USE OF STABILIZERS TO IMPROVE THE TEXTURE OF A FERMENTED BEVERAGE ELABORATED FROM MILK AND ACID WHEY

by

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ABSTRACT

Whey-milk kefir type beverages were prepared using proportions of 75% and 50% whey and stabilized using pectin (0.2% w/v) and gelatin (0.4% w/v). Physicochemical and rheological characteristics of the fermented beverages were determined and compared to a control, 0%/100% whey-milk. Rheological measurements showed that the 75%/25% whey-milk kefir beverage stabilized with gelatin had a higher viscosity than the other formulations when compared to the control. A sensory analysis was performed to determine if any differences existed between the formulations and the control. Panelists did not find any significant differences ($p<0.05$) between the 75%/25% whey-milk formulation stabilized with gelatin, the 50%/50% whey-milk formulation stabilized with pectin and the control. According to these results whey could be used in a 75% proportion when stabilized with gelatin and in a 50% proportion when stabilized with pectin when preparing a whey-milk kefir type beverage.

RESUMEN

Se prepararon unas bebidas de suero-leche tipo kefir utilizando proporciones de 75% y 50% suero y estas fueron estabilizadas con pectina (0.02% p/v) y gelatina (0.4% p/v). Se determinaron las características fisicoquímicas y reológicas de las bebidas y estas fueron comparadas a un control, 0%/100% suero-leche. Las medidas reológicas demostraron que la bebida de 75%/25% suero-leche estabilizada con gelatina tuvo una mayor viscosidad que todas las demás formulaciones cuando fue comparada con el control. Se realizó un análisis sensorial para determinar si existían diferencias entre las formulaciones y el control. Los panelistas no encontraron diferencias significativas ($p<0.05$) entre la formulación 75%/25% estabilizada con gelatina, la formulación 50%/50% estabilizada con pectina y el control. De acuerdo a estos resultados el suero puede ser utilizado en una proporción de 75% cuando es estabilizado con gelatina y en una proporción de 50% cuando es estabilizado con pectina en la elaboración de una bebida de suero-leche tipo kefir.

DEDICATION

To God

"I can do all things through Christ which strengthens me"
Philippians 4:13

To my family

Mami, Papi, Diana, Daniel and Betún.

And specially to

Tio Geto

September 5, 1949 - June 28, 2009

Who would often ask….
"Nena, pa' qué tu estudias tanto?".

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"*Now faith is being sure of what we hope for and certain of what we do not see*."

Hebrews 11:1

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1 INTRODUCTION

Milk is very commonly called the perfect food and it is known that people have consumed it since ancient times. Its high content of casein, whey proteins, calcium, phosphorus and vitamins, like vitamin D, are some of the nutrients that humans need to maintain healthy bodies (Whitney and Rady, 2005). Even though milk is known for its high nutritional value, people, for various reasons, have stopped drinking this precious fluid (Whitney and Rady, 2005). The consumption of fluid dairy products has decreased within the past decade while, at the same time, popular drinks such as sodas, juices, flavored teas and energy drinks have shown an increase in their consumption (Boor, 2001). Awareness is being made of milk and its health benefits, but the dairy industry must be able to compete with new, innovative, and convenient shelf-stable beverage products that continue to enter the market (Boor, 2001).

In 2005, the Center for Disease Control and Prevention announced that incidence of obesity among adults 18 and older increased from 56% in 1994 to 66.3% in 2004, and it was stated that the empty calories consumed in the form of beverages were the ones responsible for these results (Goel, 2007). Studies have shown that most of these calories come from a combination of soft drinks, fruit drinks and presweetened teas, all of which add calories but do not contribute significantly to the daily requirement of essential nutrients (Goel, 2007). Consequently the dairy industry is trying to attract people to consume dairy products by creating fermented products which is a means to obtain a drink with different taste, longer shelf life and a way in which lactose intolerant people can consume dairy without any problem (Tamine, 2002; Boor, 2001)

Pszczola (2008) stated that sales of dairy foods increased at a rate of 20% annually, with most of the products consumed coming from the growing category of fermented dairy products,

such as yogurt and yogurt drinks. One of these yogurt drinks that have gained popularity in the Western world has been kefir. Kefir is a fermented milk drink originated in the Caucasus Mountain in Russia. Its manufacture relies on a mixed assortment of bacteria and yeast known as "kefir-grains" to initiate the fermentation process using milk as a starter base (Hutkins, 2006). It can also be manufactured using commercially available starter culture systems formulated to contain the essential yeast and bacteria required to produce it (Hutkins, 2006). "Kefir-grains" or starter culture can successfully ferment milk from most mammals including cow, goat, and sheep, non-mammal "milks" such as soy milk, rice milk, and coconut milk and other liquids including water, fruit juice, coconut water (Hutkins, 2006) and whey (Penna et al., 2000; Almeida et al., 2009).

Whey is the by-product of the manufacture of cheese by the precipitation of casein with rennet or an acid resulting in either sweet or acid whey. Acid whey is derived from the coagulation of casein with acetic or citric acids, and is composed of 93% water and 6.35% solids, similar to sweet whey with the exception of having higher lactic acid and ash contents (Jelicic et al., 2008). This by-product results in serious disposal problems for many cheese makers because its disposal through sewage systems contaminates rivers and oceans where this waste finally ends up (IFC, 2006). The component of whey that poses the greatest disposal problem is lactose, contributing to high levels of biological oxygen demand (BOD), which results largely from the lactose content (Ghaly and Ramkumar, 1999). In the United States commercial cheese manufacturers commonly treat whey as sewage or return whey to farms to be fed to pigs or spread on fields (Balagtas et al., 2003). Even though the whey market has developed rapidly, some cheese processors still do not have the technology to process whey (Balagtas et al., 2003). In Puerto Rico 4 million tons of acid whey where produced from the manufacture of white

cheese in the year 2005 (ORIL, 2004-2005). Further usage of this whey into the preparation of a fermented beverage, like kefir, from a combination of milk and acid whey can be a means of reducing this disposal and contamination problem cheese manufactures confront (Gonzalez-Sizo, 1996).

Whey has a high water content and low total solids content thus whey-based beverages are watery in comparison to milk and other dairy products (Gallardo-Escamilla et al., 2007; Tamine 2006). For this reason these types of beverages require the addition of stabilizers to improve their texture and enhance consumer acceptability. One of the organoleptic characteristics looked upon by consumers is the texture or mouth-feel of a food product (Gallardo-Escamilla et al., 2007). Among the important purchasing criterion for foods, texture and mouth-feel are major selling points for any final product, especially for dairy products (Berry, 2006).

Stabilizers are high molecular weight hydrophilic hydrocolloids that are added to food products to control water (Roberts, 2005). Different types of stabilizers are available for the stabilization of dairy products such as pectin, gelatin, k-carrageenan, starch and xanthan gum (Berry, 2006). Although the main purpose of these is to control water in a product, the effects of viscosity, body, texture and effect on syneresis will vary depending on the type of hydrocolloid added (Berry, 2006; Ryder, 1980). Stabilization of dairy foods is of great importance and the addition of hydrocolloids will be a factor that will have a significant impact on consumer acceptability of the final kefir product. It is important to understand that the addition of stabilizers to dairy products is increasing as opportunities for product innovating present themselves in the retail, foodservice and industrial markets (Hunt and Maynes, 1997). If stabilizers are added to whey-milk beverages, higher proportions of whey may be used, thus

reducing the contents of whey disposal and would be a method of addressing this problem of the dairy industry.

1.1 Objectives

1.1.2 Primary Objective

1. Improve the viscosity of two formulations of a fermented beverage prepared from 75% acid whey and 25% milk and 50% acid whey and 50% milk by adding the commercial stabilizers Pectin (Tic Gums™) and Gelatine (Kraft Foods™).

1.1.3. Secondary Objectives

1. Determine which of these two stabilizers has a better functionality in improving the viscosity of the fermented beverages.
2. Evaluate the stabilized formulations of the fermented beverages through a sensory analysis and determine the consumer acceptability of the final product.

2 LITERATURE REVIEW

2.1 Milk Consumption

In recent years, with the creation of novelty eye catching beverages the dairy industry has experienced a decrease in the overall consumption of milk. Many people have decided to consume fortified beverages that provide them with the same nutritional value as milk but that gives them the advantage of trendy flavours or keeping for a longer time without spoiling. To the consumer, "quality" means that the product tastes good and that it keeps well in their home refrigerator (Boor, 2001). In a study conducted in the United States by the Milk Quality Improvement Program (MQIP) a direct correlation was found between milk flavor and levels of milk consumption in school-age children (Boor, 2001). In other cases, consumers simply avoid the consumption of milk because a little amount of milk's sugar, lactose, will make them sick. For this reason, in the last few years the dairy industry has had to find new ways in which to produce milk so that more people will once again consume this naturally nutritious fluid. Balagtas and others (2003) stated that with consumption of existing dairy commodities falling it was necessary to find new uses for milk as a way of increasing demand for dairy products, and that one way would be to find new, economical uses for milk. If the dairy industry wishes to gain popularity once again it has to focus on the creation of novelty products that will attract consumers to the dairy market.

In the last years, consumption of milk has declined but consumption of yogurt and other fermented milks has increased. The reason for this is that yogurt has been known throughout the ages as a health food and it is recognized as one of the first probiotic food products (Pszczola, 2008). Probiotics are live microorganisms thought to be healthy for the host organism.

According to the currently adopted definition by FAO/WHO (2002), probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, as in yogurt. Yogurt has evolved very far, not only by finding its way into the Western marketplace, but by becoming a staple in many countries (Pszczola, 2008).

2.2 Fermented Milk

Fermented milk is a dairy product prepared from milk with specific cultures in which the microflora is kept alive and may not contain any pathogenic germ (Bamforth, 2005). This type of beverage has gained popularity in the past few years possibly for various reasons described by Tamine (2002).

- The introduction of a “new” generation of yogurts with the addition of fruit and sugar has given the product an entirely fresh image and has become an inexpensive snack or dessert
- The incorporation of probiotic bacteria into the product has enhanced the health benefits of fermented milks
- Fermented dairy products don’t have to overcome the problem of spoiling too fast, they are easy to consume and are very nutritious.

Fermented dairy products such as yogurt, acidophilus milk, koumiss and kefir are seen as healthy as milk because they provide almost every nutrient needed to survive, and the addition of fermentation microorganisms will certainly improve lactose digestion among those who consume it (Hutkins, 2006).

2.2.1 Kefir

Kefir is a traditional fermented milk that originated in the Northern area of the Caucasian mountains (Delfederico et al., 2006). It is produced by the fermentative activity of "kefir grains" consisting mainly of lactococci, lactobacilli and yeasts in a protein-polysaccharide matrix (Garrote et al., 1998). The kefir grains are small, 0.5-3.5cm diameter, and irregularly shaped yellowish masses resembling florets of cauliflower. Their exact microbial composition is controversial since reports indicate that it depends strongly on the origin of the grains (Kuo and Lin, 1999; Delfederico et al., 2006). The production of this beverage has called on the attention of the scientific community due to its high nutritional value and the microorganisms ability to inhibit the development of spoilage pathogenic germs either by production of lactic acid or by the expression of antimicrobial agents (Dimitrellou et al., 2007).

Several groups have studied this complex symbiotic relationship between lactic acid bacteria and yeasts and they have been able to isolate various species. Among these are the homofermentative and heterofermentative bacteria that include: *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus kefir*, *Lactobacillus parakefiri*, *Lactococcus lactis* and *Leuconostoc mesenteroides* (Koroleva, 1982; Litopoulou-Tzanetaki and Tzanetakis 2000; Witthuhn et al., 2005b; Guzel-Seydim et al., 2005; Tamine, 2006). The viable count of lactic acid bacteria from Turkish kefir produced from kefir grains have been reported by Guzel-Seydim and others (2005) to be of ~8 Log_{10} cfu/ml and is favored over the viable count of yeast which has been ~ 6 Log_{10} cfu/ml. In the same manner yeast from kefir grains have been isolated and species such as *Kluveromyces marcianus*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Candida inconspicua*,

Candida lambica and *Candida kefir* have been found (Koroleva, 1982; Witthuhn et al., 2005b; Guzel-Seydim et al., 2005; Tamine, 2006).

The preparation of traditional kefir consists of adding kefir grains directly into pasteurized milk at 25°C. As fermentation progresses over a period of ~ 24 hours microorganisms are shed from the grains into the milk where they multiply and produce acid, flavor compounds and other physicochemical changes (Garrote et al., 1998). The resulting kefir is an acidic, mildly alcoholic dairy beverage whose specific characteristic such as taste, aroma and texture is attributed to the presence of the complex microbial population (Witthuhn et al., 2005a). It is described as a healthy drink, and its protective effect on cell damage has been studied (Guzel-Seydim et al., 2005).

Due to the complexity of kefir production from kefir grains, many starter culture companies have developed kefir starter cultures whose properties are close to those found in kefir grains. These kefir starter cultures make production less laborious, and ensure a longer shelf life, but the characteristics of the resulting product tend to be different from traditional kefir (Chen et al., 2008; Tamine, 2006). Body Ecology's Kefir Culture Starter™ is an example of the commercially available starter cultures. As declared on their label, this starter culture contains freeze-dried kefir flora containing lactic acid bacteria and yeasts. The microorganisms present in this specific starter are: *Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. cremoris, Lactobacillus kefir, Klyveromyces marxianus subsp. marxianus* and *Saccharomyces unisporus.* According to the manufacturer, this starter culture can be added to ferment milk, coconut water or whey.

