section a statement was incorporated explaining that most of the products tried in the Sensory testing (with the exception of the control) had whey in the formulation, which came from GSY production. After the statement, consumers were asked how likely they would be to purchase this type of product if it was available to them at the store where they typically shop and at the price that they typically pay for these types of products. The answers were encouraging as 40% of consumers stated that they “Probably would buy it” and 17% said that they “Definitely would buy it”. On the contrary, only 6% and 2% said that they “Probably would not buy it” and “Definitely would not buy it” respectively. The remaining 35% of the consumers, stated that they “May or may not buy it” but these can be reverted in a positive way with consumer education and understanding of acid whey properties and environmental and economical benefits of using a food by product.

Focus Group

End of shelf life

A qualitative consumer test was performed in order to evaluate acceptability of the control (45% water) and the sample with 45% YAW, after 40 days at 4°C storage, the expected shelf life of a yogurt beverage under the production conditions used. The goal was to obtain consumer feedback regarding acceptability and the associated drivers. No statistical inferences were drawn from this test as it had a limited test population (n=8).

Overall, consumers found the 45% YAW sample acceptable to drink but very *one-dimensional* in its flavor profile and only (4/8) of the respondents liked the 45% YAW sample. It was described as unbalanced and mostly *sour tasting*. On the contrary, the control was more liked and was found to be preferred overall by all the respondents. Even though consumers found the aroma character of both test products *acceptable* to them, consumers preferred the control sample as they found it more appealing and interesting compared to the single dairy notes of the 45% YAW sample.

Overall, both samples were perceived to have a similar appearance that consumers liked: creamy, smooth, appropriate viscosity and attractive pale color. The taste and flavor of both products was found to be *acceptable,* although many of the participants expressed preference for the control sample as *more balanced* of the two. Respondents found that both samples had *acceptable* consistency, appropriate for a beverage although several noted that the 45% YAW sample was somewhat thicker than the Control. This result is in accordance with physicochemical analysis as viscosity increased in samples with YAW. Most of the respondents experienced an aftertaste with both of the products tested. Most found that the aftertaste of the control sample was *acceptable* in comparison with the 45% YAW sample which had a much more pronounced aftertaste, which was not well liked.

To improve the product, the respondents suggested increasing the fruitiness of the flavor and decreasing the sharper *lingering* aftertaste that was also described as *astringent* and *building.* In terms of consistency respondents recommended a smoother and more homogenous texture and the sample to be less thick.

SHELF LIFE STUDY

In order to determine significant effects of the YAW addition and the concentration of stabilizer on the physicochemical measurements over refrigerated storage time a full factorial design with 4 levels for YAW concentration (35%, 40%, 45% and Control), 2 levels for percentage of stabilizer (0.2% and 0.4%), and 5 levels for storage time (day 0, 10, 20, 30 and 40) was built. A similar full factorial design was used to assess significant effects of concentration of stabilizer (0, 0.2 and 0.4%) and homogenization at a fixed 35% YAW addition over storage time (day 0, 10, 20, 30 and 40).

FYBs were characterized as indicated in tables 8 and 9. Table 8 describes the yogurt beverage samples containing different percentage of YAW and stabilizer while table 9 includes the samples with yogurt beverages containing 35% YAW, different percentage of stabilizer and different treatment (samples were homogenized and non homogenized). Both tables show the values at the start of the shelf-life study.

Table 2.6.Physicochemical[1] characteristics of fruit yogurt beverage samples[2] with varying concentration of yogurt acid whey (average ± standard deviation for n = 3).

Sample	Stabilizer (% w/w)	pH	Soluble Solids °(Brix)	Titratable Acidity	Viscosity (Pa·s)	WHC (%)	L*	a*	b*	C*	Hue
35%	**0.2**	4.25 ± 0.05	15.74 ± 0.24	0.701 ± 0.004	344.52 ± 10.39	94.55 ± 0.81	81.22 ± 0.46	6.77 ± 0.33	20.62 ± 0.46	21.70 ± 0.54	71.83 ± 0.47
40%		4.44 ± 0.02	15.73 ± 0.28	0.690 ± 0.011	281.26 ± 13.24	81.67 ± 1.12	81.75 ± 0.03	6.49 ± 0.13	20.26 ± 0.14	21.27 ± 0.17	72.25 ± 0.21
45%		4.42 ± 0.03	15.09 ± 0.22	0.689 ± 0.003	181.15 ± 2.34	82.78 ± 6.56	81.29 ± 0.01	6.24 ± 0.04	20.45 ± 0.01	21.38 ± 0.02	73.03 ± 0.09
Control		4.24 ± 0.04	12.14 ± 0.15	0.538 ± 0.005	184.51 ± 2.34	63.95 ± 0.83	82.07 ± 0.04	6.11 ± 0.10	19.62 ± 0.06	20.55 ± 0.08	72.69 ± 0.23
35%	**0.4**	4.36 ± 0.02	16.19 ± 0.05	0.712 ± 0.003	1857.27 ± 49.10	100.00 ± 0.00	81.23 ± 0.06	7.0 ± 0.06	21.41 ± 0.10	22.54 ± 0.10	71.75 ± 0.08
40%		4.48 ± 0.03	16.19 ± 0.09	0.695 ± 0.003	1446.4 ± 74.7	100.00 ± 0.00	81.34 ± 0.04	6.80 ± 0.09	20.88 ± 0.09	21.96 ± 0.11	71.96 ± 0.15
45%		4.40 ± 0.04	14.7 ± 0.3	0.684 ± 0.002	547.7 ± 10.3	100.00 ± 0.00	81.06 ± 0.04	6.41 ± 0.05	20.66 ± 0.06	21.64 ± 0.07	72.76 ± 0.09
Control		4.47 ± 0.09	12.76 ± 0.27	0.507 ± 0.002	521.17 ± 5.45	100.00 ± 0.00	81.88 ± 0.02	6.85 ± 0.01	20.00 ± 0.03	21.14 ± 0.03	71.10 ± 0.01

[1] Evaluated at time zero
[2] Samples 35, 40 and 45 %= contains 35, 40 and 45% yogurt acid whey; Control=contains 45% water instead of yogurt acid whey
WHC = water holding capacity

Increasing the concentration of YAW did not have a marked effect on pH, soluble solids (slight decrease), acidity (slight decrease) or color. Viscosity and water holding capacity (WHC) significantly decreased with the incremental addition of YAW, both important parameters for consumer acceptability. To determine the effect of YAW addition on the acceptability and stability of the yogurt beverage, samples with 45% YAW or 45% water (control) were compared. As expected, the control sample had lower soluble solids and lower acidity, and decreased WHC. Doubling the concentration of stabilizer from 0.2 to 0.4% had a very large effect on viscosity, resulting in 3-5 times higher viscosity values, and providing 100% WHC for all samples. Results seem to indicate that lower concentrations would have been more appropriate as the samples were too thick for a yogurt beverage.

Homogenization decreased the viscosity and improved the WHC (>80%) of all samples, thus it is recommended for the production of yogurt beverages.

Table 2.7 Physicochemical[1] characteristics of fruit yogurt beverage samples[2] treated with or without homogenization (average ± standard deviation for n = 3)

% acid whey	% of Stabilizer	pH	Soluble Solids °(Brix)	Titratable Acidity	Viscosity (Pa·s)	WHC (%)	L*	a*	b*	Chroma	Hue
35% H	**0%**	4.21 ± 0.04	15.67 ± 0.13	0.710 ± 0.002	1852 ± 60	80.07 ± 3.95	81.82 ± 0.07	7.08 ± 0.01	21.05 ± 0.06	22.21 ± 0.05	71.41 ± 0.07
35% NH		4.44 ± 0.06	14.13 ± 0.26	0.695 ± 0.015	1478 ± 52	49.63 ± 7.37	80.41 ± 0.04	6.75 ± 0.08	21.82 ± 0.15	22.84 ± 0.12	72.81 ± 0.28
35% H	**0.2%**	4.25 ± 0.05	15.74 ± 0.24	0.701 ± 0.004	345 ± 10	94.55 ± 0.81	81.22 ± 0.46	6.77 ± 0.33	20.62 ± 0.46	21.70 ± 0.54	71.83 ± 0.47
35% NH		4.40 ± 0.09	14.81 ± 0.09	0.716 ± 0.003	1020 ± 17	60.60 ± 2.40	80.96 ± 0.01	6.49 ± 0.12	21.22 ± 0.18	22.19 ± 0.21	72.99 ± 0.20
35% H	**0.4%**	4.36 ± 0.02	16.19 ± 0.05	0.712 ± 0.003	1857 ± 49	100.00 ± 0.00	81.23 ± 0.06	7.0 ± 0.06	21.41 ± 0.10	22.54 ± 0.10	71.75 ± 0.08
35% NH		4.38 ± 0.06	14.87 ± 0.16	0.703 ± 0.002	3000 ± 57	93.18 ± 3.30	80.96 ± 0.01	6.44 ± 0.04	21.21 ± 0.02	22.17 ± 0.03	73.11 ± 0.09

[1] Evaluated at time zero

[2] Samples 35% H= contains 35% yogurt acid whey and was homogenized; 35% NH= contains 35% yogurt acid whey and was not homogenized

WHC = water holding capacity

To compare the characteristics of the different yogurt beverages with added acid whey against drinkable yogurts available in the marketplace, two commercial samples were evaluated (Table 10). The values measured for one commercial sample (mango) were within the ranges of the yogurt beverage samples tested while the vanilla drinkable yogurt was more acidic, less sweet and more viscous. The vanilla yogurt acidity seemed to be too high and an informal sensory testing of the commercial vanilla yogurt indicated that the yogurt was too tart and too acidic. Janiaski et al., 2016, evaluated Brazilian commercial fermented whey beverages obtaining acidity values of 0.7 g lactic acid/100g, similar to the values obtained in this study. The mango drinkable yogurt viscosity is much closer to the values obtained for the 45% YAW and control samples which were also the most accepted samples to consume as a beverage in the consumer acceptability sensory test performed.

Table 2.8. Physicochemical characterization of two commercial drinkable yogurts. (average ± standard deviation for n = 3).

Sample	pH	Soluble Solids °(Brix)	TA (g/100 g)	Viscosity (Pa·s)	WHC (%)
Vanilla	3.86 ± 0.07	12.21 ± 0.50	1.244 ± 0.021	9696 ± 957	72.00 ± 0.41
Mango	4.24 ± 0.15	14.43 ± 0.20	0.648 ± 0.003	463 ± 18	46.43 ± 3.90

Changes in physicochemical characteristics over the refrigerated storage time

pH

pH values at days 0 and 40 of refrigerated storage are shown in Table 11, varying from 4.07 to 4.48. The pH values are within the expected range for typical drinkable yogurts between 4.0 and 4.5 (Chandan and O'Rell 2013b). It has been previously reported that American consumers prefer mild acidity (pH 4.2-4.4) products (Chandan and O'Rell 2013a). Overall, the pH decreased slightly but significantly over storage time. Similarly, samples with 35% YAW homogenized and non homogenized, had lower pH values after 40 days of refrigerated storage.

As all the samples contained yogurt, it is expected to see a pH decline over time as live LAB slowly continue the fermentation process over time, even at refrigerated conditions (González-Martínez et al. 2002). In general, samples with 0.2% of stabilizer had lower pH values than samples containing 0.4% of stabilizer. Viable cell counts of yogurt organisms can be selected as an index of quality and could be related to the sensory taste of the product during storage. The higher ionic concentration present in all samples containing YAW could influence pH over time as it inhibits starter cultures fermentative process (González-Martínez et al. 2002).

Viability of *Lactobacillus bulgaricus* and *S. thermophilus* was not verified in this study but it would be interesting to do so in order to determine if the different percentage of YAW influences the viability of LAB over time. This might be an interesting characteristic as it could extend the shelf life of products as yogurt, which is usually limited by an excessive progress of acidification (González-Martínez et al. 2002).

Table 2.9. pH[2] values for fruit yogurt beverage samples with different percentage of yogurt acid whey and stabilizer at the beginning and end of the refrigerated shelf-life study (average ± standard deviation for n = 3)[1]

Sample	0.2%		0.4%	
	Day 0	Day 40	Day 0	Day 40
35%	4.25 ± 0.05^{G}	4.23 ± 0.00^{G}	4.36 ± 0.02^{DE}	4.30 ± 0.01^{FG}
40%	4.44 ± 0.02^{ABC}	4.24 ± 0.01^{G}	4.48 ± 0.03^{A}	4.27 ± 0.02^{FG}
45%	4.42 ± 0.03^{BCD}	4.25 ± 0.04^{FG}	4.40 ± 0.04^{CD}	4.31 ± 0.01^{EF}
Control	4.24 ± 0.04^{G}	4.07 ± 0.03^{H}	4.47 ± 0.09^{AB}	4.25 ± 0.02^{EG}

[1]Values not sharing a common superscript letter represent significantly different values ($P < 0.01$) based on full factorial design and mean comparison with t-student
[2] pH measured at 5°C evaluated at time zero and after 40 days of storage

Titratable Acidity

Figure 2.11 shows changes in mean acidity values for samples with increasing percentage of YAW and control over time. Samples containing YAW had higher acidity in comparison with the control due to the added amount of lactic acid contributed by the whey.