Kefir's mixed cultures utilize lactose present in the substrate to carry out the fermentation process and produce lactic acid. Whey is a waste of insignificant cost that is rich in lactose and could be used as a raw material for kefir production (Dimitrellou et al., 2007; Paraskevopoulou et al., 2003). Different fermented whey products exist such as wine, champagne and beer, and many different ideas are arising for the production of new whey products, such as kefir (Jelicic, 2008; Hutkins, 2006).

2.3 Whey

Whey is the liquid remaining after curding of milk casein and represents about 85-95% of milk volume (Paraskevopoulou et al., 2003). It is a by-product of the elaboration of cheese with rennet or an edible acidic substance such as acetic or citric acids, resulting in either sweet or acid whey respectively. Its composition depends mainly on the technology of cheese manufacture and the quality of milk used for the production (Jelicic et al., 2008). Liquid whey consists mainly of water, lactose, vitamins, minerals and whey protein, which makes it a healthy source (**Table 1**) (Balagtas et al., 2003). Whey as raw material is reasonably priced and, on account of its content of high-grade proteins, is extremely valuable from a nutritional point of view (Jelicic, 2008). Referring to whey, Gerdes (2006) reported that it is one of the best things that yogurt manufactures can add to yogurt as it is a healthy dairy ingredient that was once considered a by-product.

Table 1: Composition of sweet and acid whey*

Component	Sweet Whey	Acid Whey
Water	93.1	93.4
Protein	0.85	0.76
Fat	0.36	0.09
Lactose	5.14	5.12
Minerals/Calcium	0.27/0.05	0.28/0.10
Vitamins	0.02	0.01

*Grams per 100 grams of product
Adapted from United States Department of Agriculture (1976)

At present, only 30% of the whey by-product is not sold and the other 70% is further processed into its components and used as a food ingredient such as an additive in some foods, for animal feedstuff and as a nutritional supplement in sport bars and drinks (Balagtas et al., 2003). Although whey protein concentrates have been used in a variety of formulations, Gallardo-Escamilla and others (2005a) reported that fermentation of liquid whey represents a more economical alternative, because the costs of evaporation and ultra-filtration are eliminated.

One of the main constituents of whey is lactose, which constitutes a serious environmental problem due to its high biological oxygen demand (BOD) (Paraskevopoulou et al., 2003). Its disposal into rivers and onto fields promotes bacterial growth and causes oxygen depletion of water and soil (Ghaly and Ramkumar, 1999). If this whey is simply dumped down the drain, a practice still common with many dairy processors, it constitutes the most potent of all dairy wastes and one of the strongest wastes of any kind in terms of its BOD (Jelen et al., 2003). Over several decades, considerable effort has been devoted to finding the least costly method of disposal of liquid whey and to identify new outlets for whey utilization, preventing the loss of potentially valuable nutrients and reducing environmental pollution (Gallardo-Escamilla et al., 2005a; González-Martínez et al., 2002).

Today with whey, and products containing whey, gaining more popularity the whey market has had to develop rapidly. One of the options considered for adding value to whey is the manufacture of beverages through lactic or alcoholic fermentations that can provide desirable sensory properties (Gallardo-Escamilla et al., 2005a; Salminen et al., 1991). This suggests the possibility of producing beverages from whey with similar sensory profiles to those of fermented milk drinks or with some flavor attributes of drinking yogurt, following manufacturing procedures conventionally used for milk (Gallardo-Escamilla et al., 2005a). Growth of the fermented milk sector represents an opportunity to advance the development of fermented milk-like products from liquid whey into products with interesting nutritional and sensory properties without requiring complicated or costly technology (Gallardo-Escamilla et al., 2005a; Sienkiewicz and Riedel, 1990).

Itara-Rogríguez (2007) reported that in the preparation of different formulations of fermented beverages from milk and whey, when whey was used in the formulations in the 50/50 and 75/25 whey/milk ratio, the viscosity of the formulations decreased and the water content increased. Due to these findings he suggested the use of stabilizers for these whey/milk ratio formulations in order to have higher viscosity values in the products and be able to use greater proportions of whey (Itara-Rodríguez 2007). During the preparation process of fermented milk drinks defects in appearance and texture are not uncommon. The most frequent and serious problem in the manufacture of many of these products is syneresis (Roberts, 2005). To many consumers, the appearance of these pools of slightly yellow-green water from the top of the product is considered unnatural and objectionable (Hunt and Maynes, 1997). To minimize these problems and improve the body and texture of the finished products stabilizers are added to enhance water- binding capacity of milk mixtures.

2.4 Stabilizers

A stabilizer is a polyssacharide or hydrocolloid used primarily to thicken and gel aqueous solutions. It also modifies and controls the flow properties and texture of liquid food and beverage products. Stabilizers often need to be added to dairy products for enhanced product functionality and in cultured dairy products to control texture and control whey separation (Singh and Heldman, 1993). For this reason the addition of stabilizers is of great importance. If properly incorporated, hydrocolloid stabilizers can provide excellent functional attributes to cultured dairy products (Roberts, 2005). Stabilizing ingredients interact with milk proteins, water, and other stabilizers to modify gel structure and immobilize water (Tunick, 2000).

Many different types of stabilizers are available in the market. Some of these include pectin, gelatin, sodium carboxymethyl cellulose, guar gum, and starch (Berry, 2006). Stabilizers in milk are usually chosen so that they can prevent milk proteins from aggregating together and prevent their sedimentation. This action is achieved by using stabilizers, such as pectin, that have electrostatic interactions with the milk protein casein (Tamine, 2006). Gelatin is also used because it improves the texture of yogurt, which results in a firmer product with fewer tendencies to syneresis (Ares et al., 2007).

According to Gallardo-Escamilla and others (2007) no studies have assessed the influence of adding hydrocolloids on the sensory properties of fermented dairy beverages elaborated with liquid whey. These types of dairy products are an emerging segment of the dairy industry that require sensory, physical and chemical characterization for quality control and product development (Gallardo-Escamilla et al., 2007). From the point of view of the consumer, lactic beverages should be, visually and in textural terms, as homogeneous as milk. And even

though most research on whey has been devoted to improving the processing methods, few studies have analyzed the impact of whey on sensory properties (Philips et al., 1995).

2.4.1 Gelatin

Gelatin is a substantially pure protein food ingredient, obtained by the thermal degradation of collagen, which is the structural mainstay and most common protein in the animal kingdom (Baziwane and He, 2003). It is a colorless or slightly yellow solid that is nearly tasteless and odorless. It contains about 98-99% protein but it has less nutritional value than many other protein sources (Alakali et al., 2008). It has great properties as a protective hydrocolloid used to modify physical properties of foods. In the food industry, gelatin is one of the water-soluble polymers that can be used as a gelling, thickening, or stabilizing agent. It is sold with a wide range of special properties such as gel strength and viscosity to suit particular applications (Baziwane and He, 2003). This stabilizer was defined in 2003 by the Food and Drug Administration (FDA) of the United States as generally regarded as safe (GRAS) for addition as a food ingredient. Gelatin has different functions. Among these are gelation, emulsification, adhesiveness, sedimentation and stabilization. Addition of gelatin will be performed depending on the desired functionality in a product. Because of its great performance as a stabilizer, gelatin it's often used in dairy products to prevent syneresis and to provide appropriate mouth-feel or viscosity to a product (Baziwane and He, 2003). Gelatin is a stabilizer which functions as a gelling agent in milk products and is used commercially at 0.3-1%. (Alakali et al., 2008).

According to studies by Fiszman and Salvador (1999) gelatin is one of the most preferable stabilizers for use in fermented milks. They reported that gelatin improved the

rheology and texture of yogurt over a great range of concentrations (Fiszman and Salvador, 1999). Ares and others (2007) studied the effect of the addition of gelatin and starch on the rheological properties and sensory texture of plain yogurt. They found that the addition of gelatin or starch to yogurt showed higher consistency coefficient values than non stabilized yogurts. They also found that in addition of gelatin and starch in yogurt at the same concentration, gelatin had a higher increase in the consistency index and a greater decrease in the flow behavior index. Their results showed a higher pseudoplastic behavior with the use of gelatin, probably due to the fact that gelatin developed a stronger three dimensional network than starch (Ares et al., 2007).

2.4.2 Pectin

Pectin is a polysaccharide found in the cell wall of most plants and contributes to many cell wall functions. It is classified as a soluble fiber and, even though it is found in most plants, it is mostly concentrated in apples and citrus fruits such as oranges, lemons and grapefruits. This polysaccharide is obtained by the aqueous extraction of citrus peels and apple pulp under mildly acidic conditions (Hunt and Maynes, 1997). In the food industry pectins are used as gelling, thickening and stabilizing agents (Roberts, 2004). Its use is required in a small dosage; allowing for economical and efficient stabilization of products that will subsequently extend the shelf life of various products (Hunt and Maynes, 1997). In acidified milk products pectins are used as stabilizing agents where, with the low pH value in yogurt drinks, they protect casein against flocculation and sedimentation (Hunt and Maynes, 1997). On account of its thickening effect, pectin is used to create a specific mouth-feel, it provides texture, prevents syneresis, and is used to regulate the viscosity of drinks.

Gallardo-Escamilla and others (2007) studied the characteristics of fermented whey after the addition of several hydrocolloids: high-methoxy pectin (HMP), propylene glycol alginate (PGA), carboxymethyl cellulose (CMC) and xanthan gum (XG). Results showed that XG did not appear to have an important effect on perceived viscosity, but CMC and HMP significantly increased the perceived thickness of the products. PGA characterized for the perceived grittiness in the product. They concluded that HMP and CMC can be used to significantly increase the instrumental and perceived viscosity of liquid whey to match the physical viscosity of lactic beverages.

Paraskevopoulou and others (2003) studied the influence of several polysaccharides (xanthan gum, guar gum and high methoxyl pectin) on the stability of a whey-milk kefir type beverage. They found that xanthan gum was the most effective of all (even an low concentrations) followed by guar gum. Pectin was less effective at stabilizing the system against the "wheying off" even when used at high concentrations. Paraskevopoulou and others (2003) also reported that the influence of pectin addition at low concentrations has not been studied. Therefore the purpose of their study was to stabilize the milk-whey kefir type drink against precipitation, improve its rheological properties and acceptability of the product by consumers and this was not possible to achieve by keeping the pectin content at low levels.

The use of stabilizers has an impact on the rheological properties of fermented milk products. One of these properties is the viscosity of the product, which can predict the stability of a fermented milk drink (Tamine, 2006). The low viscosity of fermented milk drinks is a desirable feature; however, some precautionary measures have to be considered to stabilize the protein and prevent syneresis (Tamine, 2006).

2.5 Rheology

Rheology is the study of the flow and deformation of matter. In food research, the term is often used interchangeably with texture, which refers to the flow, deformation, and disintegration of a sample under force (Tunick, 2000). Texture is included with flavor, appearance and nutrition as a principal quality factor of foods (Pollen et al., 2004). Viscosity (μ) is the tendency of a fluid to resist flow and it pertains mainly to liquid foods (Singh and Heldman, 1993). It is estimated as the cocient of shear stress (τ) and shear rate (γ) and is dependent on temperature and pressure (Hassan et al., 1996). A viscometer is the instrument used to determine the viscosity of a liquid.

The Power Law Model is very helpful in determining the rheological behavior of products (Penna et al., 2001).

$$\tau = K\gamma^n$$

In this model *K* is the consistency index, which gives us an idea of the thickness of a fluid when it is at rest (Schmidt and Smith, 1992). *n* is the flow behavior index and gives us and idea on how the fluids behave when in movement. With the determination of the flow behavior index, fluids can be classified as Newtonian or Non-Newtonian. In this model, when n=1, the fluid is known as Newtonian and if it is different than 1 it is a Non-Newtonian fluid. Non-Newtonian fluids can be classified into pseudoplastic (n<1) or dilatant (n>1) (Schmidt and Smith, 1992).

Product viscosity has been the primary property of interest in fluid food texture characterization (Pollen et al., 2004). The sensory attributes of cultured products are very important for the consumer in order to determine product acceptability (Lucey, 2004). Food can exhibit both solid and liquid characteristics, and rheology can identify the properties of such foods (Singh and Heldman, 1993).

Penna and others (2000) studied the physicochemical, sensory and rheological characteristics of five different commercial brands of lactic beverages in the Brazilian market. They found significant differences in the physicochemical characteristics of the 5 commercial brands of lactic beverages. One of them had higher sensory acceptability than all other samples. Others obtained better results for appearance than for consistency. The study showed that all of the samples behaved as non-Newtonian fluids and sensory panelists preferred lactic beverage with high consistency index and high pseudoplasticity (Penna et al., 2000).

Rheological properties of dairy products are essential for material handling, design and operation of the processing equipment used in the dairy industry (Penna et al., 2000). These parameters have been studied to a limited extent in the dairy industry but rheological parameters for lactic beverages made with whey are scarce. For this reason the specific equations and the relations between quality and the rheological properties applicable to dairy product containing whey must be established (Penna et al., 2000).