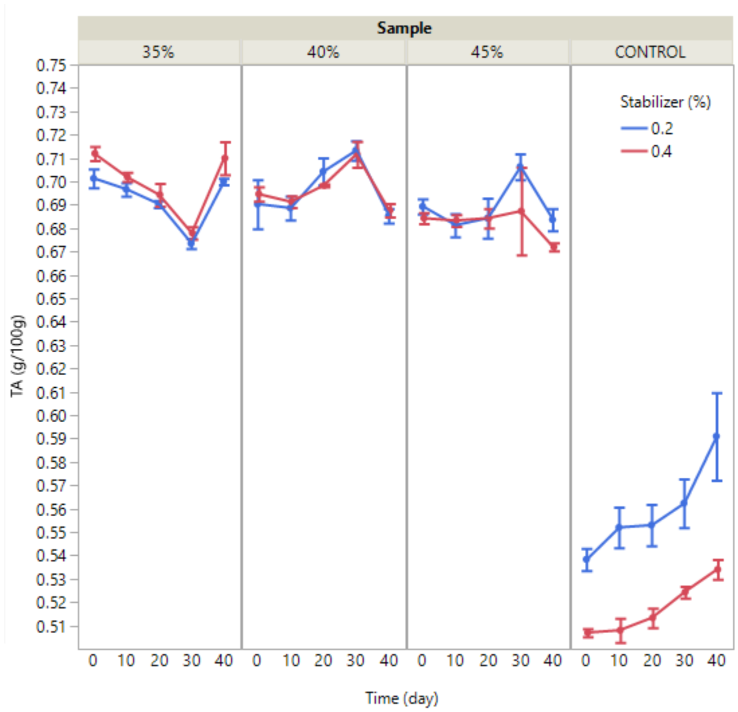

Figure 2.11. Changes in titratable acidity (TA) over refrigerated storage time for samples containing different percentage of yogurt acid whey (35, 40 and 45%) plus a control (45% water) and different levels of stabilizer. Each error bar is constructed using one standard deviation from the mean.

Table 2.10 **s**hows acidity values for samples with different percentage of stabilizer measured at the beginning and end of the refrigerated shelf-life. An increase in acidity over time was expected but this trend was only significant for the control sample ($p<0.01$). The higher ionic strength of samples containing YAW vs. the control likely contributed to inhibiting the activity of the yogurt cultures (González-Martínez et al. 2002).

Table 2.10. Titratable acidity (g lactic acid/100 g) for samples containing different percentage of yogurt acid whey plus a control (45% water) and different percentage of stabilizer evaluated at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

% acid whey	0.2 % stabilizer (% w/w)		0.4% stabilizer (% w/w)	
	Day 0	Day 40	Day 0	Day 40
35%	0.701 ± 0.004^{AB}	0.700 ± 0.001^{ABC}	0.712 ± 0.003^{A}	0.710 ± 0.007^{A}
40%	0.690 ± 0.011^{CDE}	0.685 ± 0.003^{DE}	0.695 ± 0.003^{BCD}	0.688 ± 0.003^{DE}
45%	0.689 ± 0.003^{CDE}	0.684 ± 0.005^{E}	0.684 ± 0.002^{DE}	0.672 ± 0.002^{F}
Control	0.538 ± 0.005^{H}	0.591 ± 0.019^{G}	0.507 ± 0.002^{I}	0.534 ± 0.004^{H}

[1] Values not sharing a common superscript letter represent significantly different values (P < 0.01) based on full factorial design and mean comparison with t-student

Mean acidity values for samples with 35% YAW with and without homogenization are displayed in table 2.11 Only one sample showed a minor but significant decrease in acidty over storage time (p<0.01).

Table 2.11. Titratable acidity (g lactic acid/100 g) for samples containing 35% yogurt acid whey, different percentage of stabilizer, homogenized and non homogenized, measured at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	Stabilizer (% w/w)	Storage Day	
		0	40
35% Homogenized	**0.00**	0.710 ± 0.002^{ABC}	0.714 ± 0.001^{A}
35% Non Homogenized		0.695 ± 0.015^{DE}	0.689 ± 0.005^{E}
35% Homogenized	**0.20**	0.701 ± 0.004^{CD}	0.700 ± 0.001^{CDE}
35% Non Homogenized		0.716 ± 0.003^{A}	0.678 ± 0.008^{F}
35% Homogenized	**0.40**	0.712 ± 0.003^{AB}	0.710 ± 0.007^{ABC}
35% Non Homogenized		0.703 ± 0.002^{BCD}	0.708 ± 0.006^{ABC}

[1] Values not sharing a common superscript letter represent significantly different values (P < 0.01) based on full factorial design and mean comparison with t-student

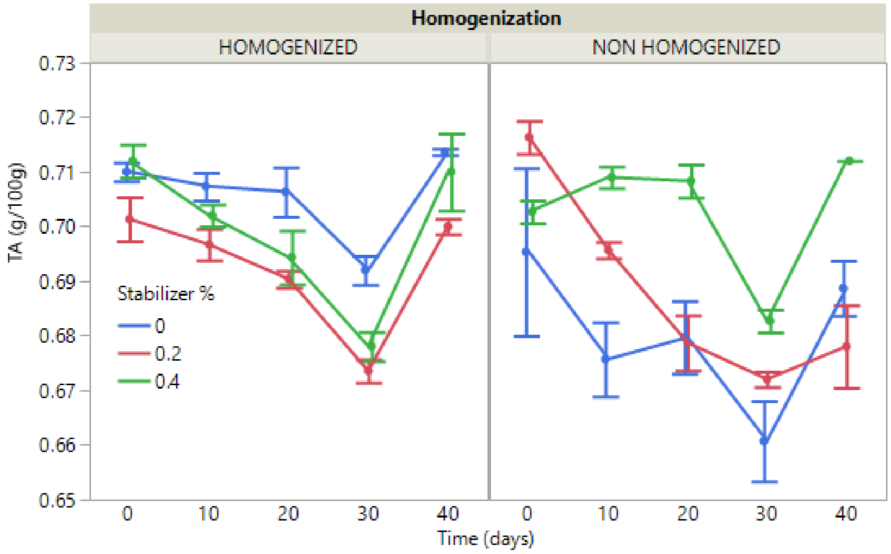

Figure 2.12. Changes in titratable acidity (TA) over refrigerated storage time for samples containing 35% yogurt acid whey, and different percentage of stabilizer, homogenized and non homogenized. Each error bar is constructed using one standard deviation from the mean.

Water holding capacity (WHC)

Whey can be expulsed from the yogurt network becoming visible as surface whey and impacting negatively consumer's perception (Lee and Lucey 2010). Spontaneous syneresis, which is the contraction of a gel without the application of any external forces (e.g., centrifugation), is related to instability of the gel network (Lucey, Munro, and Singh 1998). When drinking yogurt, a common practice is to shake the package before opening, making sure the product is homogenous and hence the consumer does not notice whey in the surface of the product. Consequently we did not measure spontaneous syneresis but WHC. Whey separation can be problematic in this type of beverages and the addition of stabilizer is normally necessary in order to prevent it (Foley and Mulcahy 1989; Tamime and Robinson 2007).

Results for changes in WHC over storage time are shown in Figures 13 and 14. As the measurements were done on individual samples every sampling time, a substantial sample to sample variation was observed (large standard deviations). WHC was significantly affected by percentage of YAW, stabilizer concentration, homogenization and their respective interactions. Storage time was not significant for either set of samples. Samples with 0.4% stabilizer had higher WHC than samples with 0.2% and 0% respectively. Same trend was seen for the non homogenized samples although WHC values were significantly smaller than homogenized samples.

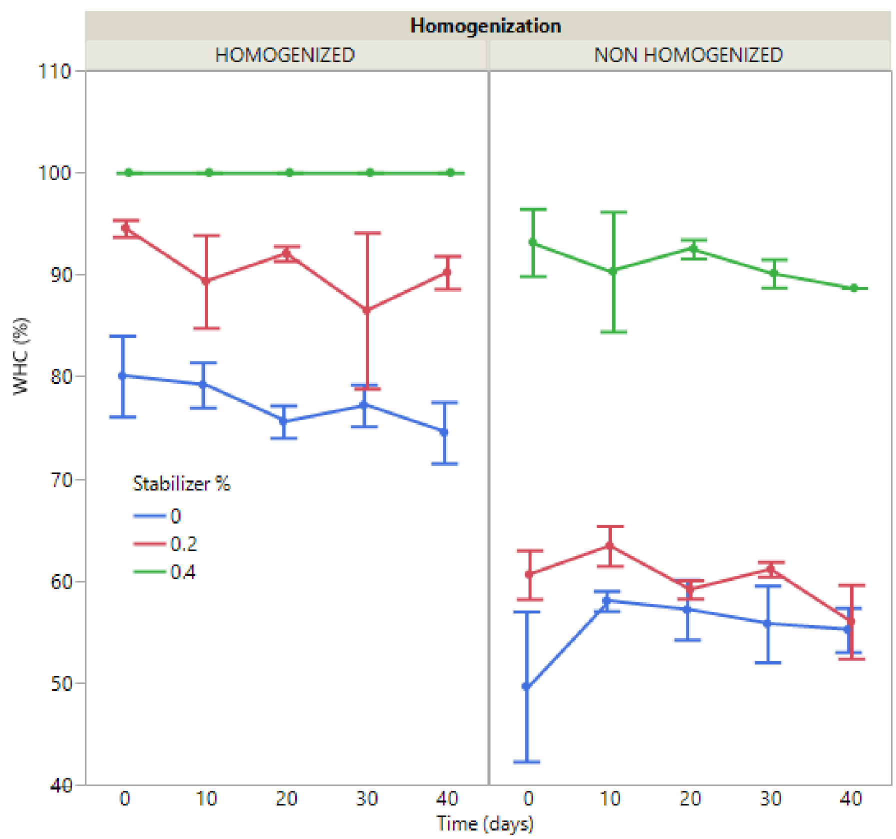

Figure 2.**13.** Changes in water holding capacity (WHC) over refrigerated storage time for samples containing 35% yogurt acid whey, and different percentage of stabilizer, homogenized and non homogenized. Each error bar is constructed using one standard deviation from the mean.

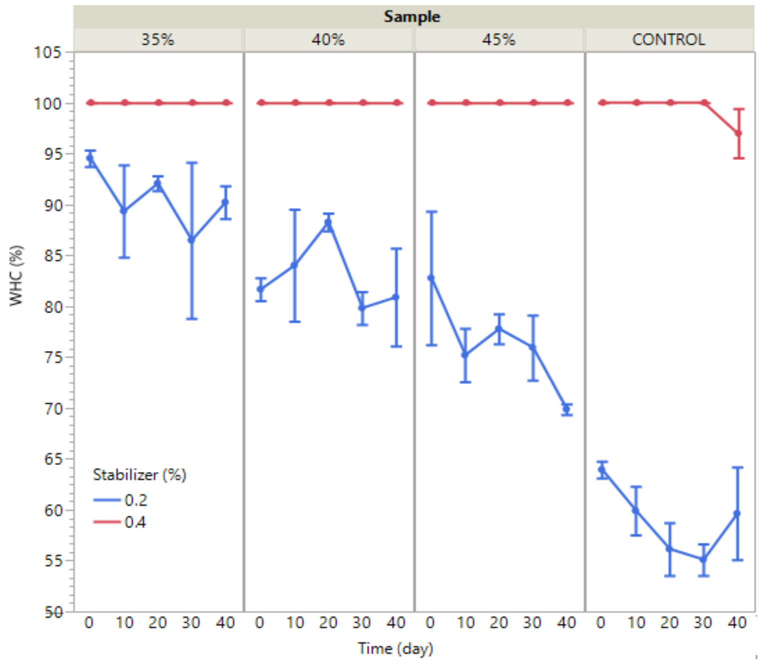

Figure 2.14. Changes in water holding capacity (WHC) over refrigerated storage time for samples containing different percentage of yogurt acid whey plus a control (45% water) and different percentage of stabilizer. Each error bar is constructed using one standard deviation from the mean.

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Table 2.12. Water holding capacity for samples containing 35% yogurt acid whey, different percentage of stabilizer (homogenized and non homogenized, measured at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	Stabilizer (% w/w)	Storage day 0	Storage day 40
35% Homogenized	**0.0**	80.07 ± 3.95^{C}	74.52 ± 3.00^{C}
35% Non Homogenized		49.63 ± 7.37^{E}	55.19 ± 2.16^{DE}
35% Homogenized	**0.2**	94.55 ± 0.81^{AB}	90.23 ± 1.61^{B}
35% Non Homogenized		60.60 ± 2.40^{D}	55.98 ± 3.62^{D}
35% Homogenized	**0.4**	100.00 ± 0.00^{A}	100.00 ± 0.00^{A}
35% Non Homogenized		93.18 ± 3.30^{B}	83.26 ± 5.46^{B}

[1]Values not sharing a common superscript letter represent significantly different values (P < 0.01) based on full factorial design and mean comparison with t-student

As can be seen in table 2.12, WHC did not change significantly over time regardless of the stabilizer level used. Homogenized samples had significantly higher WHC values compared to non homogenized samples, as the process reduces whey separation and improves stability (Lee and Lucey 2010).