3 MATERIALS AND METHODS

3.1 Milk

Milk used in the preparation of the fermented beverages was obtained from the College of Agricultural Sciences dairy farm of the University of Puerto Rico-Mayagüez, Lajas Sub-Experimental Station. Raw milk was pasteurized at 63°C for 30 minutes using a stove water bath and stirring constantly in order to heat all milk particles evenly. After pasteurization, milk was separated into cream and skim milk using a cream separator (Milk Tech, Inc.). The cream and half of the skim milk were homogenized separately using a micro homogenizer (Microfluidics™ HC-5000, Newton, Ma.) at ~63ºC and ~2,300 psi to ensure proper mixture of the fat content in them (Tamine, 2006). The other part of the skim milk was set aside for the elaboration of white cheese in order to obtain the acid whey to be used in the preparation of the beverages. Samples of homogenized fractions were taken to be analyzed for fat content and pH measurements.

The homogenized cream and skim milk were stored at refrigeration temperatures to be used later on in the elaboration of the fermented beverages. Fat content of the cream and skim milk was determined using the Monjonnier method (AOAC 989.05). This value was used in the mass balance equation in order to determine the amount of cream required to standardize the formulations of the beverages to the desired percent of fat (i.e. 3.2%).

pH of the skim milk fluctuated from 6.5 to 6.8. This measurement was used in order to standardize the acid whey to the original pH as the skim milk.

3.2 Acid Whey

In order to obtain the acid whey, the unhomogenized skim milk was further heated to 85°C and precipitation of the casein was achieved by the addition of acetic acid. This acid whey was collected, filtered and homogenized using a micro homogenizer (Microfluidics™ HC-5000, Newton, Ma.) at ~63ºC and ~2,300 psi to ensure proper mixture of the fat content in the whey (Tamine, 2006). Fat content of the whey was determined by the Mojonnier Method (AOAC 989.05) and this value was used in the mass balance equation in order to determine the amount of cream required to standardize the formulations of the beverages to the desired percent of fat (i.e. 3.2%). Whey was cooled in a refrigerator and its pH measured. The acid whey pH fluctuated from 5.4 to 5.5.

3.3 Standardization of Acid Whey

In order for the lactic acid bacteria to begin the fermentation process, acid whey used in the formulations was standardized with a 6N sodium hydroxide solution (NaOH) (Itara-Rodríguez, 2007). pH of the acid whey was adjusted to that of the milk being used in the preparation of the formulations; the pH fluctuated from 6.5-6.8 depending on the day of the milk collection.

3.4 Beverage Formulations

Mass balance equations were used to obtain three different formulations containing 3.2% fat by mixing cream, skim milk and whey. The three formulations were 0% whey/100% milk (control), 75% whey/25% milk (75/25) and 50% whey/50% milk (50/50). All three formulations were homogenized using a micro homogenizer (Microfluidics™ HC-5000, Newton, Ma.) at

~63ºC and ~2,300 psi to ensure proper mixture of the fat content in the formulations and avoid phase separation (Tamine, 2006).

The 75/25 and 50/50 formulations were each divided into 3 different previously sterilized screw cap Erlenmeyer flasks. The 0/100 (control) was added to a previously sterilized screw cap Erlenmeyer flask. All the flasks were cooled at refrigeration temperatures. The fat content of the formulations was analyzed using the Mojonnier Method (AOAC 985.05) in order to ensure that each one had a fat content of 3.2% ±0.2.

3.5 Addition of Stabilizers

Two different stabilizers, Pectin (Tic Gums™) and Gelatine (Kraft Foods™) were added separately to the beverage formulations of 75/25 and 50/50 in order to study their effects on viscosity. These stabilizers were added at 0.2% w/v of pectin, recommended inclusion for cultured dairy products (Lucey, 2004), and 0.4% w/v for gelatin (Abou-Dawood et al., 1993). A total of four different stabilized beverages were prepared: 75% whey/25% milk stabilized with pectin (75/25 P), 75% whey/25% milk stabilized with gelatin (75/25 G), 50% whey/50% milk stabilized with pectin (50/50 P) and 50% whey/50% milk stabilized with gelatin (50/50 G).

Pectin used in the formulations was previously hydrated in a glass beaker using the same refrigerated beverage formulation in which it was to be added, following manufacturers recommendations (Tic Gums™). The remaining of the beverage formulation was heated in a hot water bath at 90°C before the addition of the hydrated stabilizer (Tic Gums™). While on a hot plate, the hydrated pectin was added and mixed with a pre-sterilized magnetic agitator until it was completely dissolved.

The two formulations that contained gelatin were heated in a hot water bath at 40°C, prior to addition of the gelatin, and placed on a hot plate to maintain the temperature (Tessenderlo, 2008). Gelatin was added directly and mixed with a pre-sterilized magnetic agitator until completely dissolved.

3.6 Beverage Fermentation

A total of seven beverage formulations were prepared: 0/100 (control), 75/25, 75/25 P, 75/25 G, 50/50, 50/50 P and 50/50 G. 2.5g of a commercial kefir starter (Kefir Starter, Body Ecology™ Decatur, GA) were added as starter culture to each 500 ml Erlenmeyer flask containing the beverage formulations, following manufacturer's recommendations. All seven inoculated flasks were incubated at 25°C and pH was measured every 8 hours for the first 16 hours, and then each hour until they reached a pH of 4.4 ± 0.2 (Hutkins, 2006). Flasks were taken out of the incubator and samples were drawn for final pH, titratable acidity measurements and enumeration of microorganisms. The remaining portion of beverages was refrigerated at 5°C and stored for further analyses such as protein, total solids and viscosity.

Table 2: Abbreviations for the beverage formulations.

Formulation	Abbreviation
0% whey / 100% milk	Control
75% whey / 25% milk	75/25
75% whey / 25% milk Pectin	75/25 P
75% whey / 25% milk Gelatin	75/25 G
50% whey / 50% milk	50/50
50% whey / 50% milk Pectin	50/50 P
50% whey / 50% milk Gelatin	50/50 G

3.7 Titratable Acidity and pH

In order to evaluate the fermentation process, pH and titratable acidity (AOAC 947.05) were determined. Triplicate measures of pH were carefully taken using a properly standardized pH potentiometer with buffers 4.00, 7.00 and 10.00 (Accumet Basic, AB 15 pH Meter, Fischer Scientific). pH measurements were taken at the beginning of the fermentation process and every 8 hours during the process for the first 16 hours and then every each hour until formulations reached a pH of 4.4 ± 0.2.

Titratable acidity was measured at the beginning of the fermentation process and once all the formulations had reached a pH of 4.4 ± 0.2. Triplicate measures of titratable acidity were determined as percent of lactic acid using NaOH 0.1N.

3.8 Enumeration of microorganisms in the fermented beverages

3.8.1 Enumeration of Lactic-acid Bacteria by means of MRS-Agar

Lactic acid bacteria in the beverage formulations were enumerated using the Man, Rogosa and Sharpe (MRS) culture medium (Wehr and Frank, 2004). Serial dilutions of 10^{-2} – 10^{-5} were done for all seven-beverage formulations at the beginning of the fermentation process and serial dilutions of 10^{-4} - 10^{-7} were done at the end of the fermentation process. A 1 ml aliquot was placed in sterile Petri dishes and 20 ml of MRS medium were added using the pour plate method. Duplicate plates were incubated (Isotemp Incubator, Fisher Scientific) anaerobically at 35°C ± 2 for 48 hours using an anaerobic jar with the Gas Pack™ anaerobic generating system (BD BBL™ Plus Anaerobic System Envelopes with Palladium Catalyst, Bacton, Dickison and Company) at both the beginning and end of the fermentation process. Anaerobic strip indicators were placed to make sure an anaerobic environment was being created

(BBL™). Colonies were counted after 48 hours using a colony counter (Bantex Colony Counter, Model 920A).

3.8.2 Enumeration of Yeasts by means of PDA

Yeasts in the beverage formulations were enumerated using Potato Dextrose Agar (PDA) (pH ~3.5) culture medium (Wehr and Frank, 2004). Serial dilutions of $10^{-1} – 10^{-4}$ were done for all seven-beverage formulations at the beginning of the fermentation process and serial dilutions of 10^{-2} - 10^{-4} were done at the end of the fermentation process. A 0.1 ml aliquot was placed in sterile Petri dishes containing 20 ml of acidified PDA using the spread plate method. Duplicate plates for each beverage formulation were incubated at 25°C ± 2 for 48 hours in a dark incubator (Isotemp Incubator, Fisher Scientific) at both beginning and end of the fermentation process. Colonies were counted after 48 hours using a colony counter (Bantex Colony Counter, Model 920A).

3.9 Proximal Analysis

3.9.1 Crude Fat

Fat of fermented beverages was determined using the Mojonnier method (AOAC, 989.05). For the Mojonnier method, flasks were weighed on an analytical balance (Accu-124, Fisher Scientific) and loaded with 10 g of the fermented beverages. 1.5ml of ammonium hydroxide (NH_4OH) was added in order to neutralize any acid and casein present in the product. Three drops of phenolphthalein indicator (0.5%w/v) were added to observe the water-fat interface during the extraction. Addition of 10 ml of ethyl alcohol, 25 ml of diethyl ether and 25 ml of petroleum ether induced the separation of the fat from the solution. After every addition

flasks were vigorously agitated for approximately 1 min. After the addition of all reagents, flasks were left to rest for 30 minutes. At that time, the organic phase was extracted and placed in a clean, previously weighed and labeled, glass plate. This extraction process was repeated two times for all 7 fermented beverages, in triplicate measures.

Once the organic phase was in the glass plate, it was left to rest for 30 minutes in a ventilated hood. At that time, when most of the solvent had evaporated, plates were placed in an oven for 15 minutes at 100°C ±1°C, and placed in a desiccator for 30 minutes to cool down. Plates were then weighed using an analytical balance (Accu-124, Fisher Scientific) and the percent of crude fat was determined by mass difference.

3.9.2 Proteins

The Kjeldahl method (AOAC 920.105) was used for the determination of protein content of fermented beverages. Between 0.120 and 0.150 g of the samples were weighed in triplicate in 100 ml digestion tubes. Approximately 0.6 g of the catalyzing agent of potassium sulfate (K_2SO_4) and copper sulfate ($CuSO_4$) was added to each tube, along with 7 ml of sulfuric acid (H_2SO_4). Tubes were placed in a digestion block (Digestion System 40-1016 Digestor, Tecator) and heated to 350°C for 3 hours until the samples became colorless. Once digestion ended, tubes were placed on a cooling rack and let cool. 60 ml of distilled water were added, in three stages, to the cooled tubes with the digested sample in order to dissolve the crystals that were formed. The solution in the digestion tubes was transferred to distillation tubes, 50 ml of 40% NaOH were added to the samples and immediately distilled in a distillator for 6 minutes (Distillation Unit 100, Fisher Scientific). Erlenmeyer flasks containing 25 ml of 4% boric acid were used to trap ammonia gas produced from the distillation. The resulting solution from the distillation was

titrated with a 0.1N HCl solution. The amount of nitrogen in the sample was determined with a stereochemistry reaction between the moles of boric acid and moles of nitrogen. Total protein content in the samples was calculated using the conversion factor for milk products, 6.38.

3.9.3 Total Solids

The percent of total solids (AOAC 925.23) was determined by drying 3g of the fermented beverage in disposable aluminum plates. Triplicate samples were placed in a vacuum oven for 16 hours at 100°C. After 16 hours, plates were cooled in a desiccator and weighed using an analytical balance, (Accu-124, Fisher Scientific). Percent of total solids was calculated by difference in mass.

3.10 Rheology

A viscometer (Cannon LV 2000) was used to determine the viscosity of fermented beverages. Triplicate measures were taken at five different revolutions per minute (RPM) using different spindles as required for the different fermented beverages. Spindles used for viscosity measurements of formulations were the following: Spindle L3 for the 0/100 (control); spindle L1 for the 75/25 and 75/25 P; and spindle L2 for the 75/25 G, 50/50, 50/50 P and 50/50 G. Twenty milliliter samples of each fermented beverage were placed in the sample tubes and placed in a 500 ml beaker with ice in order to maintain a temperature of 5°C ± 2, and viscosity measurements were taken at 3, 6, 12 and 30 RPM to determine the flow behavior index, *n,* and the consistency index, *K* (Pa*s^n), values.

3.11 Sensory Analysis

Sensory analysis was performed to determined if panelists could find any differences between the control and three of the fermented beverages: 75/25 G, 50/ 50 P and 50/50 G formulations. The 75/25, 50/50 and 75/25 P formulations were not taken into account for the sensory evaluation due to their obvious watery appearance.

A Different from Control test was conducted. Each of the 48 untrained panelists, which included students, faculty and personnel from the University of Puerto Rico-Mayaguez, were asked to evaluate the sample labeled "control" and four samples, coded with random three digit numbers, with respect to how different (overall) each was from the "control" (Meilgaard et. al., 2007). These coded samples were a "blind control" and the previously mentioned fermented beverages: 75/25 G, 50/50 P and 50/50 G formulations. Evaluation took place in the sensory analysis room of the Agro-Industrial Innovation and Technology Center of the University of Puerto Rico-Mayaguez. Panelists were presented with minimum instructions, water and crackers to cleanse their palate between samples, an evaluation sheet and a pencil in order to evaluate the control and the four coded samples. The evaluation sheet consisted of a 7-point category: (no difference, very slight difference, slight difference, moderate difference, large difference, great difference and extreme difference). These were then converted to a numeric scale for statistical analysis in which the no difference received a score of 0 and the extreme difference received a score of 6.