All samples with 0.4% stabilizer had 100% WHC throughout the shelf-life study except for the control which had a significant decrease in WHC at 40 days.

Table 2.13. Water holding capacity for samples containing different percentage of yogurt acid whey plus a control (45% water) and different percentage of stabilizer evaluated at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

% acid whey	Stabilizer (% w/w)	Storage Day	
		0	40
35%	**0.2**	94.55 ± 0.81^{BC}	90.23 ± 1.61^{C}
40%		81.67 ± 1.12^{D}	80.9 ± 4.80^{D}
45%		82.78 ± 6.56^{D}	69.88 ± 0.52^{E}
Control		63.95 ± 0.83^{F}	59.65 ± 4.55^{G}
35%	**0.4**	100.00 ± 0.00^{A}	100.00 ± 0.00^{A}
40%		100.00 ± 0.00^{A}	100.00 ± 0.00^{A}
45%		100.00 ± 0.00^{A}	100.00 ± 0.00^{A}
Control		100.00 ± 0.00^{A}	96.99 ± 2.42A^{B}

[a] Values not sharing a common superscript letter represent significantly different values (P < 0.01) based on full factorial design and mean comparison with t-student

Samples with 0.2% of stabilizer and 35-40% YAW did not show significant differences for WHC values measured at the beginning and at the end of the storage time. Samples containing 45% YAW or 45% water (control) showed significant decreases in WHC over time (see table 2.13) as the higher YAW content implies less yogurt in the formulation and therefore less gel structure to retain whey. Moreover all samples containing YAW had higher WHC values than the control, indicating that the whey reduces syneresis.

Viscosity

Viscosity mean values over time can be visualized in figures 2.15 and 2.16. It is clear that viscosity was influenced by percentage of YAW, stabilizer content, homogenization, storage time and their respective interactions (P <0.01).

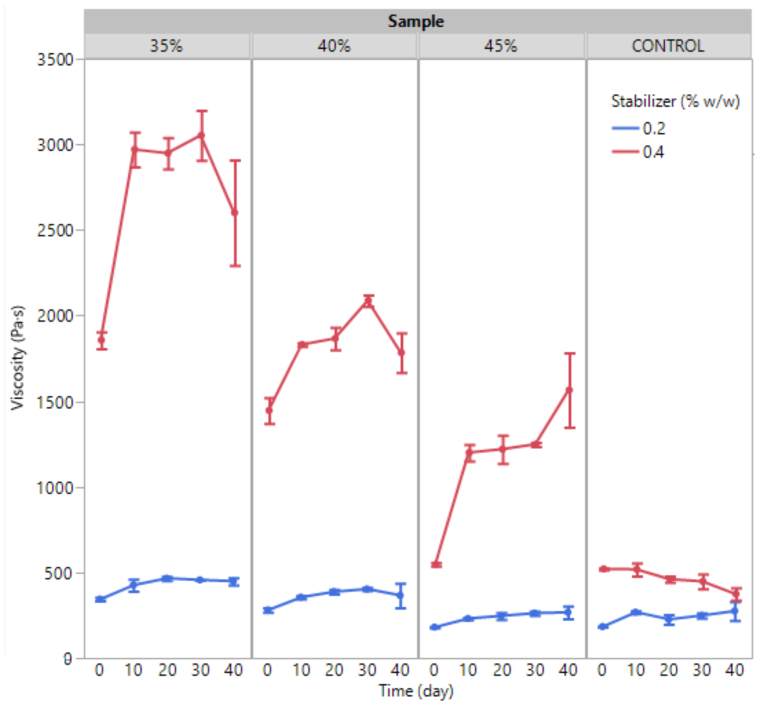

Figure 2.15. Changes in viscosity over refrigerated storage time for samples containing different percentage of yogurt acid whey plus a control (45% water) and different percentage of stabilizer. Each error bar is constructed using one standard deviation from the mean.

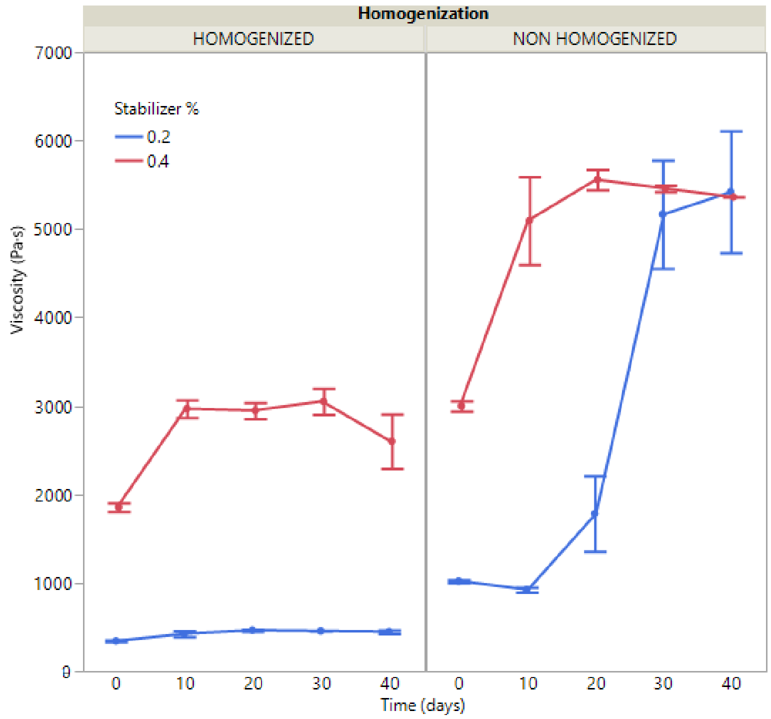

Figure 2.16. Changes in viscosity over refrigerated storage time for samples containing 35% yogurt acid whey and different percentage of stabilizer, homogenized and non homogenized. Each error bar is constructed using one standard deviation from the mean.

Mean values of samples at the beginning and end of the shelf-life are shown in Tables 2.14 and 2.15 Viscosity values for beverages with 0.2% stabilizer were significantly lower than for samples containing 0.4% stabilizer. For samples containing 0.2% of stabilizer, viscosity values increased over time but the differences between first and last day of storage time were not significant. For samples with 0.4% stabilizer, viscosity increased significantly over time, except for the control which showed no significant change (Table 2.14).

Table 2.14. Viscosity values for samples containing different percentage of yogurt acid whey plus a control (45% water) and different percentage of stabilizer evaluated at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	Stabilizer (% w/w)	Sotrage Day	
		0	40
35%	0.20	344.52 ± 10.39^{FG}	449.12 ± 21.44^{DEF}
40%		281.26 ± 13.24^{EGH}	366.22 ± 71.36^{EFG}
45%		181.15 ± 2.34^{H}	267.60 ± 37.85^{GH}
Control		184.51 ± 2.34^{H}	275.27 ± 55.82^{FGH}
35%	0.40	1857.27 ± 49.10^{B}	2601.60 ± 307.73^{A}
40%		1446.40 ± 74.67^{C}	1784.18 ± 115.36^{B}
45%		547.74 ± 10.30^{D}	1565.86 ± 218.10^{C}
Control		521.17 ± 5.45^{DE}	375.02 ± 36.52^{DEFG}

[1] Values not sharing a common superscript letter represent significantly different values (P < 0.01) based on full factorial design and mean comparison with t-student

For samples with no stabilizer, homogenized and non homogenized, viscosity increased significantly over time. For homogenized samples with 0.2% and 0.4% stabilizer the increase in viscosity over time was not significant but it was significant for non homogenized beverages.

Table 2.15. Viscosity values for samples containing 35% yogurt acid whey, different percentage of stabilizer, homogenized and non homogenized, measured at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	Stabilizer (% w/w)	Storage Day	
		0	40
35% Homogenized	0.20	344.52 ± 10.39^{F}	449.12 ± 21.44^{EF}
35% Non Homogenized		1020.16 ± 16.93^{DEF}	5422.93 ± 691.51^{A}
35% Homogenized	0.40	1857.27 ± 49.10^{CD}	2601.60 ± 307.73^{BC}
35% Non Homogenized		3000.53 ± 57.27^{B}	4968.47 ± 444.81^{A}

[a] Values not sharing a common superscript letter represent significantly different values (P < 0.01) based on full factorial design and mean comparison with t-student.

Note: Control samples containing 35% acid whey and 0% stabilizer were analyzed but due to experimental error, viscosity results for these samples were dismissed.

Soluble solids

Changes in soluble solids over time can be seen in Tables 2.16 and 2.17. For samples containing 35%, 40%, 45% added YAW and the control, percentage of YAW was the most significant factor followed by the interaction between percentage YAW and stabilizer. There were not significant changes over time except for the sample with 40% YAW and 0.2% stabilizer which showed a slight decrease.

Table 2.16. Soluble solids (°Brix) for samples containing different percentage of acid plus a control (45% water) and different percentage of stabilizer evaluated at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

% acid whey	Stabilizer (% w/w)	Storage Day	
		0	40
35%	**0.20**	15.74 ± 0.24^{B}	15.54 ± 0.73^{BC}
40%		15.73 ± 0.28^{B}	15.28 ± 0.18^{CD}
45%		15.09 ± 0.22^{CDE}	14.90 ± 0.19^{DE}
Control		12.14 ± 0.15^{G}	12.09 ± 0.34^{G}
35%	**0.40**	16.19 ± 0.05^{A}	16.19 ± 0.20^{A}
40%		16.19 ± 0.09^{BC}	15.75 ± 0.20^{B}
45%		14.73 ± 0.3^{E}	15.07 ± 0.14^{CDE}
Control		12.76 ± 0.27^{F}	12.34 ± 0.74^{F}

[1]Values not sharing a common superscript letter represent significantly different values ($P < 0.01$) based on full factorial design and mean comparison with t-student.

Table 2.17. Soluble solids (°Brix) for samples containing different percentage of acid plus a control (45% water) and different for samples containing 35% yogurt acid whey, different percentage of stabilizer, homogenized and non homogenized, measured at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	Stabilizer (% w/w)	Storage Day	
		0	40
35% Homogenized	0.00	15.67 ± 0.13^{C}	15.78 ± 0.15^{BC}
35% Non Homogenized		14.13 ± 0.26^{E}	14.43 ± 0.10^{DE}
35% Homogenized	0.20	15.74 ± 0.24^{C}	15.54 ± 0.73^{C}
35% Non Homogenized		14.81 ± 0.09^{D}	14.75 ± 0.43^{D}
35% Homogenized	0.40	16.19 ± 0.05^{AB}	16.19 ± 0.20^{A}
35% Non Homogenized		14.87 ± 0.16^{D}	14.83 ± 0.08^{D}

[1] Values not sharing a common superscript letter represent significantly different values ($P < 0.01$) based on full factorial design and mean comparison with t-student

Color

In products like yogurt, color is a very important attribute and it is influenced by the ingredients used (Aryana and Mcgrew 2007). Mango yogurt was common to all of the samples and light orange was the prevalent color among all samples. Color components for the yogurt beverage samples are shown in Tables 2.18 and 2.19. Even though significant differences were found based on composition and treatment, all samples looked very similar and is was not possible for the consumers to differentiate samples based on color alone.

Table 2.18. Color components for samples containing different percentage of yogurt acid whey plus a control (45% water) and different percentage of stabilizer evaluated at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	stabilizer (%)	L*		a*		b*		C*		Hue	
		Day of Measurement		Day of Measurement		Day of Measurement		Day of Measurement		Day of Measurement	
		0	40	0	40	0	40	0	40	0	40
35%	0.20	81.22 ± 0.46^{GH}	81.87 ± 0.85^{C}	6.77 ± 0.33^{C}	7.23 ± 0.08^{A}	20.62 ± 0.46^{FGH}	21.52 ± 0.20^{AB}	21.70 ± 0.54^{FG}	22.64 ± 0.24^{AB}	71.83 ± 0.47^{EFGH}	71.94 ± 0.87^{GHI}
40%		81.75 ± 0.03^{F}	82.63 ± 0.07^{B}	6.49 ± 0.13^{DEFG}	6.71 ± 0.11^{CDE}	20.26 ± 0.14^{IJ}	20.86 ± 0.01^{DEF}	21.27 ± 0.17^{H}	21.92 ± 0.03^{EF}	72.25 ± 0.21^{CDE}	72.17 ± 0.27^{DEF}
45%		81.29 ± 0.01^{G}	82.29 ± 0.09^{C}	6.24 ± 0.04^{GH}	6.50 ± 0.29^{DEF}	20.45 ± 0.01^{GHI}	20.93 ± 0.21^{DE}	21.38 ± 0.02^{GH}	21.92 ± 0.29^{EF}	73.03 ± 0.09^{A}	72.74 ± 0.57^{AB}
Control		82.07 ± 0.04^{DE}	83.05 ± 0.25^{A}	6.11 ± 0.10^{H}	6.47 ± 0.13^{EFG}	19.62 ± 0.06^{K}	20.41 ± 0.11^{GHI}	20.55 ± 0.08^{I}	21.41 ± 0.11^{GH}	72.69 ± 0.23^{ABC}	72.42 ± 0.36^{BCD}
35%	0.40	81.23 ± 0.06^{GH}	82.29 ± 0.04^{C}	7.0 ± 0.06^{AB}	7.30 ± 0.05^{A}	21.41 ± 0.10^{B}	21.85 ± 0.06^{A}	22.54 ± 0.10^{BC}	23.03 ± 0.05^{A}	71.75 ± 0.08^{FGH}	71.53 ± 0.17^{GHI}
40%		81.34 ± 0.04^{G}	82.37 ± 0.03^{C}	6.80 ± 0.09^{BC}	6.82 ± 0.28^{BC}	20.88 ± 0.09^{DEF}	21.22 ± 0.27^{BC}	21.96 ± 0.11^{DEF}	22.29 ± 0.33^{CD}	71.96 ± 0.15^{DEFG}	72.18 ± 0.52^{DEF}
45%		81.06 ± 0.04^{H}	81.97 ± 0.03^{E}	6.41 ± 0.05^{FG}	6.73 ± 0.03^{CD}	20.66 ± 0.06^{EFG}	21.08 ± 0.05^{CD}	21.64 ± 0.07^{FG}	22.13 ± 0.04^{DE}	72.76 ± 0.09^{AB}	72.28 ± 0.08^{BCDE}
Control		81.88 ± 0.02^{EF}	82.82 ± 0.28^{A}	6.85 ± 0.01^{BC}	6.88 ± 0.07^{BC}	20.00 ± 0.03^{J}	20.25 ± 0.15^{HI}	21.14 ± 0.03^{H}	21.34 ± 0.22^{GH}	71.10 ± 0.01^{I}	71.30 ± 0.13^{HI}

[1] Values not sharing a common superscript letter represent significantly different values ($P < 0.01$) based on full factorial design and mean comparison with t-student

Table 2.19. Color components for samples containing different percentage of yogurt acid whey plus a control (45% water), different percentage of stabilizer, homogenized (H) and non homogenized (NH), measured at time zero and after 40 days of refrigerated storage (average ± standard deviation for n = 3).[1]

Sample	Stabilizer (%)	L*		a*		b*		C*		Hue	
		Day of Measurement		Day of Measurement		Day of Measurement		Day of Measurement		Day of Measurement	
		0	40	0	40	0	40	0	40	0	40
35% H	0.00	81.82 ± 0.07^{CD}	82.86 ± 0.07^{A}	7.08 ± 0.01^{B}	7.31 ± 0.06^{A}	21.05 ± 0.06^{D}	21.72 ± 0.06^{B}	22.21 ± 0.05^{DE}	22.91 ± 0.06^{AB}	71.41 ± 0.07^{E}	71.40 ± 0.17^{E}
35% NH		80.41 ± 0.04^{H}	81.58 ± 0.11^{DE}	6.75 ± 0.08^{C}	6.68 ± 0.09^{CD}	21.82 ± 0.15^{B}	22.13 ± 0.05^{A}	22.84 ± 0.12^{ABC}	23.12 ± 0.07^{A}	72.81 ± 0.28^{C}	73.21 ± 0.20^{AB}
35% H	0.20	81.22 ± 0.46^{G}	81.87 ± 0.85^{B}	6.77 ± 0.33^{C}	7.23 ± 0.08^{AB}	20.62 ± 0.46^{E}	21.52 ± 0.20^{BC}	21.70 ± 0.54^{F}	22.64 ± 0.24^{BC}	71.83 ± 0.47^{D}	71.94 ± 0.87^{DE}
35% NH		80.96 ± 0.01^{G}	81.52 ± 0.25^{EF}	6.49 ± 0.12^{DE}	6.30 ± 0.11^{E}	21.22 ± 0.18^{CD}	21.38 ± 0.06^{C}	22.19 ± 0.21^{E}	22.29 ± 0.08^{DE}	72.99 ± 0.20^{BC}	73.57 ± 0.23^{A}
35% H	0.40	81.23 ± 0.06^{FG}	82.29 ± 0.04^{B}	7.0 ± 0.06^{B}	7.30 ± 0.05^{AB}	21.41 ± 0.10^{C}	21.85 ± 0.06^{AB}	22.54 ± 0.10^{CD}	23.03 ± 0.05^{AB}	71.75 ± 0.08^{DE}	71.53 ± 0.17^{DE}
35% NH		80.96 ± 0.01^{G}	82.03 ± 0.13^{BC}	6.44 ± 0.04^{E}	6.62 ± 0.04^{CDE}	21.21 ± 0.02^{CD}	21.53 ± 0.16^{BC}	22.17 ± 0.03^{E}	22.53 ± 0.15^{BCDE}	73.11 ± 0.09^{BC}	72.91 ± 0.16^{ABC}

[1] Values not sharing a common superscript letter represent significantly different values ($P < 0.01$) based on full factorial design and mean comparison with t-student

Particle Size

Particle size distribution was evaluated after 10 days of refrigerated storage, in samples containing 35% YAW with 0, 0.2 and 0.4% stabilizer, homogenized and non homogenized, to assess differences based on experimental conditions. As expected, homogenized samples had smaller particles and tighter size distribution than non homogenized samples regardless of the concentration of stabilizer (Figures 2.17, 2.18 and 2.19), thus improving physical stability over time and providing a smoother mouthfeel.

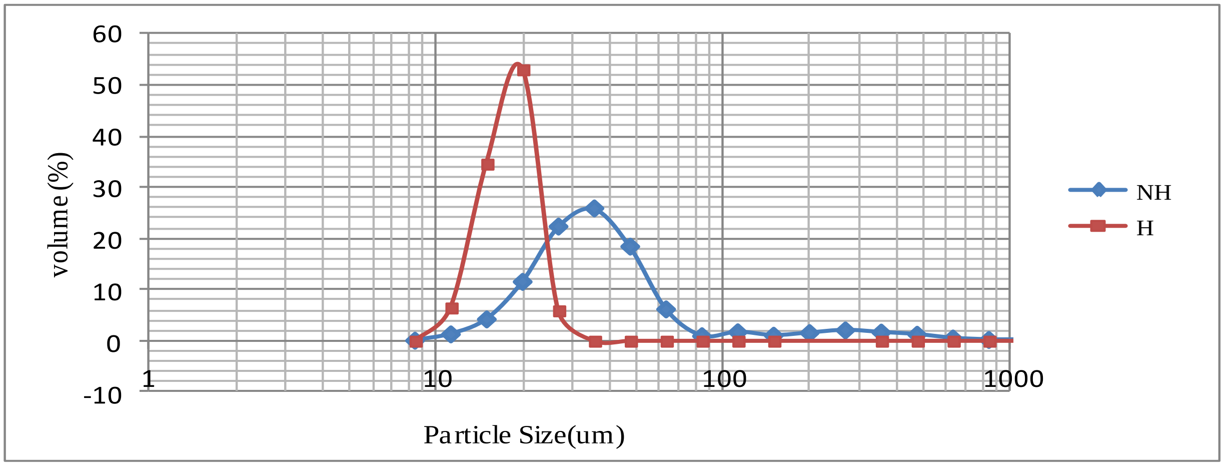

Figure 2.17. Particle size distribution for fruit yogurt beverage samples containing 35% yogurt acid whey and 0.2% stabilizer, homogenized (H) and non homogenized (NH). Evaluated after 10 days of refrigerated storage.

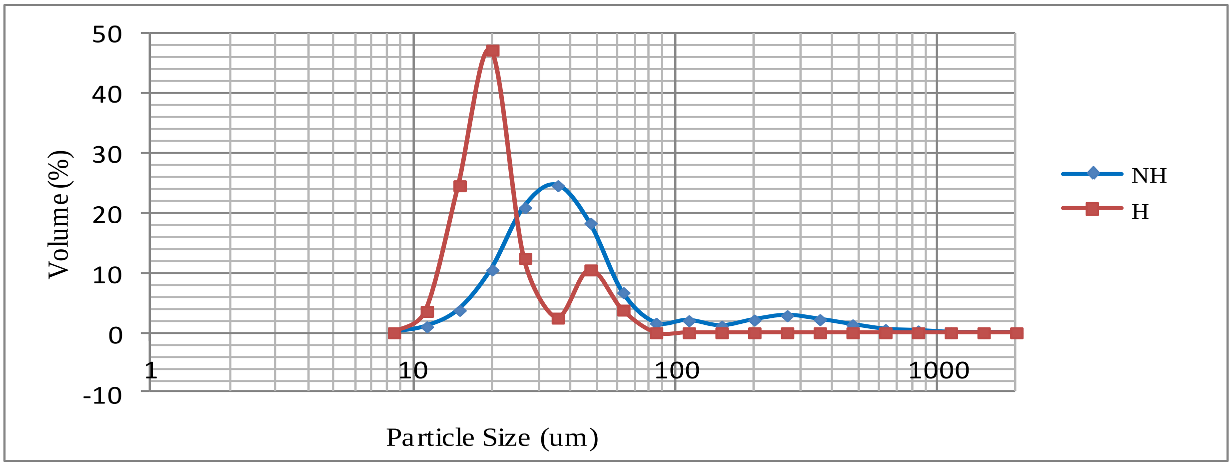

Figure 2.18. Particle size distribution for fruit yogurt beverage samples containing 35% yogurt acid whey and 0.4% stabilizer, homogenized (H) and non homogenized (NH). Evaluated after 10 days of refrigerated storage.

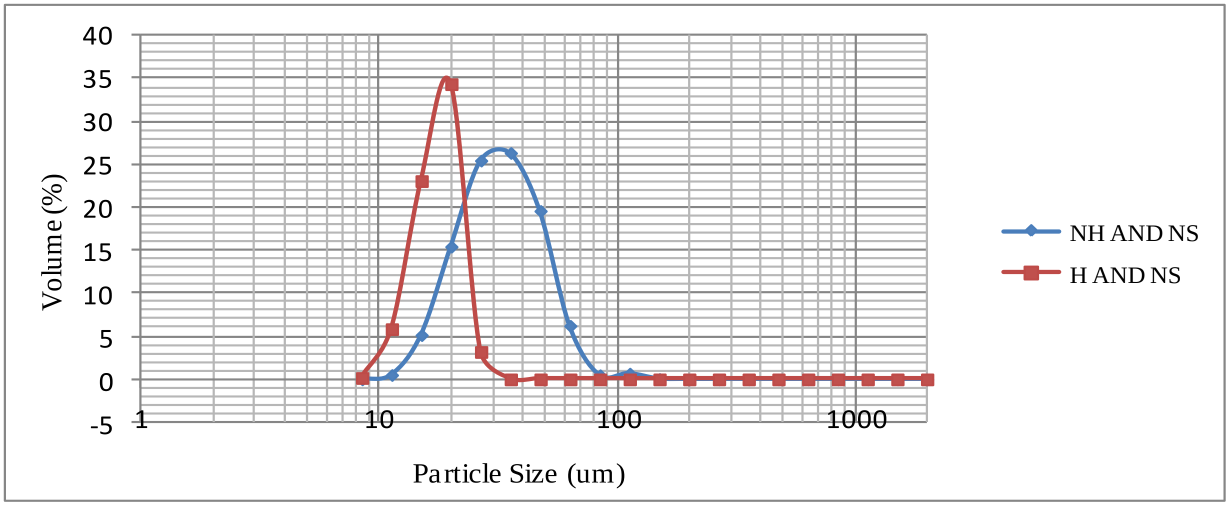

Figure 2.19. Particle size distribution for fruit yogurt beverage samples containing 35% yogurt acid whey and no stabilizer (NS=No stabilizer), homogenized (H) and non homogenized (NH). Evaluated after 10 days of refrigerated storage.

Table 2.20 shows the average particle size and width or span of the particle size distribution at the beginning and at the end of the shelf-life study. Homogenization had a significant effect by producing smaller particles and reduced span with minimum changes over the 40 days of refrigerated storage.

Table 2.20. Changes in particle size and distribution span over refrigerated shelf-life for fruit yogurt beverage samples containing 35% yogurt acid whey, homogenized and non homogenized, with different concentrations of stabilizer (average ± standard deviation for n = 3).

Treatment	**Stabilizer %w/w**	**Day 1** Diameter µm	**Day 1** Span µm	**Day 40** Diameter µm	**Day 40** Span µm
Homogenized	0	20.97 ± 0.05	0.507 ± 0.003	21.015 ± 0.006	0.505 ± 0.001
	0.2	21.75 ± 0.06	0.47 ± 0.01	33.16 ± 18.81	1.07 ± 1.03
	0.4	34.64 ± 0.61	1.62 ± 0.05	34.24 ± 0.16	1.67 ± 0.01
Non Homogenized	0	50.18 ± 0.08	1.32 ± 0.02	59.67 ± 6.09	1.51 ± 0.12
	0.2	71.26 ± 8.34	1.99 ± 0.59	72.58 ± 4.23	2.64 ± 0.19
	0.4	72.23 ± 2.84	2.63 ± 0.15	82.88 ± 5.91	4.46 ± 0.48

CONCLUSIONS

In this study, we were able to determine consumer acceptability through sensory tests for FYB samples containing YAW as part of the formulation. In addition, we were able to assess that consumers do not object to a FYB formulated with YAW as there was no significant difference from the preference test between freshly prepared samples with 45% YAW or 45% water (control),

FYBs formulated with 25-45% YAW varied in perceived texture, a key attribute for a drinkable product, from too thick to too thin, with the 35% sample preferred overall. Minor changes in formulation and processing can be applied to optimize the beverage to meet consumers' expectations. Based on the results of this study, 35-45% YAW is the acceptable range to formulate FYBs. Regarding acid whey distinctive flavor, consumers accepted the beverage and did not object to its aftertaste, an encouraging result for the food industry interested in acid whey utilization.