3.12 Statistical Analysis

All physicochemical, microbiological, and rheology measurements collected during the experimental phase were analyzed using the Statistical Analysis System (SAS) version 9.1, 2005.

The experiment was a completely randomized block design. The experiment was replicated three times (blocks) with triplicate measurements each time for the physicochemical and rheology parameters and in duplicate for the microbiological parameters. The differences between means were determined by Tukey's multiple range test at a 95% confidence interval. The sensory analysis data was analyzed using ANOVA for two factors (treatments and panelists) without replication to determine the difference between the treatments and the control. In order to determine which treatments were significantly different a Z-test two sample for means was performed (Meilgaard et al., 2007).

4 RESULTS AND DISCUSION

4.1 Physicochemical characteristics

4.1.1 Fat

Fat content of fermented beverages was measured using the Mojonnier method. ANOVA results are presented in **Table 3** as the mean of the three experimental measures with their corresponding standard deviation. There were no significant differences ($P>0.05$) in the total content of fat. The amount of fat in commercial kefir usually ranges from 1.5 to 2.0 % of fat, and the addition of fat content is usually determined by the manufacturer preparing the product (Tamine, 2006). Milk has a 3.25% fat content, the percent of fat required by law for whole milk in Puerto Rico (ORIL, 2004). The main steps in the manufacture of fermented milk products include standardization of milk fat, heat treatment, homogenization, addition of starter culture, fermentation, cooling and storage (Lucey, 2004). For purposes of this experiment the percent of fat in the beverage formulations was standardized to ~3.2% and these were homogenized to reduce fat globules (<2μm) in order to reduce the tendency of cream layer formation and whey separation (Lucey, 2004). Tukey's mean comparison test shows that there were no significant differences in terms of the fat content of the fermented beverages; all are similar to the control. The choice of milk and standardization of fat is one of the factors that need to be controlled in fermented milk manufacturing to obtain high quality products (Tamine, 2006). Philips and others (1995) found that textural properties and viscosity of milk were influenced by fat percentage. For this reason it was important to have all beverages to the same percent of fat to make sure that this factor did not affect the functionality of the stabilizers added, pectin and gelatin, and would not affect the viscosity of the fermented beverages.

Table 3: Crude fat of the fermented beverages.

Formulations	% Fat
Control	$3.23^{a} \pm 0.035$
75/25	$3.23^{a} \pm 0.030$
75/25 P	$3.23^{a} \pm 0.030$
75/25 G	$3.23^{a} \pm 0.030$
50/50	$3.24^{a} \pm 0.011$
50/50 P	$3.24^{a} \pm 0.011$
50/50 G	$3.24^{a} \pm 0.011$
P-value	0.5984

Values in the same column with different letters are significantly different (Tukey test at α=0.05).
Values are given as means with standard deviation values.

4.1.2 Total Solids

The percent of total solids of fermented beverages was measured. ANOVA results are presented in **Table 4** as the mean of three experimental measures with their corresponding standard deviation. There were significant differences ($p<0.05$) in the total solids content of the beverage formulations. Total solids in milk are composed of fat, proteins, lactose and minerals and whole milk for beverage use should contain ~12.41% total solids (Broster et al., 1981). On the other hand, whey has a total solids content of ~6.25% (USDA, 1976). In Tukey's mean comparison test it was found that all beverage formulations were different from the control, which had a total solids content of 11.24%. Significant differences were also found between formulations. The highest value of total solids was for the 50/50 G (9.89%) formulation, and the 75/25 (9.25%) formulation had the lowest total solids content.

Whey is approximately 93% water and contains approximately 50% of the total solids present in milk, in which lactose is the main constituent (Jelicic et al., 2008) thus it was expected to see a pattern in reduction of total solids content in the formulations with higher proportions of whey. The theoretical yield of total solids for the 75/25, 75/25 P, 75/25 G, 50/50, 50/50 P and 50/50 G formulations were calculated and these were 10.1%, 10.3%, 10.5%, 11.5%, 11.7% and

11.5% respectively (Apendix 15). When compared to the experimental values on **Table 4**, it can be seen that these show a similar pattern where the formulations with a higher proportion of milk (50%) had a higher yield in the total solids content than the ones who contained less milk (25%) in the formulation; as the amount of milk in the formulations increased the total solids content also increased.

The total solids content may have a significant effect on the viscosity of whey-based beverages due to the low total solids content of whey (Gallardo-Escamilla et al., 2007). The mouthfeel of fermented whey beverages is poor and watery in comparison with that of fermented milks and for that reason those types of beverages require the use of hydrocolloids to improve its texture (Gallardo-Escamila et al., 2007). In studies by Tamine and Deeth (1980) they reported that by increasing the total solids content during the manufacture of fermenting milks, including yogurt, improved the textural characteristics and sensory properties of the products. This can be seen on the results obtained from the Power Law Model (**Table 11**) where the formulations with lower total solids content had a lower consistency index when compared to those who had higher total solids contents, thus a higher consistency index. It can also be seen that the addition of stabilizers had a significant increase in the consistency index of the formulations.

Table 4: Total solids content of the fermented beverages.

Formulations	% Total solids
Control	$11.24^{a} \pm 0.485$
75/25	$9.25^{c} \pm 0.453$
75/25 P	$9.37^{c} \pm 0.669$
75/25 G	$9.58^{bc} \pm 0.462$
50/50	$9.56^{bc} \pm 0.233$
50/50 P	$9.71^{bc} \pm 0.187$
50/50 G	$9.89^{b} \pm 0.109$
P-value	**<0.0001**

Values in the same column with different letters are significantly different (Tukey test at α=0.05).
Values are given as means with standard deviation values.

4.1.3 Protein

The amount of protein on the fermented beverages was measured. ANOVA results are presented in **Table 5** as a mean of three experimental measures with their corresponding standard deviation. There were significant differences ($p<0.05$) in the protein content of fermented beverages. The composition of milk may vary depending on a variety of factors like feeding regimens, individual animals and breed. Thus, the protein content of milk may fluctuate from 2.9% to 3.3%, in which casein is the main component (Marth and Steele, 2001). Whey in the contrary has a protein content of less than 1% and its main constituents are beta-lactoglobulin, alpha-lactalbumin, immunoglobulins, serum albumin, and proteose peptone (Jelicic et al., 2008). This low percentage of protein content in whey is one of the factors that contribute to the low content of total solids seen in the formulations (**Table 4**) as the amount of whey increases. As seen on **Table 5** the control had the highest protein content (2.93%), this falls within the amount of protein that milk should contain and as reported by Tamine (2006) kefir prepared with milk should not contain less than 2.7% protein. In Tukey's mean comparison test it can be seen that all the beverage formulations were different from the control, the protein content of the formulations was lower. The formulation with the highest protein content was the 50/50 G (1.93%) and it was significantly different from all other formulations, an expected result since this formulation contained more milk and was stabilized with gelatin. A theoretical yield for protein content was calculated for the 75/25 P, 75/25 G, 50/50 P, 50/50 G and these resulted in 1.21%, 1.60%, 1.82% and 2.20% respectively (Apendix 16). From these values it can be seen that the theoretical yield for the formulation with lower proportions of whey was higher, and the theoretical yield of the formulations stabilized with gelatin was higher than those stabilized with

pectin. Which can be expected since gelatin has a protein content of 98-99% (Alakali et al., 2008) and pectin has a protein content of 0% (Tic Gums™). The results on **Table 5** show the same pattern where the formulations with higher proportions of whey had lower protein content and the formulations with lower proportions of whey had higher protein content. In the same matter the formulations stabilized with gelatin had a higher protein content than those stabilized with pectin.

Table 5: Protein content of the fermented beverages.

Formulations	% Protein
Control	$2.93^{a} \pm 0.557$
75/25	$0.66^{f} \pm 0.194$
75/25 P	$0.73^{ef} \pm 0.196$
75/25 G	$1.07^{de} \pm 0.205$
50/50	$1.47^{c} \pm 0.216$
50/50 P	$1.41^{cd} \pm 0.162$
50/50 G	$1.93^{b} \pm 0.084$
P-value	**<0.0001**

Values in the same column with different letters are significantly different (Tukey test at α=0.05).
Values are given as means with standard deviation values.

4.1.4 pH

The pH of beverage formulations was measured at the beginning and the end of the fermentation process. ANOVA results are presented in **Table 6** as a mean of three experimental measures with their corresponding standard deviation.

Significant differences ($p<0.05$) were found on the initial pH of the beverage formulations. The beverages showed an initial pH ranging from 6.35 to 6.67. In Tukey's mean comparison test it can be seen that all formulations were different from the control, which had the highest pH value (6.67). There were significant differences between the 75/25 formulations where the two formulations that contained a stabilizer, 75/25 P and 75/25 G, had a lower pH

value than the 75/25 formulation. This tendency was also observed with the 50/50 formulations where the 50/50 P and 50/50 G had a lower pH value than the 50/50 formulation. This tendency could be do to the fact that both of the stabilizers added had a pH of 4.8 (Tic Gums™; Alakali et al., 2008), which once added into the formulations had a lowering effect on their pH. From the results on **Table 6** it can also be observed that the beverage formulations with a higher proportion of whey (75%) were significantly different from formulations with lower proportions of whey (50%). Where the formulations with higher proportions of whey had a lower pH value than those with lower proportions of whey and higher proportions of milk in their formulation.

In various studies it has been found that an important factor in the processing of milk products is the equilibrium between soluble and colloidal salts. The salt content of milk exists in a dynamic equilibrium and any alteration in one salt, or the form in which it exists, causes a shift in the balance and/or form of other salts. It is known that the factor that shows a direct effect on processing is the change in the form of calcium in a milk product. It is important to know that the result of the reduction in colloidal calcium phosphate is an increase in calcium ions; and that an increase in colloidal calcium phosphate implies, as equilibrium is established, a decrease in calcium ions. There are series of factors that may influence the equilibrium between soluble and colloidal salts that may affect milk processing (Fox, 1985; Davis and Macdonald 1953; Jenness and Patton 1959; Wasltra and Jenness 1984):

- Addition of an acid
    - As pH is lowered, progressively more colloidal calcium phosphate is solubilized. In acid coagulation of casein, most of the colloidal calcium phosphate is lost from casein, remaining in acid whey.
- Addition of an alkali

- As pH is raised, more and more of the soluble calcium phosphate is precipitated in colloidal form.

- Addition of colloidal phosphate
  - As colloidal phosphate is added to milk content of soluble phosphate is lowered, and pH decreases.

The results in **Table 6** show that the initial pH of the beverage formulations had a small decreased with the proportion of whey added whether it was 75% or 50%. The formulations with a higher proportion of whey (75%) had a slightly lower pH value than those with a smaller proportion of whey (50%). From the previously mentioned scientific findings it can be hypothesized that the significant differences found in initial pH of the beverage formulations could have been due to an increase in the amount of colloidal phosphate when NaOH standardized whey was mixed with fresh milk resulting in a decrease of soluble phosphate thus a decrease in pH (Fox, 1985, Davis and Macdonald 1953; Jenness and Patton 1959; Wasltra and Jenness 1984). Gallardo-Escamilla and others (2007) reported that whey-based lactic beverages constitute an emerging segment of dairy products that require chemical, physical and sensory characterization for quality control and product development. Thus, further studies should be performed on the use of NaOH standardized acid whey and the addition of stabilizers on the pH of whey/milk mixtures used for fermented beverages.

There were significant differences found ($p<0.05$) in the final pH of the fermented beverages. The final pH fluctuated between 4.39 to 4.44 suggesting that the bacteria used the lactose present in the beverages to carry out the fermentation process. The control (4.44) and the 75/25 (4.44) were significantly different from the 75/25 G (4.41) and the 50/50 G (4.39); but there were no significant differences between the control and all the other formulations.

Body Ecology's Kefir Starter Culture™ recommends fermenting the inoculated milk for a period of 18 to 24 hours and coconut water for a period of 36 to 48 hours. In this experiment, ferments were mixtures of whey and milk thus it was important to monitor the pH measurements because there was an uncertainty on the time it would take to reach a desired pH of 4.4 ± 0.2. Commercial kefir may have a pH of 4.2 - 4.6, and even though there were significant differences in the final pH, the pH of all fermented beverages was in this recommended range (Hutkins, 2006). Thus pH was measured every 8 hours for the first 16 hours and then every hour until formulations reached the desired pH. Beverage formulations with the lowest initial pH reached the desired pH in a longer time than those with the higher initial pH. The 75/25 formulations reached the desired pH in ~ 36 hours, the 50/50 formulations in ~25 hours and the control, which had the highest initial pH, in ~18 hours (data not shown). Many factors affect the activities of fermenting cultures during a fermentation process and one of these is the pH. It is of great importance to maintain the pH (~6.6) of the growth medium at the optimum level in order to increase the number of bacterial cells and allow the fermentation process to proceed (Salminen et al., 2004). Even though there were no significant differences ($p>0.05$) found in the lactic acid bacteria and yeast count of the beverages there was a difference in the time it took the beverages to reach the desired pH. Bacteria in the beverage formulations with the lower pH took a longer time to begin and proceed with the fermentation process than those who had the pH at an optimum level for their growth.