The limited focus group evaluation performed with samples after 40 days of refrigerated storage seems to indicate that samples with 45% YAW or 45% water were acceptable to drink, but the 45% YAW was dominated by heavy fermented dairy notes and lingering sour/astringent aftertaste. As these descriptors were not perceived by consumers when testing the fresh samples, a formal sensory study should be performed every 7-10 days to determine the end of the shelf life.

In this Study we were able to measure physicochemical characteristics of refrigerated FYB over time. Increasing the concentration of YAW did not have a marked effect on pH, soluble solids (slight decrease), acidity (slight decrease) or color. Viscosity and water WHC significantly decreased with the incremental addition of YAW, both important parameters for consumer acceptability. The control sample had lower soluble solids and lower acidity, and decreased WHC. Doubling the concentration of stabilizer from 0.2 to 0.4% had a very large effect on viscosity, resulting in 3-5 times higher viscosity values, and providing 100% WHC for all samples. Results seem to indicate that lower concentrations would have been more appropriate as the samples were too thick for a yogurt beverage.

Homogenization is recommended for the production of yogurt beverages as it decreased the viscosity and improved the WHC (>80%) of all samples. Moreover, homogenized samples had smaller particles and tighter size distribution than non homogenized samples regardless of the concentration of stabilizer, improving physical stability over time and providing a smoother mouthfeel.

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CHAPTER 3

Development of an Acid Whey-Based Sports Drink Beverage

INTRODUCTION

Whey beverages

Acid whey has the potential to be used as the main component of beverages because of its nutritional composition. Moreover, beverages with numerous applications and functionalities can be formulated with whey and whey based products (Chavan et al. 2015)

Whey beverages usually refer to beverages in which the main component is whey in its liquid form, excluding those that have acid whey components such as lactose and whey protein (Jelen 2009). Even though dairy whey seems to be an ideal raw material for creating nutritious dairy beverages, whey-based beverages are not popular in the market place and constitute a small segment of the global market (Jelen 2009). The successful acid whey beverage, that has been in the market since 1952 is the Swiss product Rivella, manufactured by a specialized beverage manufacturing company, and formulated as 1/3 whey, water and fruit juice (Jelen 2009).

Developing whey beverages through fermentation and/or without its one of the most attractive approaches for the utilization of whey (Yadav, Yadav, and Kalia 2010). Sady et al. 2013 developed an orange juice beverage containing acid whey, a by-product of twarog (quark) cheese production. The sensory evaluation showed that the beverage with acid whey had a negative effect on taste and odour compared to the control (same beverage without acid whey), being more noticeable after six month of storage with an overall liking 2-10% lower than the control;

nevertheless, in a 5 point scale for overall sensory quality, the scores obtained were very good to good.

Sayd et al. 2013, developed a refrigerated functional beverage containing dry sweet whey from cheese production (6 g of spray dried whey in 100 ml distilled water), mango powder, flax seed oil and pectin. Researchers reported that the beverage developed was an excellent source of antioxidants and that color, flavor and taste were stable during 15 days of storage.

A whey-based banana herbal beverage containing mint extract was prepared by Yadav et al, 2010. Whey was obtained in the lab by filtering milk through cheese cloth after heating, acidifying with citric acid and stirring until complete coagulation. Percentage of whey used in the beverage was 80% (whey pH = 5.5). They reported desirable consumer acceptability after 15 days of refrigeration without any chemical preservatives. Baccouche et al, 2013 reported on the physicochemical stability of whey-based prickly pear beverage utilizing approximately 50% whey from mozzarella processing (whey pH= 4.7-4.9), but did not perform any sensory studies to determine the acceptability of the beverages. Chatterjee et al., 2015 developed whey based orange beverages utilizing concentrated whey. Whey was obtained after acidifying milk with 2% calcium lactate solution, stirring until complete coagulation and filtering with cheese cloth. Concentrated whey was prepared in a rotary vacuum pan evaporator at 50°C to about 50% of original volume. Researchers of this study stated that using concentrated whey helps removing typical whey off-flavor, improving the sensory quality of the beverage. A sensory evaluation study helped to determine that the optimal beverage formulation has a 3 to 2 ratio of concentrated liquid whey and orange juice respectively. The beverage remained in good condition for up to 11 days at room temperature and up to 3 months under refrigeration with addition of sodium benzoate. Singh et al. 2014, use lactose hydrolyzed whey from the manufacture of cheddar cheese and paneer to develop a lemon based whey beverage with more than 80% hydrolyzed whey. The pH of the beverage was adjusted to 3.8. Researchers from this study determined the most acceptable formula for the beverage although no shelf life study was performed. They obtained a maximum of 85-90% hydrolysis in whey stating that the sweetness of whey due to lactose hydrolysis would help its utilization in dairy products such as flavoured milk, fermented milk and whey-based fruit beverages. Dhamsaniya et al 2013, developed a whey based banana and *Mentha arvensis* beverage. They obtained the whey following a similar procedure as stated above by Yadav et al. 2010. The beverage contained 15 ml banana juice, 3 ml M. arvensis extract, 8 g sugar powder and 77 ml milk whey per 100 ml and researchers stated having optimum physicochemical characteristics and organoleptic quality.

Sports drinks

The main objectives when developing a sports beverage are to avoid dehydration, provide carbohydrates to increase available energy, replenish electrolytes lost because of excessive sweating, be in compliance with regulatory requirements and last but not least, develop a highly palatable beverage (Coombes and Hamilton 2000). Sports drinks are mainly composed by water, carbohydrates (low carbohydrate concentration <10%, or high carbohydrate concentration >10%) and electrolytes. Electrolytes such as sodium, potassium and chloride are added not only to create an isotonic beverage with respect to the plasma, but also to improve palatability (Coombes and Hamilton 2000). In addition, a Mintel consumer survey (Sport drink, Mintel database, Sept 2012) reported that consumers' priorities when purchasing sport drinks are mainly flavor, brand, size and price.

According to "Nutritional and performance drinks", Mintel database, January 2015, dollar sales of nutritional and performance drinks grew by 42% from 2009-2014, to reach an estimated $11.5 billion. It is expected that the category of sports drinks, performance drinks, and nutritional drinks will remain a solid performer because of continued interest in health, convenience and function.

Acid whey contains water, electrolytes and carbohydrate and hence, seems appropriate to be used in the development of natural and clean label sports drink beverages. Top claims for sports drinks in 2011 were functional benefits, environmentally-friendly packaging, no additives/preservatives and "all natural" (Sport drink, Mintel database, Sept 2012) which can be aligned with using acid whey, a by-product from Greek style yogurt (GSY) production.

However, the current beverage market is extremely competitive and it will not be easy for a beverage to be accepted by consumers if it does not satisfy desirable sensory quality, thirst-quenching effectiveness, favorable price and positive health image (Chavan et al. 2015).

The objective of this study was to develop a yogurt acid whey based, clean label sports drink that matches the nutrition profile of commercial sports drink and determine consumer acceptability in a formal sensory study.

MATERIALS AND METHODS

Ingredients and materials

Yogurt acid whey (YAW) from GSY processing was provided by Byrne Hollow Farm (Cortland, NY). YAW was pasteurized at 72°C for 15 seconds in compliance with Grade "A" Milk Ordinance provided by FDA and stored at 4° C until use.

Shelf-stable Organic Lime Juice was purchased at a local grocery store (Santa Cruz Natural Incorporated, CA, USA).

Mint was purchased at a local grocery store. Mint leaves were extracted with 96% ethanol at room temperature (ratio 1:1). After 24 h the liquid from extraction was filtered through cheesecloth and ethanol was evaporated in a rotavap (Buchi Rotavapor R-200, New Castle, USA) at 54°C to preserve flavor. Volume was reduced to achieve 6X concentration. Finally, the mint extract was filtered through Whatman filter paper N°41 and stored at 4°C until use.

Ginger root was purchased at a local grocery store. After peeling and washing, the ginger was juiced. 5% lime juice was added to the ginger juice to avoid browning reactions and was stored at 4°C until use.

Simple syrup was prepared by adding water and sugar in the same proportion until sugar was completely dissolved.

Chemicals

NaOH 0.1N standard solution (VWR analytical, Radnor, PA, USA).

Buffer, reference standard pH 7.00 ± 0.01 and pH 4.00 ± 0.01 (VWR analytical, Radnor, PA, USA).

Physicochemical measurements

pH

pH was measured at 5°C using a Thermo Scientific Orion 2 Star pH meter (Thermo Fisher Scientific, Beverly, MA) calibrated using standardized pH 4.00 and 7.00 buffers (VWR analytical, Radnor, PA, USA).

Titratable Acidity

Titratable acidity (TA) was determined using a G20 compact titrator (Mettler Toledo, Schwerzenbach, Switzerland). TA was expressed as g of lactic acid/100 g of sample and was obtained by titrating 10 g of sample diluted with 50 ml of deionised water with 0.1N NaOH.

Soluble solids

The soluble solids content were determined as degree Brix with a Leica Auto Abbe refractometer model 10500-802 (Leica Inc., Buffalo, NY).

Beverage formulation and Sample preparation

A variety of fruit juices, fresh herbs, and aromatic extracts were combined with 40-90% YAW to determine flavor compatibility. Beverages with flavors from blueberry-lime to pineapple-cucumber were filtered through 25 μm pore filter paper, pasteurized to 85°C, cooled in ice water baths, and informally tested for flavor and aroma. Post-pasteurization, all beverages were turbid, with varying degrees of sedimentation and off-odors. Switching from YAW from full-fat yogurt to zero-fat yogurt reduced turbidity and sediment formation, eliminated off odor from fat oxidation and increased shelf-stability. Even though zero-fat YAW had a milder odor than the full-fat, the distinctive aroma of dairy remained in all formulations. To address this challenge, prototypes with infusions of cilantro, mint (leaves and extract) and ginger root were tested to mask the diary notes and to compliment the fruit juice flavor profile. The best formulation with the highest percentage of YAW is shown in Table 3.1.

To evaluate the acceptability of the formulated beverage, a formal sensory test was conducted. Samples for sensory evaluation were prepared one day in advanced. Ingredients were weighted following the proportions listed in table 3.1 and immediately vacuum filtered using Whatman paper N° 41. Afterwards, the samples were heated in a kettle at 74°C to pasteurize the drink, hot packed in sterile bottles and cooled down in an ice bath. After preparation, samples were stored at 4°C until use.

Table 3.1. Final formulation for a yogurt acid whey-based sports drink beverage.

Ingredients	% w/w
Acid Whey	60.00
Water	19.50
Lime juice	11.00
Simple syrup (50 % sugar/water)	8.00
Ginger juice	0.75
Mint extract	0.75

The samples were served cold at approximately 7°C, in identical 60 ml plastic cups with lids. Water and unsalted crackers were provided for palate cleansing. Each sample was labeled using four-digit random numbers.

Consumers

Consumers were recruited from the Cornell Sensory testing database to participate in a Sports drink sensory test. Participants were scheduled using doodle poll with a maximum of 8 participants each 15 min section. Walk-ins were also allowed in this study as there was no particular requirement to participate except that participant must have no allergies or intolerances to dairy products/ingredients.

Sensory evaluation was conducted at the Cornell Sensory testing facility with 120 participants (69% females and 31% males) between the ages of 18-60.

Test design and Evaluation

Sensory evaluation was conducted following the guidelines and policies of the Cornell Institutional Review Board for Human Participants. RedJade software (RedJade ®, Redwood Shores, CA, USA) was used to design the test, questionnaires and to collect data.

At the beginning of the test, each participant had to accept the participant study agreement in order to continue with the study. In this agreement, participants had a description of the research, risk and benefits of performing the study, confidentiality protection and contact emails in case of any concern.

The study consisted of an acceptability test and each participant had to rate the sports drink on a 9-point hedonic scale for four liking attributes (overall liking, color, aroma and flavor) and on a Just About Right (JAR) 5-point scale for three attributes (sweetness, tartness and serving temperature). In addition, the study also included questions about aftertaste acceptability, acid whey familiarity, purchase intent before and after reading the ingredient list for the sports drink from this study and from a commercial lemon-lime sports drink. Participants were also asked to complete demographic questions and received a $5 reward for their participation.

Statistical Analysis

RedJade software (RedJade ®, Redwood Shores, CA, USA) was used to analyse data. Means were compared using Tukey's test. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The physicochemical characteristics for the acid whey-based (AWB) sports drink developed in this study and a commercial lemon-lime sports drink are shown in Table 23. The AWB sports drink had 2.5 times higher TA than the commercial sports beverage as it contained lactic acid from YAW plus citric acid from lime juice, whereas the commercial drink only had citric acid added. Even though the AWB sports drink had significantly higher acidity, the pH was higher due to the buffer capacity of the ingredients used. The amount of lime juice added at 11% was selected to aid in balancing the dairy flavor notes of the YAW and to match the requirements for acidity in lemonade of at least 0.7 g/100 mL (21CFR146.120).