Table 6: pH of the fermented beverages.

Formulations	Initial$_a$ pH	Final$_b$ pH
Control	$6.67^{a} \pm 0.146$	$4.44^{a} \pm 0.019$
75/25	$6.47^{d} \pm 0.131$	$4.44^{a} \pm 0.027$
75/25 P	$6.38^{e} \pm 0.110$	$4.42^{ab} \pm 0.042$
75/25 G	$6.35^{e} \pm 0.092$	$4.41^{b} \pm 0.049$
50/50	$6.60^{b} \pm 0.092$	$4.41^{ab} \pm 0.011$
50/50 P	$6.54^{bc} \pm 0.064$	$4.42^{ab} \pm 0.057$
50/50 G	$6.53^{c} \pm 0.081$	$4.39^{b} \pm 0.071$
P-value	**<0.0001**	0.1709

Values in the same column with different letters are significantly different (Tukey test at α=0.05).
Values are given as means with standard deviation values.
$_A$ Measurements taken at the beginning of the fermentation process.
$_B$ Measurements taken at the end of the fermentation process.

4.1.5 Titratable acidity

The titratable acidity of the beverage formulations was measured at the beginning and end of the fermentation process. ANOVA results are presented in **Table 7** as the mean of the three experimental measures with their corresponding standard deviation.

There were significant differences ($p<0.05$) found in the initial titratable acidity of the beverage formulations. Titratable acidity values fluctuated between 0.115% to 0.166%. In Tukey's mean comparison test it can be seen that there were significant differences between the control (0.166%) and the 75/25 (0.115%) and 75/25 P (0.132%) formulations. The titratable acidity of fresh milk should be around 0.14%-0.16% and this is expressed in terms of lactic acid. Lactic acid is the principal acid produced by fermentation after milk is drawn from the udder, thus fresh milk contains traces of it (Wasltra et al., 1999). Higher values of titratable acidity in fresh milk products (before a fermentation process) are considered to result from lactic acid produced by bacterial action, which could reflect a milk supply of poor quality (Fox, 1985; Davis and Macdonald, 1953; Jenness and Patton, 1959; Walstra and Jenness, 1984). The results on **Table 7** show that the beverage formulations were from a fresh milk supply.

There were no significant differences found ($p>0.05$) between the control and beverages formulations, or between formulations. The final titratable acidity values fluctuated between 0.675% to 0.803%. Titratable acidity of milk is expressed as percent of lactic acid. Lactic acid bacteria use lactose found in milk as substrate and convert it into lactic acid, thus this measurement was taken to determine the indirect fermentative activity of lactic acid bacteria during a fermentation process (Jay et al., 2005). Tamine (2006) reports that the final titratable acidity of a commercial kefir should be no less than 0.6%, and all fermented beverages had values in this range

Table 7: Titratable acidity of the fermented beverages.

Formulations	Initial % TA	Final % TA
Control	$0.166^{a} \pm 0.035$	$0.803^{a} \pm 0.130$
75/25	$0.115^{c} \pm 0.016$	$0.675^{a} \pm 0.113$
75/25 P	$0.132^{bc} \pm 0.011$	$0.683^{a} \pm 0.213$
75/25 G	$0.160^{ab} \pm 0.031$	$0.675^{a} \pm 0.105$
50/50	$0.140^{abc} \pm 0.014$	$0.794^{a} \pm 0.119$
50/50 P	$0.166^{a} \pm 0.016$	$0.713^{a} \pm 0.119$
50/50 G	$0.162^{ab} \pm 0.019$	$0.703^{a} \pm 0.113$
P-value	**<0.0001**	0.1967

Values in the same column with different letters are significantly different (Tukey test at $\alpha=0.05$).
Values are given as means with standard deviation values.
$_{A}$ Measurements taken at the beginning of the fermentation process.
$_{B}$ Measurements taken at the end of the fermentation process

4.2 Enumeration of Microorganisms

Lactic acid bacteria (LAB) and yeasts were enumerated at the beginning and end of the fermentation process. ANOVA results are presented in **Table 10** as a mean of two experimental measures with their corresponding standard deviation.

4.2.1 Lactic-acid bacteria

There were no significant differences ($p>0.05$) in the enumeration of LAB at the beginning of the fermentation process of the beverages formulations. The bacterial count at the beginning of the fermentation fluctuated between 6.13 Log_{10} CFU/ml in the 75/25 P formulation to 6.22 Log_{10} CFM/ml in the 50/50 G formulation. The beverage formulations started the fermentation process with a similar amount of inoculum. This measurement was taken in order to make sure that the starter culture was active and the fermentation process would proceed. In studies by Liu and Lin (2000) the initial count of lactic-acid bacteria in milk inoculated with kefir grains was of 6.0 Log_{10} CFU/ml. This measurement was an indication that part of the microflora contained in kefir grains is transferred to milk immediately after inoculation. Thus in this experiment even though a kefir starter culture was used instead of kefir grains, the use of the starter culture produced similar log cycles of lactic-acid bacteria in the beverage formulations as those reported by Liu and Lin (2000).

There were no significant differences ($p>0.05$) in the enumeration of LAB at the end of the fermentation process. The bacterial count at the end of the fermentation fluctuated between 8.15 Log_{10} CFU/ml for the 75/25 G formulation to 8.27 Log_{10} CFU/ml for the 50/50 G. The microbiological composition of kefir grains is still controversial since different reports indicate that microflora strongly depends on the origin of the grains (Lin and Kuo, 1999). Thus the population of lactic-acid bacteria will depend on the composition of the kefir grains or starter culture that are used for fermentation. **Table 8** shows the lactic-acid bacterial count for kefir prepared with kefir grains from different country locations. It can be seen that the LAB count varies according to the kefir grains' country of origin, which can be due to the climate of the country, the cultivation of kefir grains, and the microbial flora found in such

grains (Kroger, 1993; Seydim et al., 2005; Mann, 1989; Witthuhn et al., 2004; Loretan et al., 2003; Kuo and Lin, 1999; Liu and Lin, 2000, Garrote et al., 1998). When comparing LAB to that of the whey-milk formulations studied, it can be seen that the starter culture produced similar log cycles to the kefir from Russia (Kroger, 1993), Taiwan (Liu and Lin, 2000), and South Africa (Witthuhn et al., 2004). Thus the use of the starter culture incorporated in the elaboration of the beverage formulations showed to be as effective in the production of lactic-acid bacteria as the kefir grains from four different countries.

Table 8: Enumeration of lactic-acid bacteria isolated from kefir elaborated with kefir grains from different locations.

Location	Substrate	Source	Lactic Acid Bacteria Log_{10} CFU/ml
Russia	milk	kefir grains	8.0 - 9.0
Turkey	milk	kefir grains	8.6
Czechoslovakia	milk	kefir grains	9.0
South Africa[1]	milk	kefir grains	4.8 - 8.9*
South Africa[2]	milk	kefir grains	9.0
Taiwan[1]	milk	kefir grains	9.9 – 11.5*
Taiwan[2]	milk / soy milk	kefir grains	8.2 / 9.0
Argentina	milk	kefir grains	8.0
USA-PR+	whey-milk mixture	starter culture	8.1 – 8.2++

[1 S.A.] Witthuhn et al., 2004; [2 S.A.] Loretan et al., 2003; [1TW] Kuo and Lin, 1999; [2TW] Liu and Lin, 2000
* grains collected from different households
+Current investigation; ++ Formulations with different whey-milk proportions

The kefir starter is a mixture of microorganisms that effectively metabolize lactose and produce a refreshing fermented beverage through a yeast-lactic fermentation (Tamine, 2006). The kefir starter used contains the Gram + bacteria *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Lactobacillus kefyr* and *Leuconostoc mesenteroides subsp*. *cremoris* and they activate at different times during the fermentation process (Kefir Starter, Body Ecology™; Koroleva, 1982). The *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus kefyr* develop during the first hours of the fermentation allowing the rapid formation of acids.

These are known as homofermentatives, they utilize lactose to produce lactic acid as the end product of the fermentation process (Jay et al., 2005). As the fermentation progresses *Leuconostoc mesenteroides subsp. cremoris* starts to develop; producing aroma compounds slowly and gradually growing during the later stages of the fermentation (Koroleva, 1982). This genus is known as heterofermentative, which produce lactate, carbon dioxide, and ethanol as end products of the fermentation process (Koroleva, 1982).

4.2.2 Yeast

There were no significant differences ($p>0.05$) in the yeast content at the beginning of the fermentation process. All fermented beverages were similar to the control and between each other. The yeast count at the beginning of the fermentation process fluctuated from 3.61 Log_{10} CFU/ml in the 50/50 formulation to 3.75 Log_{10} CFU/ml in the 75/25 and 75/25 P formulations. The beverage formulations started the fermentation process with an adequate amount of inoculum. This measurement was taken in order to make sure that the starter culture was active and the fermentation process would proceed. In studies by Liu and Lin (2000) the initial count of yeasts in milk inoculated with kefir grains was of 5.8 Log_{10} CFU/ml. This measurement was an indication that part of the microflora contained in kefir grains is transferred to milk immediately after inoculation. In this experiment the use of a kefir starter culture produced ~2.1 log cycles lower of yeast than those reported by Liu and Lin (2000).

There were no significant differences ($p>0.05$) in the yeast content at the end of the fermentation process. All fermented beverages were similar to the control and between each other. The yeast count at the end of the fermentation fluctuated from 4.70 Log_{10} CFU/ml in

the 7/25 G formulation to 4.95 Log_{10} CFU/ml in the 75/25 and 75/25 P. The microbiological composition of kefir grains is still controversial since different reports indicate that microflora strongly depends on the origin of the grains (Lin and Kuo, 1999). Thus the population of lactic-acid bacteria will depend on the composition of the kefir grains or starter culture that are used for fermentation. **Table 9** shows the yeast count for kefir prepared with kefir grains from different country locations. It can be seen that the yeast count varies according to the kefir grains' country of origin, which can be due to the climate of the country, the cultivation of kefir grains, and the microbial flora found in such grains (Kroger, 1993; Seydim et al., 2005; Mann, 1989; Witthuhn et al., 2004; Loretan et al., 2003; Kuo and Lin, 1999; Liu and Lin, 2000, Garrote et al., 1998). When comparing yeast count from the kefir of the different countries to that of the whey-milk formulations studied, it can be seen that the starter culture produced similar log cycles to the kefir from Russia (Kroger, 1993). Thus the use of the starter culture incorporated in the elaboration of the beverage formulations showed to be as effective in the production of yeasts as the kefir grains from three different countries.

Table 9: Enumeration of yeast isolated from kefir elaborated with kefir grains from different locations.

Location	Substrate	Source	Yeast Log_{10} CFU/ml
Russia	milk	kefir grains	4.0-5.0
Turkey	milk	kefir grains	6.1
Czechoslovakia	milk	kefir grains	7.0
South Africa[1]	milk	kefir grains	5.1 – 8.5*
South Africa[2]	milk	kefir grains	8.0
Taiwan[1]	milk	kefir grains	5.3 - 6.8*
Taiwan[2]	milk / soy milk	kefir grains	6.6 / 5.6
Argentina	milk	kefir grains	7.0
USA-PR+	whey-milk mixture	starter culture	4.7 - 4.9++

[1 S.A.] Witthuhn et al., 2004; [2 S.A.] Loretan et al., 2003
[1TW] Kuo and Lin, 1999; [2TW] Liu and Lin, 2000
* grains collected from different households
+Current investigation; ++ Formulations with different whey-milk proportions

The choice of yeast strains is important in order to contribute to the typical flavor and aroma of kefir. The yeasts in the starter culture were similar to those that may be found in the actual kefir grains used to produce traditional kefir (Simova et al., 2002). These are *Klyvromyces marxianuns* var. *marxianus* and *Saccaromyces unisporus* (Kefir Starter, Body Ecology™). *Klyveromyces marxianus* var. *marxianus* ensures the metabolism of lactose through alcohol fermentation and the formation of the typical yeasty flavor and aroma of kefir (Simova et al., 2002). *Saccaromyces unisporus* is a non-lactose fermenting yeast that produces alcohol and carbon dioxide from glucose. Yeasts have a much slower growth rate than the lactic acid producers and start a slow production of aroma compounds at the later stages of the fermentation process (Witthuhn et al., 2005a).

The addition of a starter culture was the key factor in the elaboration of the fermented beverages. The microorganisms included in the starter were the ones responsible for imparting the characteristics of kefir to the fermented beverage formulations. The properties of lactic beverages, such as acidity level and sensory profile are important traits of the product; and these aspects are influenced by the chemical composition of the milk base, processing

conditions, and the activity of starter culture during the incubation period (Mahdian and Mazaheri, 2007).

Table 10: Enumeration of lactic-acid bacteria and yeast in the fermented beverages.