Table 3.2. Physicochemical characterization of the sports drink beverage containing yogurt acid whey, water, lime, sugar, ginger and mint, and a commercial lemon-lime flavored sports drink (mean ± standard deviation, n = 3).

Sample	Titratable acidity (g of citric acid/100 g)	pH	Soluble Solids (°Brix)
Sports drink from study	0.7810 ± 0.0006	3.38 ± 0.06	9.32 ± 0.04
Commercial sports drink	0.2990 ± 0.0006	2.99 ± 0.05	6.37 ± 0.02

Figure 3.1 compares nutritional labels for both sports drinks, showing similar caloric content (150 kcal for AWB sports drink vs. 130 kcal for commercial). Total carbohydrate content was higher for the AWB sports drink (13 % vs. 11%) but since both drinks were above 10% total carbohydrates, they are considered to have high carbohydrate concentration (Coombes and Hamilton 2000).

Sodium was significantly lower for the AWB sports drink (135 mg vs. 270 mg). Coombes and Hamilton, 2000 compared many popular sports drinks' nutritional content and determined that the range of sodium for a beverage of serving size 591 ml is between 57-260 mg. The AWB drink is right in the middle of the range, thus indicating that even a much lower percentage of YAW in the final formulation would meet the expected electrolyte concentration. The AWB sports drink also provided 45% calcium and 35% vitamin C as added benefit.

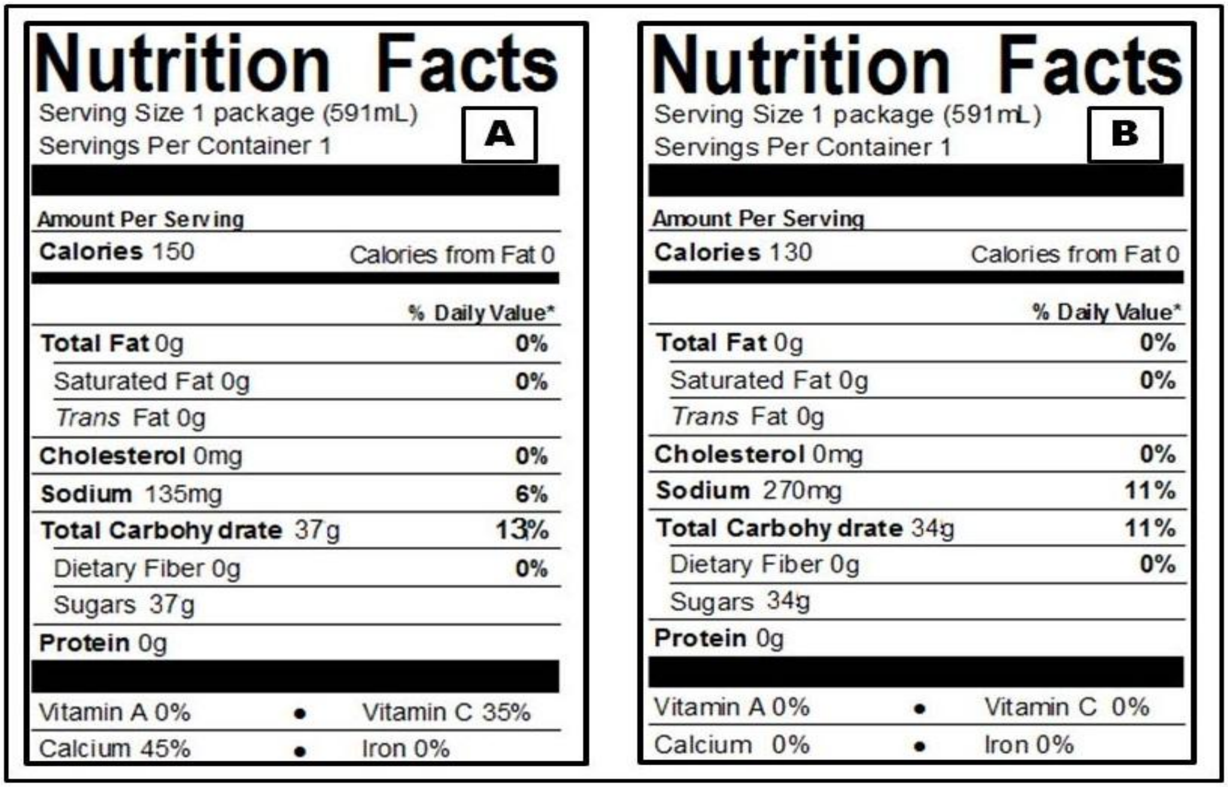

Figure 3.1. Figure A[1] on the left corresponds to Nutritional label of sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint. Figure B on the right corresponds to nutritional label of a commercial lemon-lime flavored sports drink.

[1] Also contains phosphorus 238 mg, potassium 574 mg, magnesium 37 mg and chloride 307 mg

Consumer Acceptability Test

Consumers rated the AWB sports drink on a 9-point hedonic scale for four liking attributes (overall liking, color, aroma and flavor). As can be seen in Figure 3.2, 35% of consumers (n=119) stated to have liked the product overall and 18% of consumers dislike it. “Refreshing” was the most common observation as liking driver from consumers input which correlates with figure 3.3 showing that 47% of consumers agree that the sports drink is refreshing. Only 12% of the consumers disagree with this statement.

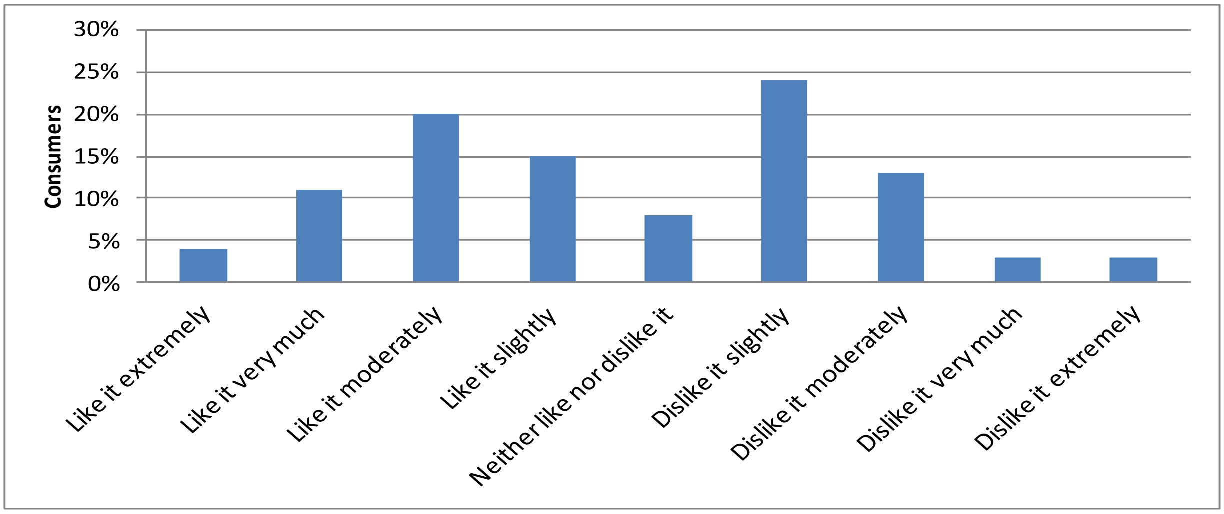

Figure 3.2. Consumer (n=119) response for overall liking on a 9-point hedonic scale (1=dislike extremely to 9=like extremely) of sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

This positive result is key towards the development of this sport drink as "thirst quencher" and "refreshing beverage" are attributes that people who exercise will be seeking when consuming this kind of beverage. Mintel launched a survey for internet users, aged 18+ who consumed any sports drink, and 64% of the respondents stated to consume sport drinks as thirst quencher/refreshing beverages (n=880; ages 18+) ("Sports Drinks", Mintel database September 2012)

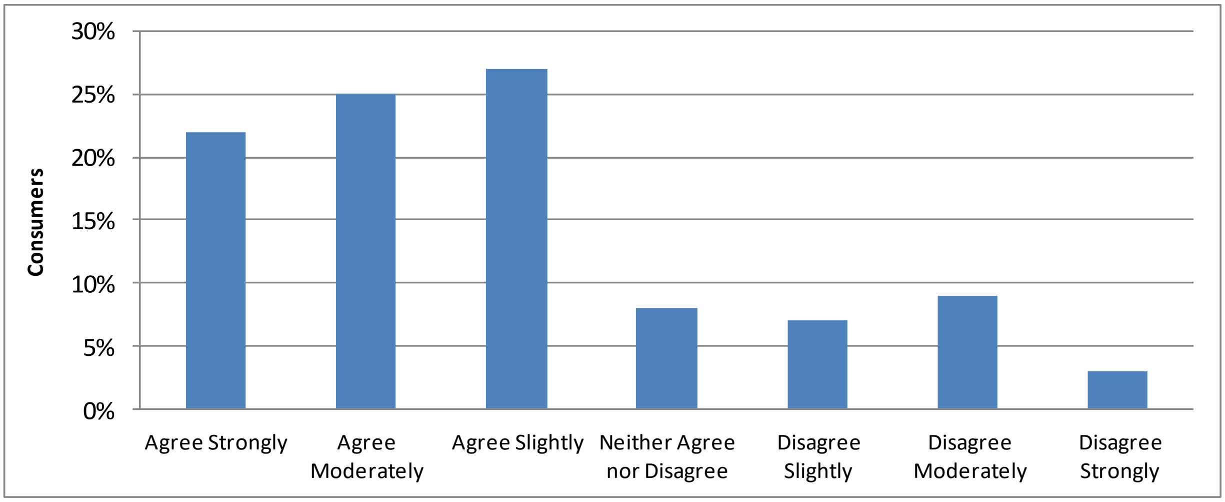

Figure 3.3. Consumer (n=119) response for refreshing sense on a 7-point hedonic scale (1=disagree strongly to 7= agree strongly) of sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

Acid whey has a naturally neon yellow/green color due to its riboflavin (vitamin B6) content, an advantage in the development of the lime based sports drink as there was no need to use artificial colors. Consumers were asked their opinion on a 9-point hedonic scale for color liking without yet knowing the ingredients or the flavor of the beverage.

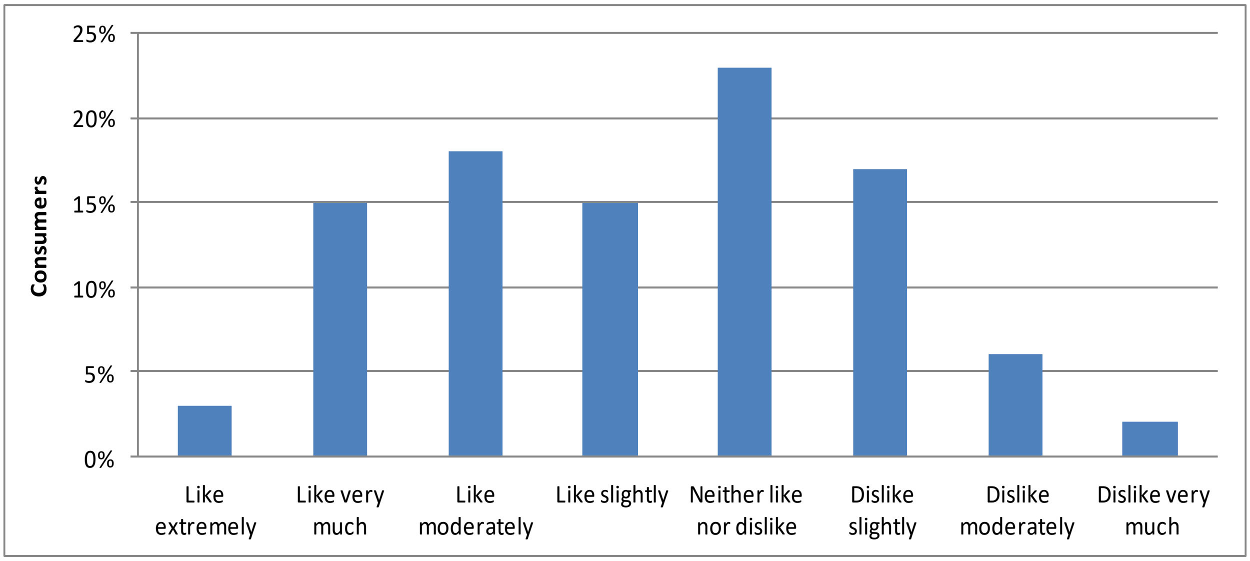

Figure 3.4. Consumer (n=119) response for color liking on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) of sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

As can be seen in Figure 3.4, 36% of consumers liked the color of the sample. Moreover, consumers stated to have ranked the sample positively in the 9-point hedonic scale, because it had the appearance of lemonade and/or commercial lemon base sports drinks.