Formulations	Lactic Acid Bacteria Log_{10} CFU/ml		Yeast Log_{10} CFU/ml	
	$Initial_A$	$Final_B$	Initial	Final
Control	$6.18^a \pm 0.034$	$8.22^a \pm 0.123$	$3.74^a \pm 0.167$	$4.86^a \pm 0.138$
75/25	$6.13^a \pm 0.055$	$8.19^a \pm 0.136$	$3.75^a \pm 0.223$	$4.95^a \pm 0.258$
75/25 P	$6.13^a \pm 0.089$	$8.19^a \pm 0.099$	$3.75^a \pm 0.077$	$4.95^a \pm 0.186$
75/25 G	$6.20^a \pm 0.121$	$8.15^a \pm 0.176$	$3.69^a \pm 0.117$	$4.70^a \pm 0.182$
50/50	$6.20^a \pm 0.089$	$8.20^a \pm 0.113$	$3.61^a \pm 0.092$	$4.81^a \pm 0.269$
50/50 P	$6.16^a \pm 0.105$	$8.24^a \pm 0.132$	$3.77^a \pm 0.123$	$4.93^a \pm 0.116$
50/50 G	$6.22^a \pm 0.102$	$8.27^a \pm 0.099$	$3.66^a \pm 0.109$	$4.76^a \pm 0.224$
P-values	0.6477	0.4157	0.4399	0.2273

Values in the same column with different letters are significantly different (Tukey test at α=0.05).
Values are given as means with standard deviation values.
$_A$ Measurements taken at the beginning of the fermentation process.
$_B$ Measurements taken at the end of the fermentation process.

4.3 Rheological Properties of the Fermented Beverages

Rheological measurements were taken at 5°C for all the fermented beverages and consistency index values (*K*) and flow behavior index values were determined using the Power Law Model. ANOVA results are presented in **Table 11** as the mean experimental measures with their corresponding standard deviation.

4.3.1 Consistency Index

There were significant differences ($p<0.05$) in the values of *K* for the fermented beverages and these values fluctuated from 2.35 to 3.74 Pa s^n. In Tukey's mean comparison test, it can be

seen that all beverage formulations were different from the control, which had the highest consistency index of 3.74 Pa s^n. The 75/25 formulation had the lowest consistency index (2.35 Pa s^n) and was significantly different from all other beverage formulations. 75/25 contained the highest proportion of whey and no stabilizer.

There were significant differences between the 75/25 (2.35 Pa s^n), 75/25 P (2.62 Pa s^n) and 75/25 G (3.21 Pa s^n). These formulations had the same proportion of whey and *K* increased with the addition and type of stabilizer added. There were significant differences between the 75/25 G (3.21 Pa s^n) and the 50/50 G (3.41 Pa s^n) which had the same stabilizer but different proportions of whey. *K* increased with a decrease in the proportion of whey. The 50/50 (3.09 Pa s^n) and 50/50 P (3.22 Pa s^n) formulations were significantly different to the 50/50 G (3.41 Pa s^n) where the proportion of whey was the same and *K* increased with the type of stabilizer added.

Results show that the consistency index increased when the proportion of whey decreased. This behavior was also seen with the type of stabilizers added in which the formulations stabilized with gelatin had a higher consistency index than those that were stabilized with pectin or that did not have any stabilizers added. In studies by Ares and others (2007) it was reported that gelatin had a higher increase in the consistency index and a decrease in the flow behavior index because gelatin may have developed a stronger three-dimensional network in yogurt. **Table 12** shows the consistency index of lactic beverages and yogurts studied by a series of investigators. The yogurt from Domagala's study (2008) was prepared with whole milk and had a consistency index similar to the control (3.74 Pa s^n) from this current study. The yogurt from Ares and collaborators (2007) was stabilized with gelatin and its consistency index was fairly similar to that of the 75/25 P (2.62 Pa s^n).

Table 11: *n* and *K* values for the fermented beverages Obtained from the Power law model.

Formulation	*n*	K (Pa*s^n)
Control	$0.448^{ab} \pm 0.059$	$3.74^{a} \pm 0.037$
75/25	$0.488^{a} \pm 0.087$	$2.35^{e} \pm 0.171$
75/25 P	$0.440^{ab} \pm 0.096$	$2.62^{d} \pm 0.124$
75/25 G	$0.457^{ab} \pm 0.030$	$3.21^{c} \pm 0.056$
50/50	$0.367^{bc} \pm 0.077$	$3.09^{c} \pm 0.137$
50/50 P	$0.365^{bc} \pm 0.035$	$3.22^{c} \pm 0.049$
50/50 G	$0.297^{c} \pm 0.031$	$3.41^{b} \pm 0.030$
P-value	**<0.0001**	**<0.0001**

Values in the same column with different letters are significantly Different (Tukey test at α=0.05).

In reports by Penna and others (2000) they defined lactic beverages as a series of products that are prepared with milk and whey. They studied the rheological parameters of five commercial lactic beverages that currently exist in the Brazilian market. Consistency indices were obtained for the five commercial lactic beverages and these varied significantly. The results were: 1.155 Pa s^n, 1.177 Pa s^n, 2.496 Pa s^n, 0.601 Pa s^n and 0.304 Pa s^n. When comparing the commercial samples with the *K* value of 2.496 Pa s^n with the 75/25 P (2.62 Pa s^n) formulation, it can be seen that these values were fairly similar. All other *K* values for the beverage formulations from the current study were higher than the commercial sample. According to Keogh and O'Kennedy (1998) yogurts may have a variation in viscosity due to a series of factors which include: fat (Philips et al., 1995) and total solids content, addition of hydrocolloids and its rate of addition, processing, incubation and storage conditions. In studies by Aportela-Palacios and others (2005) they reported that an increase in total solids contents increased the consistency index (*K*). This tendency can be observed from the results in **Table 11** where it can be seen that the *K* values increased as the total solids content of the formulations decreased (**Table 4**).

Table 12: Consistency index for lactic beverages and yogurt in different studies.

Studies	Yogurt	Milk and whey	Consistency Index Pa s^n
Penna et al., 2000		x	0.304-2.496
Domagala, 2008	x		3.97
Ares et al., 2007	x		2.67
Aportela-Palacios et al., 2005	x		1.03
Current study, 2010		x	2.35-3.74

4.3.2 Flow Behavior Index

There were significant differences ($p<0.05$) in the values of *n* for the fermented beverages and these values fluctuated from 0.297 (50/50 G) to 0.488 (75/25). In Tukey's mean comparison test it can be seen that there were significant differences between the control (0.448) and the 50/50 G (0.297) formulation; but there were no significant differences between the control and the other formulations. Significant differences were found between the 75/25 (0.488) formulation and the 50/50 (0.367), 50/50 P (0.365) and 50/50 G (0.297) formulations. All beverage formulations behaved as pseudoplastic fluids ($n<1$).

Table 13 shows the flow behavior index of lactic beverages and yogurts studied by a series of investigators. The yogurt from Domagala's study (2008) was prepared with whole milk and had a flow behavior index similar to the 50/50 (0.367) and 50/50 P (0.365) from this current study. The yogurt from Ares and collaborators (2007) was stabilized with gelatin and its flow behavior index was much higher than those from the current study. Flow behavior indices were obtained for the five commercial lactic beverages and these varied significantly (Penna et al., 2000). The results were: 0.433, 0.398, 0.302, 0.463 and 0.578. When compared to the *n* values for the current study the 50/50 G (0.297) is lower than those *n* values reported by Penna and others (2000). All samples in the studies behaved as pseudoplastic fluids ($n<1$).

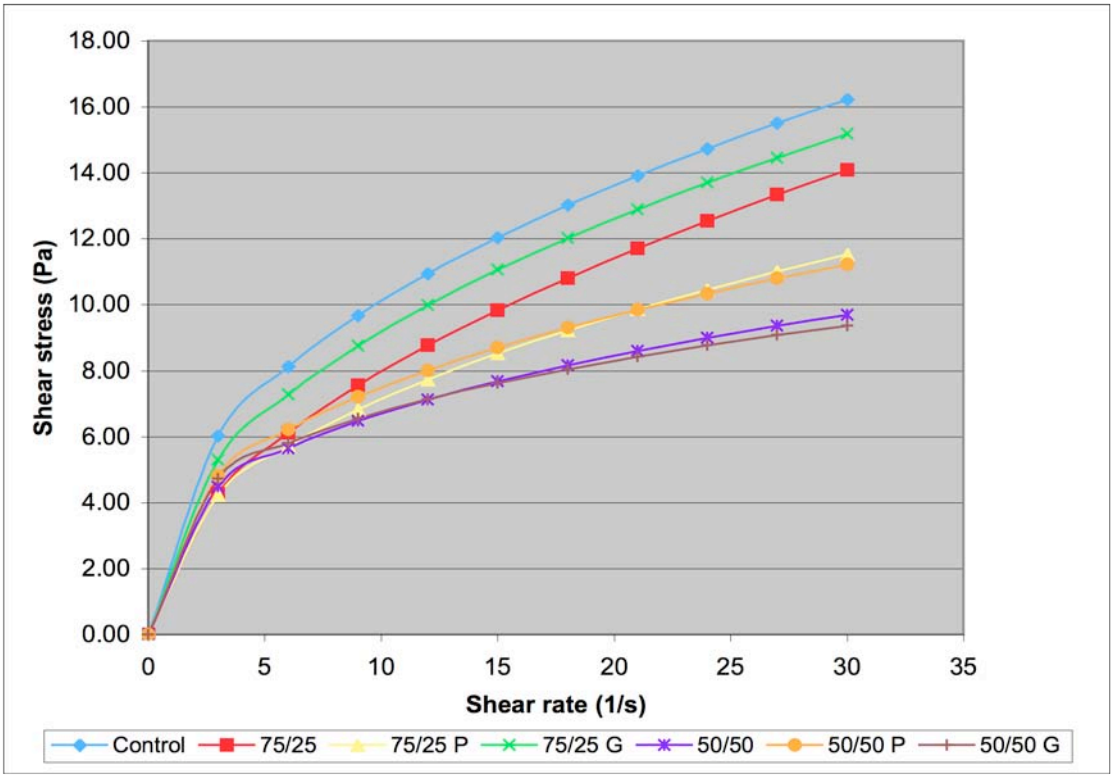
The results in **Table 11** do not show obvious tendencies with proportions of whey in the formulations or type of stabilizer added, but it can be seen that the formulation with highest

proportion of whey (75/25) and without addition of stabilizer had the highest *n* value (0.488). In reports by Itara-Rodríguez (2007) the flow behavior index for beverages with whey/milk mixtures increased with higher proportions of whey showing a pseudoplastic behavior. **Figure 1** shows the shear stress/shear rate relationship flow curves for all six formulations and the control at 5°C, which shows an upward curve in the graph. As seen on the graph all formulations behaved as non-Newtonian fluids, pseudoplastic type and the 75/25 G formulation curve is the one that shows a closer similarity to the control curve. Even though a series of published results exist about the rheological properties of dairy products, rheological parameters for dairy beverages made with whey are scarce (Penna et al., 2000). Thus more studies are necessary to establish the relations between quality and rheological properties of dairy products prepared with whey.

Table 13: Flow behavior index for lactic beverages and yogurt in different studies.

Studies	Yogurt	Milk and whey	Flow Behavior Index
Penna et al., 2000		x	0.302-0.578
Domagala, 2008	x		0.380
Ares et al., 2007	x		0.660
Aportela-Palacios et al., 2005	x		0.570
Current study, 2010		x	0.297-0.488

Figure 1: Apparent viscosity estimates based on the Power Law Model for the beverage formulations (T = 5°C)



4.4 Sensory evaluation of the fermented beverages.

A sensory evaluation was performed for the control, 75/25 G, 50/50 P, and 50/50 G. The type of test used was a Different From Control in which 48 non-trained panelists evaluated the samples. Results were analyzed using ANOVA (**Table 14**) for two factors for the scores given by the panelists on the four fermented beverages analyzed. The test showed significant differences between the different samples tested ($p<0.05$). This shows that panelists were able to detect a difference between the samples tested and the control. A Z-test was performed to determine the degree of differences between the control and the samples. The control (1.83) was significantly different from the 50/50 G (2.92) formulation. There were no significant differences ($p>0.05$) between the control, 75/25 G (2.33), and 50/50 P (2.52) formulations.

Results show that panelists found the greatest difference between the 50/50 P and the control. When *K* and *n* values for the 75/25 G and 50/50 P formulations were compared, these did not show significant differences among each other, but shear stress values at the same shear rate were lower for the 50/50 P. When comparing the sensory analysis results with the apparent viscosity curve it can be seen that the 75/25 G is the formulation that shows more similarity to the control in both parameters, thus its use can be recommended in the elaboration of a kefir type beverage. In studies by Kilcast and Clegg (2002) they reported that sensory matching could not be limited to rheological measurements only, but they had to be related to the perceived sensory characteristics of a product as well. Gallardo-Escamilla and others (2005b) reported that although research on whey has been devoted to the improvement of processing methods for whey, research on the sensory evaluation of liquid whey should be further evaluated.

Table 14: Sensory evaluation: Different from Control Test scores for the fermented beverages.

Formulations	Test Score
Control	$1.83^{b} \pm 1.74$
75/25 G	$2.33^{ab} \pm 1.36$
50/50 P	$2.52^{ab} \pm 1.53$
50/50 G	$2.92^{a} \pm 1.41$

Values in the same column with different letters are significantly different (Tukey test at α=0.05).

5 CONCLUSION

The effect of the stabilizers pectin and gelatin were analyzed on 75/25 and 50/50 whey-milk formulations. The objectives were to improve the texture of theses formulations and find which stabilizer had a greater effect in doing this for the different whey-milk proportions. A sensory analysis was performed to determine the acceptability of consumers of these stabilized products.

A kefir type beverage was elaborated with the addition of a starter culture to 0% / 100% whey-milk mixture and was used as control. This beverage had a chemical composition similar to kefir. According to Zubillaga and others (2001) the chemical composition of kefir will vary depending on the source of milk and its fat content, the composition of the grains or starter cultures, and its elaboration process. As reported by Otles and Cagindi (2003) traditional kefir has a chemical composition of: water (87.5%), total solids (12.87%), fat (3.5%), protein (3.3%) and pH (4.2-4.6); and a lactic acid bacteria content of 10^8 cfu/ml (Garrote et al., 1998). The control had a very close similarity in chemical composition to the traditional kefir: water (89.1%), total solids (11.24), fat (3.2%), protein (2.93%), and pH (4.4); and lactic acid bacteria counts were 10^8 cfu/ml.

When the control was compared to the beverage formulations the 50/50 G showed more similarity in chemical composition was the 50/50 G: water (90%), total solids (9.89%), fat (3.2%), protein (1.93%) and pH (4.4); and a lactic acid bacteria counts of 10^8 cfu/ml. The other five fermented beverages had similar water and fat content, pH, and lactic acid bacteria but had lower total solids and protein content.

When rheological measurements were taken all formulations became more pseudoplastic with the addition of a stabilizer. The 75/25 G formulation's consistency index and flow behavior index showed more similarity to the control when looked at together. From the apparent viscosity curves it was concluded that the 75/25 G was the formulation that showed a higher viscosity than all other formulations when compared to the control. From this it can be determined that gelatin can be used as a stabilizer to improve the viscosity of a fermented beverage prepared with a 75% whey proportion.

The sensory analysis showed that panelists did not find differences between the control and the 75/25 G and 50/50 P, but the 50/50 G was different from all other formulations. From this analysis it can be determined that whey can be used in a 75% proportion when stabilized with gelatin and in a 50% proportion when stabilized with pectin.

The addition of stabilizers improved the viscosity of the products and whey could be used in higher proportions than those recommended by Itara-Rodríguez (2007). This could then help reduce the amounts of whey disposed by cheese manufacturers and would help resolve the problem the dairy industry confronts today.

Paraskevopoulou and others (2003) reported that when panelists tasted the whey-milk kefir prepared by fermentation with kefir cells, they found it more acceptable by taste when compared to whey. When the whey-milk kefir was compared to traditional kefir, this beverage was very far away from being characterized as a kefir drink. From our study we can determine that the addition of pectin and gelatin improved the viscosity of the beverage formulations since panelists did not find differences in the beverages when compared to the control. Our study concludes that the addition of stabilizers improved the texture of the whey-milk kefir prepared with a starter culture.

6 RECOMMENDATIONS

- Addition of flavors and sweeteners to make it more acceptable to the consumers.
- Microbiological and physicochemical analysis to determine the shelf life of the product.

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8 APPENDIX

Apendix 1: ANOVA Statistical Analysis

ANOVA. DISEÑO DE BLOQUES COMPLETOS ALEATORIZADOS

Class Level Information

Class	Levels	Values
BLOCK	3	1 2 3
TRAT	7	1 2 3 4 5 6 7

Data for Analysis of pHI
pHF ATI ATF FAT PROT ST

Number of Observations Read	63
Number of Observations Used	63

Data for Analysis of
LABI LABF LEVI LEVF

Number of Observations Read	63
Number of Observations Used	42

Dependent Variable: pHI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1.26603492	0.15825437	97.28	<.0001
Error	54	0.08785079	0.00162687		
Corrected Total	62	1.35388571			

R-Square	Coeff Var	Root MSE	pHI Mean
0.935112	0.619939	0.040334	6.506190

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	2	0.53683810	0.26841905	164.99	<.0001
TRAT	6	0.72919683	0.12153280	74.70	<.0001

Dependent Variable: pHF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.09437460	0.01179683	18.68	<.0001
Error	54	0.03411111	0.00063169		

Corrected Total	*62*	*0.12848571*			

R-Square	*Coeff Var*	*Root MSE*	*pHF Mean*
0.734514	*0.568567*	*0.025133*	*4.420476*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*
BLOCK	*2*	*0.07580000*	*0.03790000*	*60.00*	*<.0001*
TRAT	*6*	*0.01857460*	*0.00309577*	*4.90*	*0.0005*

Dependent Variable: ATI

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	*8*	*0.02218878*	*0.00277360*	*5.78*	*<.0001*
Error	*54*	*0.02591137*	*0.00047984*		
Corrected Total	*62*	*0.04810015*			

R-Square	*Coeff Var*	*Root MSE*	*ATI Mean*
0.461304	*14.72300*	*0.021905*	*0.148783*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*
BLOCK	*2*	*0.00086929*	*0.00043465*	*0.91*	*0.4103*
TRAT	*6*	*0.02131948*	*0.00355325*	*7.41*	*<.0001*

Dependent Variable: ATF

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	*8*	*0.38037907*	*0.04754738*	*3.20*	*0.0048*
Error	*54*	*0.80260594*	*0.01486307*		
Corrected Total	*62*	*1.18298501*			

R-Square	*Coeff Var*	*Root MSE*	*ATF Mean*
0.321542	*16.91072*	*0.121914*	*0.720929*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*
BLOCK	*2*	*0.21705677*	*0.10852839*	*7.30*	*0.0016*
TRAT	*6*	*0.16332229*	*0.02722038*	*1.83*	*0.1102*

Dependent Variable: FAT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.00614286	0.00076786	1.34	0.2442
Error	54	0.03094286	0.00057302		
Corrected Total	62	0.03708571			

R-Square	Coeff Var	Root MSE	FAT Mean
0.165639	0.740452	0.023938	3.232857

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	2	0.00332381	0.00166190	2.90	0.0636
TRAT	6	0.00281905	0.00046984	0.82	0.5594

Dependent Variable: PROT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	33.91780859	4.23972607	66.46	<.0001
Error	54	3.44473385	0.06379137		
Corrected Total	62	37.36254244			

R-Square	Coeff Var	Root MSE	PROT Mean
0.907802	17.30421	0.252570	1.459584

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	2	0.62079148	0.31039574	4.87	0.0114
TRAT	6	33.29701711	5.54950285	86.99	<.0001

Dependent Variable: ST

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	28.10939811	3.51367476	33.53	<.0001
Error	54	5.65925691	0.10480105		
Corrected Total	62	33.76865502			

R-Square	Coeff Var	Root MSE	ST Mean
0.832411	3.303778	0.323730	9.798779

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	2	3.96575257	1.98287628	18.92	<.0001

TRAT	*6*	*24.14364555*	*4.02394092*	*38.40*	*<.0001*

Dependent Variable: LABI

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	*8*	*0.08239174*	*0.01029897*	*1.46*	*0.2094*
Error	*33*	*0.23275439*	*0.00705316*		
Corrected Total	*41*	*0.31514612*			

R-Square	*Coeff Var*	*Root MSE*	*LABI Mean*
0.261440	*1.357862*	*0.083983*	*6.184950*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*
BLOCK	*2*	*0.04842007*	*0.02421004*	*3.43*	*0.0442*
TRAT	*6*	*0.03397166*	*0.00566194*	*0.80*	*0.5749*

Dependent Variable: LABF

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	*8*	*0.25913147*	*0.03239143*	*2.57*	*0.0268*
Error	*33*	*0.41603713*	*0.01260719*		
Corrected Total	*41*	*0.67516860*			

R-Square	*Coeff Var*	*Root MSE*	*LABF Mean*
0.383803	*1.369905*	*0.112282*	*8.196312*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*
BLOCK	*2*	*0.15685649*	*0.07842825*	*6.22*	*0.0051*
TRAT	*6*	*0.10227498*	*0.01704583*	*1.35*	*0.2628*

Dependent Variable: LEVI

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	*8*	*0.25964094*	*0.03245512*	*2.07*	*0.0681*
Error	*33*	*0.51750703*	*0.01568203*		
Corrected Total	*41*	*0.77714797*			

R-Square	*Coeff Var*	*Root MSE*	*LEVI Mean*
0.334095	*3.380457*	*0.125228*	*3.704467*

Source	*DF*	*Type I SS*	*Mean Square*	*F Value*	*Pr > F*

BLOCK	2	0.14575653	0.07287826	4.65	0.0167
TRAT	6	0.11388442	0.01898074	1.21	0.3257

Dependent Variable: LEVF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.96975806	0.12121976	4.76	0.0006
Error	33	0.83997757	0.02545387		
Corrected Total	41	1.80973563			

R-Square	Coeff Var	Root MSE	LEVF Mean
0.535856	3.286945	0.159543	4.853829

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BLOCK	2	0.61131896	0.30565948	12.01	0.0001
TRAT	6	0.35843910	0.05973985	2.35	0.0535

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	2	0.61131896	0.30565948	12.01	0.0001
TRAT	6	0.35843910	0.05973985	2.35	0.0535

Tukey's Studentized Range (HSD) Test for pHI

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.001627
Critical Value of Studentized Range	4.33055
Minimum Significant Difference	0.0582

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	6.67444	9	1
B	6.60000	9	5
B			
C B	6.54222	9	6
C			
C	6.53111	9	7
D	6.46667	9	2
E	6.37556	9	3
E			
E	6.35333	9	4

Tukey's Studentized Range (HSD) Test for pHF

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.000632
Critical Value of Studentized Range	4.33055
Minimum Significant Difference	0.0363

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	4.44333	9	2
A			
A	4.44333	9	1
A			
B A	4.42333	9	6
B A			
B A	4.42111	9	3
B A			
B A	4.41333	9	5
B			
B	4.40556	9	4
B			
B	4.39333	9	7

Tukey's Studentized Range (HSD) Test for ATI

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.00048
Critical Value of Studentized Range	4.33055
Minimum Significant Difference	0.0316

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	0.16632	9	1
A			
A	0.16632	9	6
A			
B A	0.16160	9	7
B A			
B A	0.15971	9	4
B A			
B A C	0.13988	9	5
B C			
B C	0.13232	9	3
C			
C	0.11532	9	2

Tukey's Studentized Range (HSD) Test for ATF

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.014863
Critical Value of Studentized Range	4.33055

Minimum Significant Difference 0.176

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	0.80343	9	1
A			
A	0.79379	9	5
A			
A	0.71326	9	6
A			
A	0.70284	9	7
A			
A	0.68323	9	3
A			
A	0.67544	9	2
A			
A	0.67450	9	4

Tukey's Studentized Range (HSD) Test for FAT

Alpha 0.05
Error Degrees of Freedom 54
Error Mean Square 0.000573
Critical Value of Studentized Range 4.33055
Minimum Significant Difference 0.0346

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	3.24000	9	5
A			
A	3.24000	9	6
A			
A	3.24000	9	7
A			
A	3.23333	9	1
A			
A	3.22556	9	4
A			
A	3.22556	9	2
A			
A	3.22556	9	3

Tukey's Studentized Range (HSD) Test for PROT

Alpha 0.05
Error Degrees of Freedom 54
Error Mean Square 0.063791
Critical Value of Studentized Range 4.33055
Minimum Significant Difference 0.3646

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	2.9311	9	1
B	1.9323	9	7
C	1.4691	9	5
C			
D C	1.4128	9	6
D			
D E	1.0748	9	4
E			
F E	0.7314	9	3
F			
F	0.6657	9	2

Tukey's Studentized Range (HSD) Test for ST

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.104801
Critical Value of Studentized Range	4.33055
Minimum Significant Difference	0.4673

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	11.2373	9	1
B	9.8894	9	7
B			
C B	9.7096	9	6
C B			
C B	9.5810	9	4
C B			
C B	9.5635	9	5
C			
C	9.3654	9	3
C			
C	9.2452	9	2

Tukey's Studentized Range (HSD) Test for LABI

Alpha	0.05
Error Degrees of Freedom	33
Error Mean Square	0.007053
Critical Value of Studentized Range	4.43649
Minimum Significant Difference	0.1521

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	6.22312	6	7
A			
A	6.20035	6	2
A			
A	6.20008	6	5
A			
A	6.19738	6	4
A			
A	6.18313	6	1
A			
A	6.16005	6	6
A			
A	6.13053	6	3

Tukey's Studentized Range (HSD) Test for LABF

Alpha	0.05
Error Degrees of Freedom	33
Error Mean Square	0.012607
Critical Value of Studentized Range	4.43649
Minimum Significant Difference	0.2034

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	8.26987	6	7
A			
A	8.23740	6	6
A			
A	8.21960	6	1
A			
A	8.19595	6	5
A			
A	8.19103	6	3
A			
A	8.14787	6	4
A			
A	8.11247	6	2

Tukey's Studentized Range (HSD) Test for LEVI

Alpha	0.05
Error Degrees of Freedom	33
Error Mean Square	0.015682
Critical Value of Studentized Range	4.43649
Minimum Significant Difference	0.2268

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	3.77465	6	6
A			

A	3.74922	6	3
A			
A	3.73998	6	1
A			
A	3.70352	6	2
A			
A	3.68983	6	4
A			
A	3.66425	6	7
A			
A	3.60982	6	5

Tukey's Studentized Range (HSD) Test for LEVF

Alpha	0.05
Error Degrees of Freedom	33
Error Mean Square	0.025454
Critical Value of Studentized Range	4.43649
Minimum Significant Difference	0.289