Even thought it was very challenging to mask the YAW odor in the drink and hence the acceptability of the beverage was uncertain, 46% of consumers (Figure 3.5) stated liking the aroma of the sample and 39% (Figure 3.6) liking the flavor and taste of the sports drink. When developing the beverage, one of the main goals was to mask the dairy aroma and flavor of the YAW. Analysing the consumer answers when asked why they did not like the aroma of the beverage, only 2 (n=119) consumers stated that the sample smelled like sour milk.

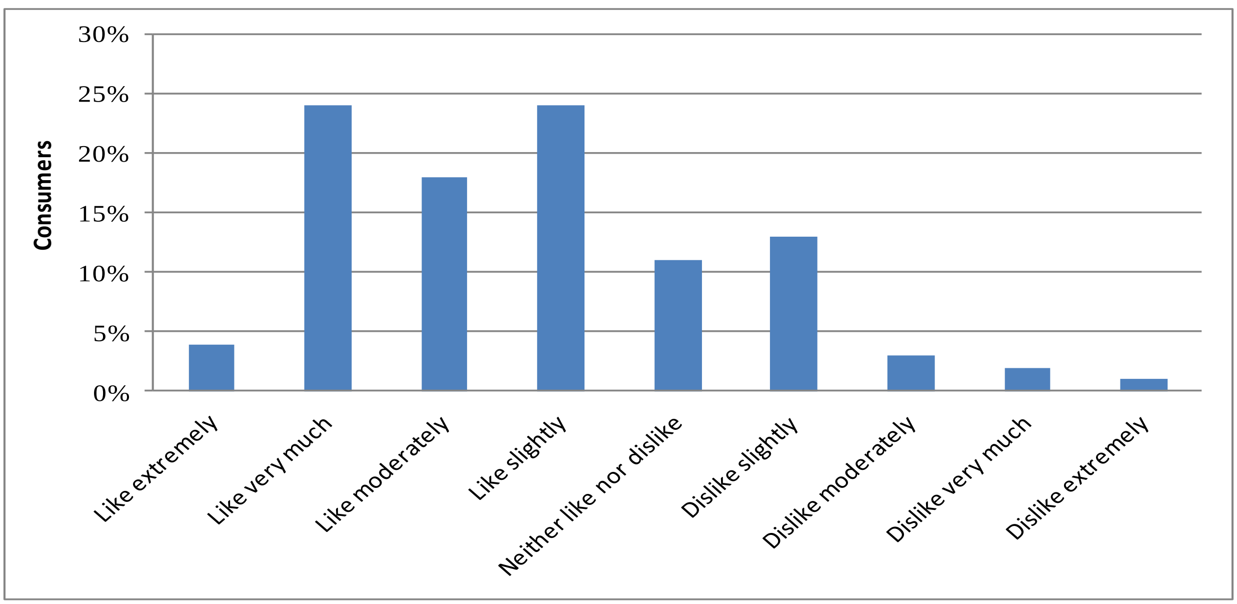

Figure 3.5 Consumer (n=119) response for aroma liking on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) of sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

Additionally, only 8 consumers (< 7%) mentioned dairy when asked to describe the flavor and taste of the sports drink. The small percentage of consumers detecting dairy aroma and flavor was extremely important for the development of this product as consumers do not expect to smell or taste dairy in a shelf stable sports drink.

A variety of fruit juices, fresh herbs and aromatic extracts were combined with YAW to determine flavor compatibility: berries, mango, pineapple, cucumber, ginger, cilantro, mint, among others. From prototyping, it was determined that spearmint was good at masking the dairy aroma and taste. However, we were not able to find a commercial spearmint extract to perform as well as fresh leaves as the commercial extract tested influenced negatively the flavor of the beverage. Consequently we proceeded to prepare a fresh mint extract. Since there are many different types of spearmints, it was difficult to standardize the product but using alcohol as the solvent for extraction worked well. As YAW flavor and aroma is so peculiar and difficult to mask, it would be extremely important and interesting to determine which compounds in spearmint are the responsible for masking the YAW odor.

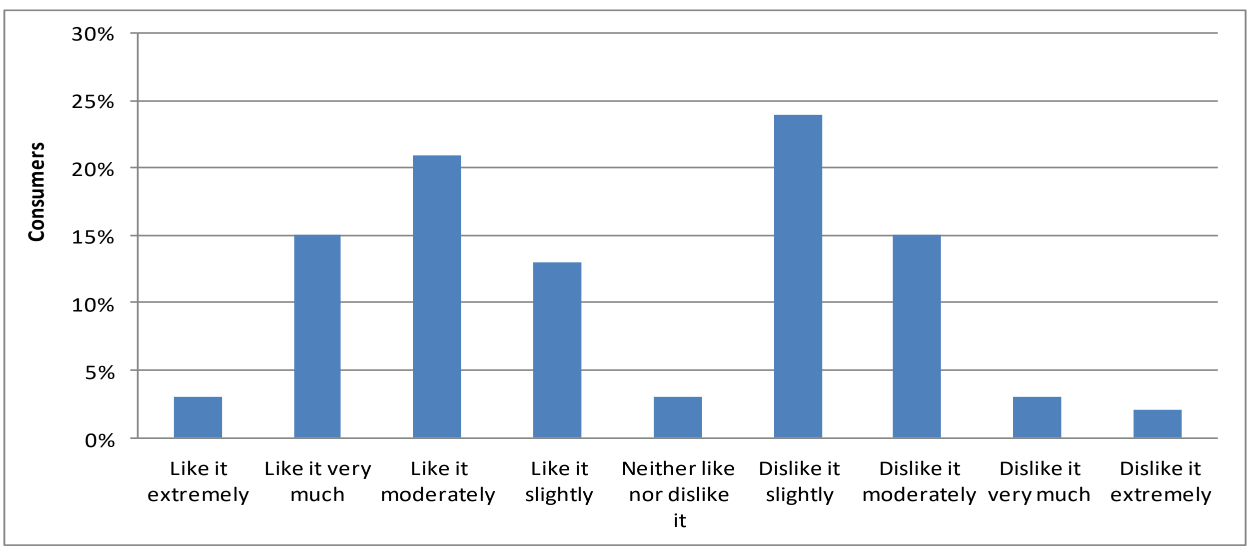

Figure 3.6 Consumer (n=119) response for flavor liking on a 9-point hedonic scale (1=dislike extremely to 9= like extremely) of sport drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

Penalty Analysis

Penalty analysis or mean drop analysis is used by the food industry when developing new products, in order to optimize formulations (Narayanan et al. 2014). Penalty analysis combines just-about-right (JAR) and overall liking tests to correlate a decrease in consumer acceptance to attributes that are not at the JAR level and penalizes products in terms of penalty or deviation from JAR (Lawless and Heymann 2010).

Penalty analysis showed that consumers perceived the sports drink as JAR in Sweetness as shown in figure 3.8.

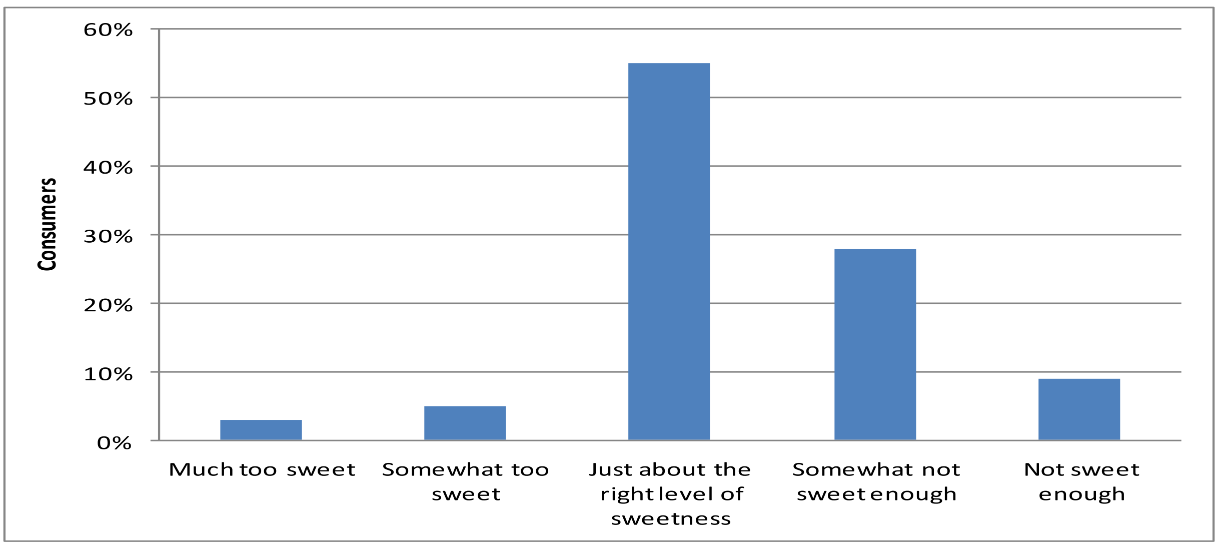

Figure 3.6. Consumer (n=119) response sweetness liking on a Just About Right (JAR) scale for sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

On the other hand, consumers rated the sample as being too tart. This can be better visualised in the assessed penalty graph, figure 3.7. Penalty report plots the mean decrease in overall liking (assessed penalty) versus percentage of not-JAR evaluations (percentage of consumers that rated an attribute as not-JAR) and the preferred attributes are located at the lower left section of the plot (Narayanan et al. 2014). Attributes that received at least 20% responses in any of the not-JAR categories ("much too low" and "somewhat too low" responses) need to be taken into consideration for optimization (Narayanan et al. 2014). Moreover, a penalty of 2.0 in a 9-point scale is considered as a challenge to review in formulation.

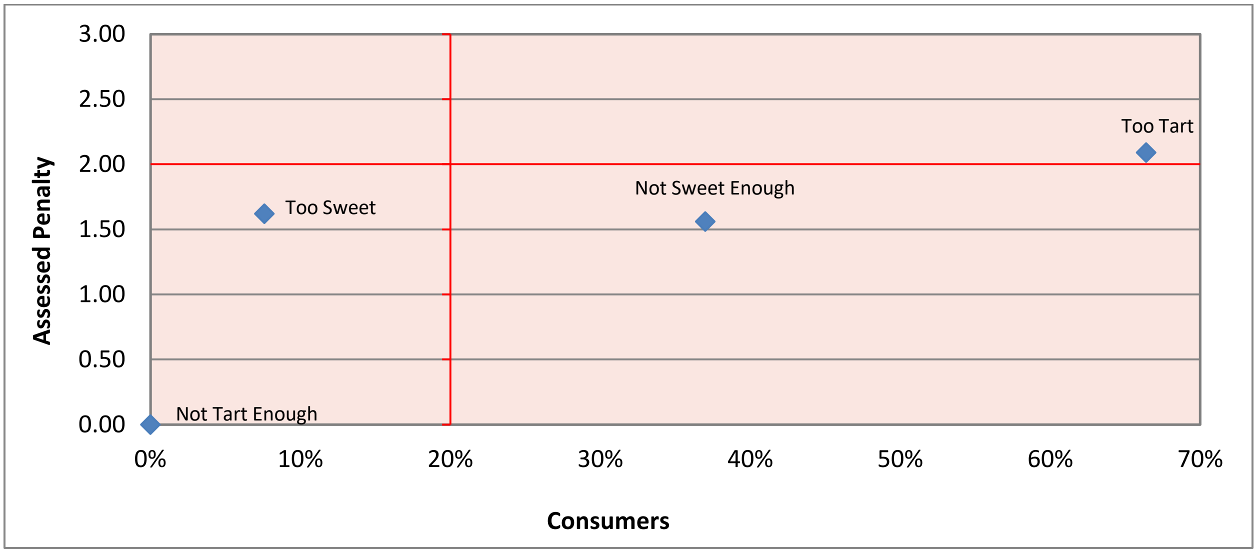

Figure 3.7. Assessed penalty report for sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

As seen in Figure 3.7, approximately 66% of consumers gave a penalty of more than 2.0 for tartness attribute. Tartness is inherent to acid whey and it was a challenging attribute to balance while developing the beverage while trying to mask the YAW taste. The beverage flavor selected was lime which also contributed to the tartness of the beverage. When developing the product, many trials with less lime juice were tested but lowering the lime percentage compromised the overall acceptability of the beverage.

Other challenge when developing the beverage was aftertaste. Acid whey is astringent and its lingering acidity dries the tongue, which can be perceived as the opposite of thirst quenching, an expected quality of a sports beverage. Even though there was no significant difference between consumers who did and did not notice an aftertaste ($p<0.05$) there was also no significant difference among consumers who accepted and did not accepted the aftertaste of the sample.

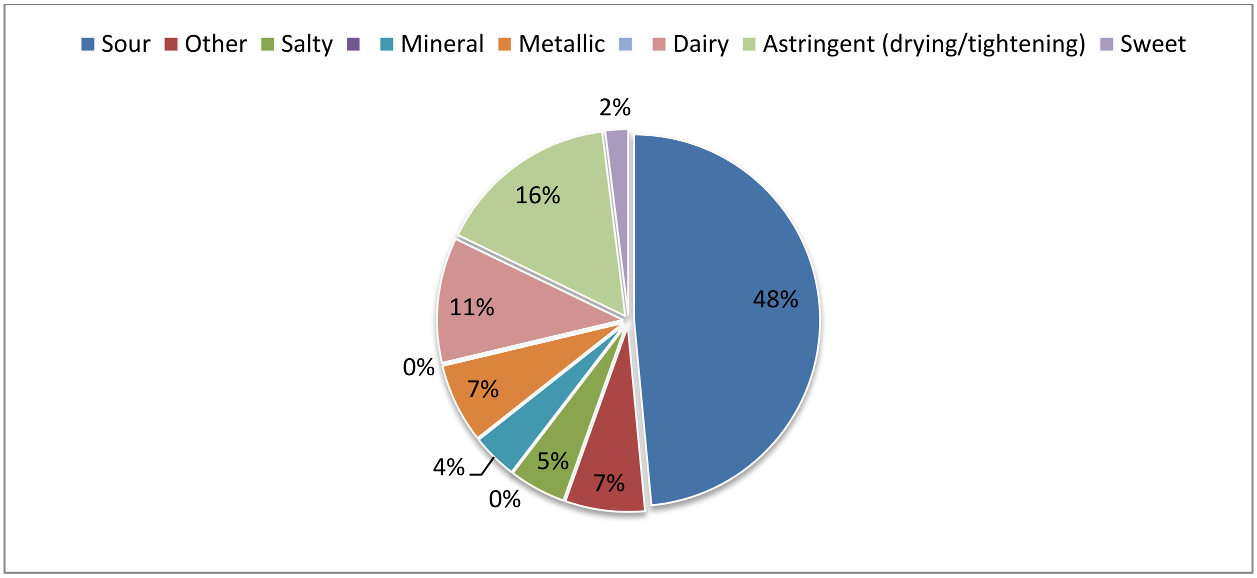

Figure 3.9. Aftertaste descriptors for sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

In Figure 3.9 shows the aftertaste descriptors for the AWB sports drink. As expected, a higher percentage of consumers stated sour to be the best description for the aftertaste, followed by 16% who stated to be astringent. Only 12% of consumers chose dairy as the best descriptor. This result is in accordance with the low percentage of consumers selecting dairy as part of the flavor, taste and aroma as described before. Metallic aftertaste is a common issue in this type of beverages due to salt or mineral content but only 7% stated a metallic aftertaste in the sports beverage.

Informal testing was conducted by tasting the product at room temperature and dairy aromas/flavors were more noticeable than at cold temperature. This issue is not minor as this product should be adequate to be consumed at any temperature, especially considering that consumers might take the sports drink in their gym bags and drink it after exercising.

Purchase Intent

In the study, consumers were told to read a small paragraph stating general information about sports drinks and sports drink benefits. Afterwards, they were asked how likely they would be to purchase this beverage, if it was available to them where they regularly shop at the price they typically pay for a similar beverage.

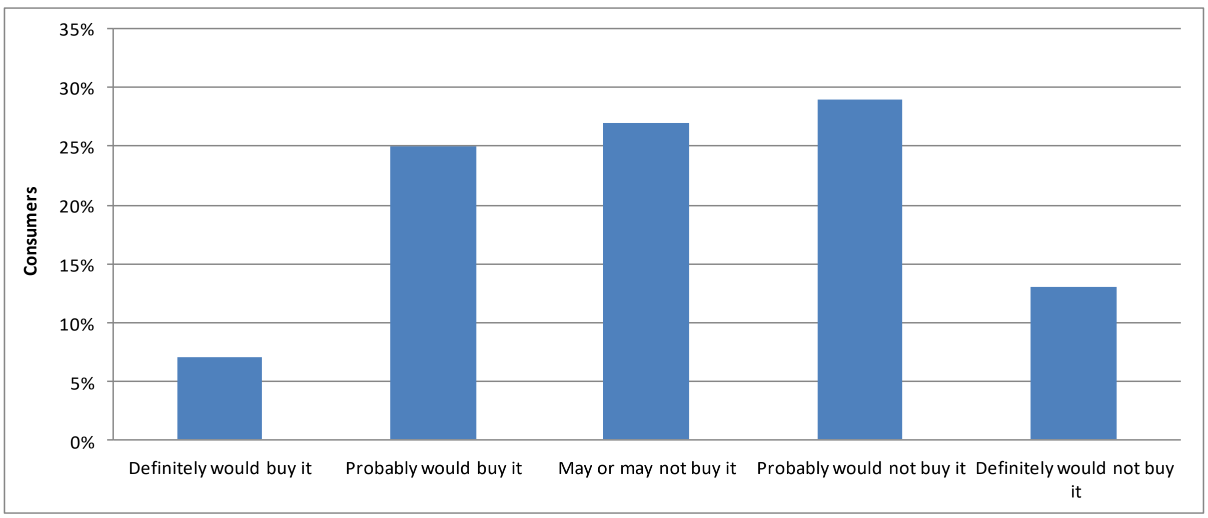

Figure 3.10. Purchase intent before reading ingredient list of beverage for sports drink containing yogurt acid whey, water, lime, sugar, ginger and mint.

Figure 3.10 shows that consumers are slightly more likely to not buy this beverage if available where they typically shop at a reasonable prize. Nevertheless, after this question, consumers were told to read two labels (Figure 3.11). Label A, on the right stated the ingredients of the AWB sports beverage whereas label B, on the left, listed the ingredients of a popular sports drink currently on the market. Afterwards, consumers were asked if they were more or less likely to purchase the AWB sports drink after seeing the label information.

(A)-Ingredient list of the Sports drink you have tasted	**(B)-Ingredient list of a popular sports drink, currently on the market**
Whey, water, organic lime juice, sugar, ginger, natural mint extract	Water, sugar, dextrose, citric acid, natural flavor, salt, sodium citrate, monopotassium phosphate, gum arabic, sucrose acetate isobutyrate, glycerol ester of rosin, yellow 5, yellow 6.

Figure 3.11. Label A: ingredient list of sports drink of the study containing yogurt acid whey, water, lime, sugar, ginger and mint. Label B: Ingredient list of a popular sports drink, currently on the market.

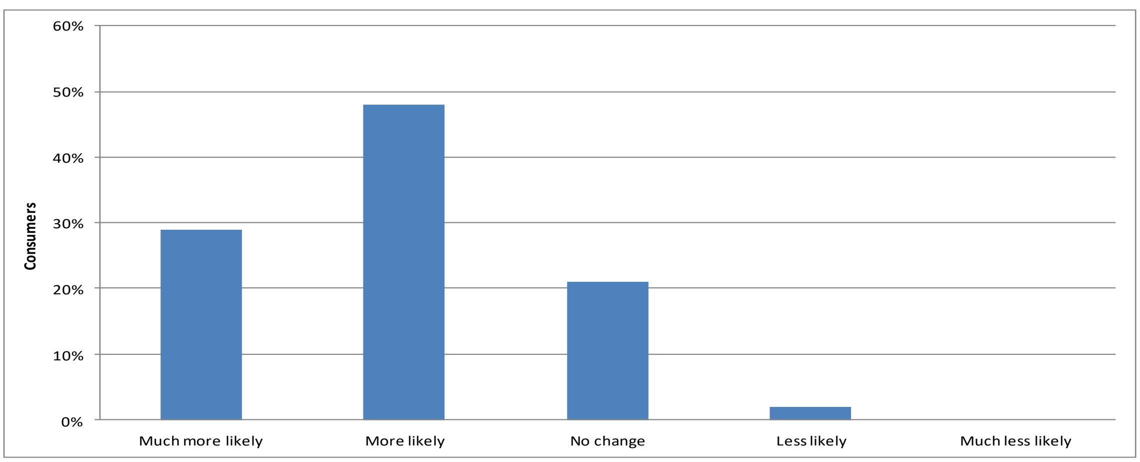

Figure 3.12. Purchase intent after reading ingredient list of sport drink from the study containing yogurt acid whey, water, lime, sugar, ginger and mint and a commercial sport drink.

Figure 3.12 clearly shows that consumers are more likely to buy the AWB sports beverage after comparing the two labels. It is known that clean label is an on-going trend and highly influences consumers' decisions. In addition, Lu Ann Williams, 2016 reported that consumers' awareness for clean and less processed claims are still growing, thus the need for product formulations with natural ingredients.

Most consumers stated appreciating the clean label of the AWB sports beverage in comparison with the currently marketed sports beverage's ingredient list. Moreover, consumers stated that facts as natural ingredients and no chemicals or unknown ingredient names in label would make them more likely to buy this product instead of the other. This information is encouraging and should be taken into consideration to further investigation and development of natural and clean labeled products.

Consumers' Acid Whey Knowledge

Consumers where asked if they were familiar with acid whey and 61% vs 39% were not. They were also asked to state any knowledge about acid whey, and what were their feelings/thoughts about it. Many consumers stated that acid whey is a by-product of a process, without stating which process. Some of them knew that it came from GSY but many others stated it came from cheese or did not mention any process as source of acid whey.

From the answers received, a large percentage of consumers indicated to be in favor of the idea of adding value to a waste product and stated this to be the reason for being more likely to consume the AWB product knowing that it contained acid whey. It is encouraging for food scientists or product developers to know that consumers are receptive to the idea of buying products made from food by-products. Furthermore, after being informed that acid whey was the main ingredient of this beverage, 65% of consumers stated that knowing this did not change the likelihood of purchasing this sports drink and 23% indicated to be more likely to buy the product. Nevertheless, about 10% of these positive reactions were based on assuming a high protein content in the beverage. Whey protein powder mixes and drinks have become popular products among athletes, especially within body builders (Functional Beverages, Mintel database, May 2010) and hence consumers associate whey with high protein content. In GSYprocessing, whey protein denatures because of the high temperatures applied to the milk during the processing, causing the protein to unfold and be part of the gel structure (Smith, Smith, and Drake 2016b). Therefore protein is retained in the yogurt and not in the acid whey, leaving protein content in YAW almost at zero percent.

Tartness Reduction

Preliminary efforts were to reduce the acidity of the beverage included the addition of sodium bicarbonate and powdered whey protein. Perceived astringency and tartness decreased in all informal testing conducted when adding these two ingredients to the beverage. It was determined that 0.08% $NaHCO_3$ or 2% protein powdered lowered TA from 0.75-0.80 to 0.60-0.70 g of citric acid/100 g beverage. Further investigation is needed to optimize the percentage of $NaHCO_3$ and/or protein powder and to evaluate the new beverage formula in a consumer acceptability sensory testing. This preliminary result is encouraging to address the sports beverage perception of being too tart. The beverage could be marketed as a sports drink with protein content. Nonetheless, even though whey protein powder has nutritional potential, it may contribute to undesirable flavors to finished products (Smith, Smith, and Drake 2016b; Wright et al. 2009; Evans et al. 2010; Oltman et al. 2015; Carunchia Whetstine, Croissant, and Drake 2005), increase sedimentation problems during heat treatment (Baccouche et al. 2013), requiring a stabilizer or an extra step in the processing diagram to be incorporated into the formulation.

CONCLUSIONS

In this study we were able to develop a whey based sports drink beverage containing 60% YAW. Nutritional label obtained for this beverage was comparable to the nutritional label of leading sports drinks available in the market. The product’s overall liking was positive for 35% of consumers (n=119) whereas aroma, flavor/taste and color were liked by 46, 39 and 36% of consumers respectively. Consumers likelihood for purchasing the product increased after reading the product ingredient list vs. an already in the market leading sports drink due to consumers attitudes toward clean label and all natural ingredients is continuously growing. Consumers penalized the product as being too tart and further research is needed to overcome this attribute.

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Recommendations and Future work

Findings from the two studies conducted will be useful for the food industry interested in acid whey utilization.

Fruit yogurt beverages containing 35 to 45% yogurt acid whey (YAW) were preferred by consumers. Nevertheless, consumers perceived beverages with 35% YAW as too thick and not acceptable as a drink for a large percentage of consumers. In addition, consumers stated that beverages with 45% YAW were not sweet enough and did not have enough fruity flavor. To overcome this issue, we recommend changes in the formulation by adding fruit juice to beverages containing 35% YAW to decrease the thickness, and mango puree to the ones containing 45% YAW too add more fruity flavor. It would be interesting to further optimize these formulations and evaluate them in a formal sensory study to confirm ideal ranges. In addition, it will be very important to perform a formal sensory study every 7-10 days to determine the end of the shelf life of the final refrigerated product.

The viability of *Lactobacillus bulgaricus* and *S. thermophilus* was not verified in this study but it would be interesting to do so in order to determine if the different percentage of YAW influences the viability and growth of LAB over time. If certain concentration of YAW limits LAB growth, it could be used in the formulation of yogurt beverages with YAW incorporated, as an interesting characteristic to extend the refrigerated shelf life, which is usually limited by an excessive progress of acidification (González-Martínez et al. 2002).

For the YAW based sports drink, it was a real challenge to mask the strong dairy flavor from the addition of 60% YAW. For this reason, this product should be reformulated, reducing the percentage of YAW to 40-50% based on consumer acceptability in a formal sensory study.

Consumers penalized the product for being too tart and further research is needed to overcome this issue. Reducing the YAW concentration will be the first step, followed by optimizing the formulation to achieve the best flavor profile while meeting the basic requirements of a sports drink. Moreover, stability and shelf life of this shelf-stable drink over time should be determined.

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