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRAT
A	4.96008	6	2
A			
A	4.95297	6	3
A			
A	4.92762	6	6
A			
A	4.86260	6	1
A			
A	4.80907	6	5
A			
A	4.75985	6	7
A			
A	4.70462	6	4

ANOVA: DISEÑO DE BLOQUES COMPLETOS ALEATORIZADOS

Class Level Information

Class	Levels	Values
block	3	1 2 3
Trat	7	1 2 3 4 5 6 7

Number of Observations Read	63
Number of Observations Used	63

Dependent Variable: n

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.26477255	0.03309657	8.29	<.0001
Error	54	0.21560531	0.00399269		

Corrected Total	62	0.48037787

R-Square	Coeff Var	Root MSE	n Mean
0.551176	15.45340	0.063188	0.408892

Source	DF	Type I SS	Mean Square	F Value	Pr > F
block	2	0.01957304	0.00978652	2.45	0.0957
Trat	6	0.24519951	0.04086659	10.24	<.0001

Dependent Variable: k

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	11.94647875	1.49330984	162.29	<.0001
Error	54	0.49688528	0.00920158		
Corrected Total	62	12.44336403			

R-Square	Coeff Var	Root MSE	k Mean
0.960068	3.103250	0.095925	3.091110

Source	DF	Type I SS	Mean Square	F Value	Pr > F
block	2	0.07144228	0.03572114	3.88	0.0266
Trat	6	11.87503647	1.97917275	215.09	<.0001

PRUEBAS DE TUKEY

Tukey's Studentized Range (HSD) Test for n

Alpha	0.05
Error Degrees of Freedom	56
Error Mean Square	0.0042
Critical Value of Studentized Range	4.32468
Minimum Significant Difference	0.0934

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Trat
A	0.48829	9	2
A			
B A	0.45653	9	4
B A			
B A	0.44792	9	1
B A			
B A	0.44012	9	3
B			
B C	0.36723	9	5
B C			
B C	0.36517	9	6

C
C 0.29698 9 7

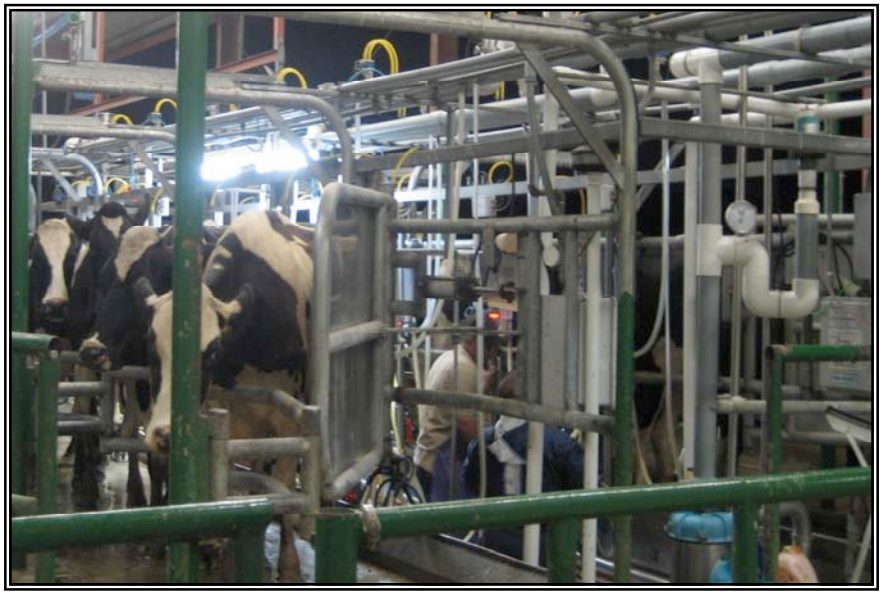
Tukey's Studentized Range (HSD) Test for k

Alpha	0.05
Error Degrees of Freedom	56
Error Mean Square	0.010149
Critical Value of Studentized Range	4.32468
Minimum Significant Difference	0.1452

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Trat
A	3.73674	9	1
B	3.40761	9	7
C	3.22204	9	6
C			
C	3.21181	9	4
C			
C	3.08924	9	5
D	2.61998	9	3
E	2.35033	9	2

Apendix 2: Milking cows in the Sub-Experimental Station in Lajas, P.R.



Apendix 3: Milk Tank.

Apendix 4: Stove water bath for milk pasteurization.

Apendix 5: Cream Separator, Milk Tech-Inc.

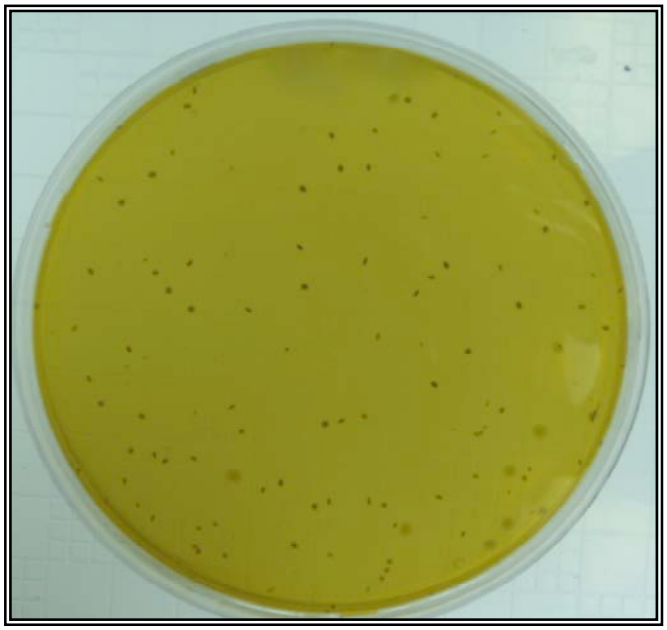
Apendix 6: Skim milk (left) and Cream (right).

Apendix 7: Microfluidics™ HC-5000 micro-homogenizer.

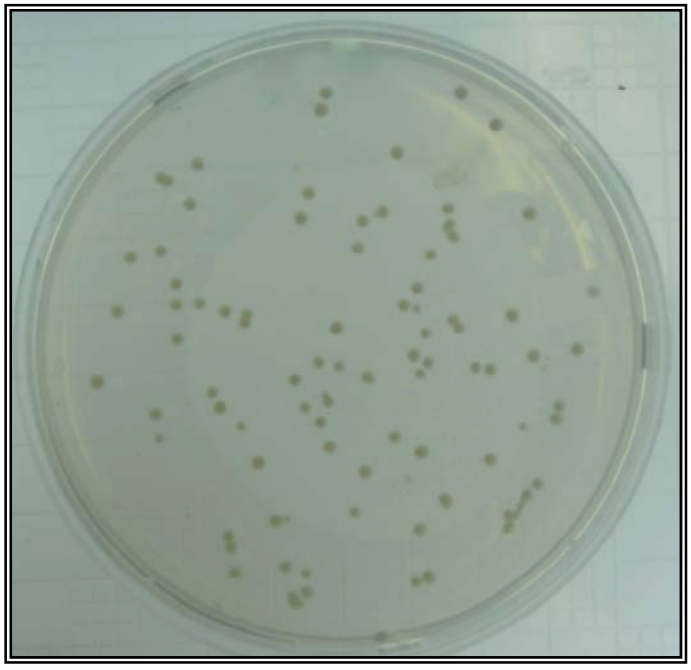
Apendix 8: Protein Analysis using the Kehjdal Method

Apendix 9: Viscosemeter, Cannon LV-2000

Apendix 10: Lactic acid bacteria colonies on MRS medium.



Apendix 11: Yeast colonies on PDA medium.



Apendix 12: Approvement letter for sensory analysis from the Committee for the Protection of Human Beings in Investigations

UNIVERSIDAD DE PUERTO RICO EN MAYAGÜEZ
DECANATO DE ASUNTOS ACADÉMICOS
COMITÉ PARA LA PROTECCIÓN DE LOS SERES HUMANOS EN LA INVESTIGACIÓN
(CPSHI/IRB-- 00002053)

09-08-LS- 02

26 de marzo del 2009

Srta. Lurdes Siberio Pérez
73 Calle Artocarpus
Isabela, PR 00662

Estimada Srta. Sibero:

El comité revisó su propuesta: Use of stabilizers to texture of a fermented beverage elaborated from milk and acid y luego de las correcciones aprueba gustosamente su investigación.

La aprobación de su propuesta de investigación se extiende desde el 26 de marzo del 2009 hasta el 26 de marzo del 2010. Le recuerdo que cualquier modificación de su proyecto necesitaría pasar por una nueva revisión por parte de este Comité.

Le deseo mucho éxito en su trabajo de investigación y quedo a sus órdenes para cualquier pregunta o clarificación ulterior que estimase necesaria.

Cordialmente,

Dafne Javier, D.B.A.
Presidenta CPSHI
UPR, Mayagüez

Apendix 13: Evaluation sheet used in the Different from Control Test

Prueba Diferente al Control

Número de panelista _____

Instrucciones:

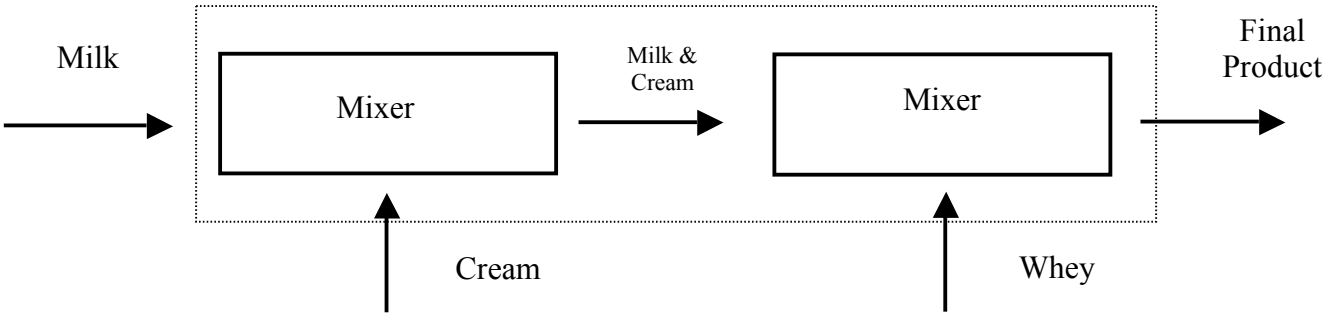
1. **Usted recibirá 5 muestras, una identificada como el control y 4 muestras identificadas con números aleatorios de tres dígitos.**
    a. **Escriba el número de sus muestras en orden de izquierda a derecha en los encacillados provistos.**
2. **Pruebe y evalúe el control.**
    a. **Luego evalúe las muestras por separado de izquierda a derecha, pausando 30 segundos entre las muestras.**
        i. **Entre cada muestra limpie su paladar tomando agua y comiendo galleta.**
3. **Utilizando la escala provista debajo, determine la magnitud de las diferencias de las muestras con respecto al control.**
    a. **Marque con una *X* para cada número de muestra correspondiente la diferencia que existe entre la muestra evaluada y el control.**
4. **Si tiene algún comentario lo puede añadir en el espacio provisto al final de la hoja.**

Número de muestra										
No hay diferencia										
Diferencia leve										
Poca diferencia										
Diferencia moderada										
Bastante diferencia										
Gran diferencia										
Diferencia extrema										

Comentarios:_____
_____
_____
_____.

Apendix 14: Sensory evaluation

Apendix 15: Theoretical Mass Balance of Solids in Whey-Milk Samples



1. Equations to be used:
    a. Total mass balance around the dashed line:
        1. $m_{milk} + m_{cream} + m_{whey} = m_{final\ product}$
    b. Mass balance of solids around dashed line:
        1. $x_{milk}m_{milk} + x_{cream}m_{cream} + x_{whey}m_{whey} = m_{final\ product}$
2. where x_{milk}, x_{cream}, and x_{whey} are the theoretical percentages of the solids in each given substances.
3. Theoretical percent of solids in the final product:

$$\frac{m_{solids\ final\ product}}{m_{final\ product}}\ x\ 100\% \qquad \frac{362.73}{3600.0}\ x\ 100\% = 10.1\%$$

75% Whey/25%Milk Mixture

	Mass used (g)	Theoretical % of Solids	Mass of solids in sample
Cream	272.42	41.61	113.4
Milk	627.58	12.41	77.88
Whey	2700.0	6.35	171.45
Total	3600.0		362.73

4. Percent yield

$$\frac{Experimental\ yield\ of\ solids}{Theoretical\ yield\ of\ solids}\ x\ 100\% \qquad \frac{9.25}{10.1}\ x\ 100\% = 92.0\%$$

50% Whey/50%Milk Mixture

	Mass used (g)	Theoretical % of Solids	Mass of solids in sample
Cream	260.5	41.61	108.4
Milk	1539.5	12.41	191.1
Whey	1800.0	6.35	114.3
Total	3600.0		413.8

1. Theoretical percent of solids in the final product:

$$\frac{m_{solids\ final\ product}}{m_{final\ product}}\ x\ 100\% \qquad \frac{413.8}{3600.0}\ x\ 100\% = 11.5\%$$

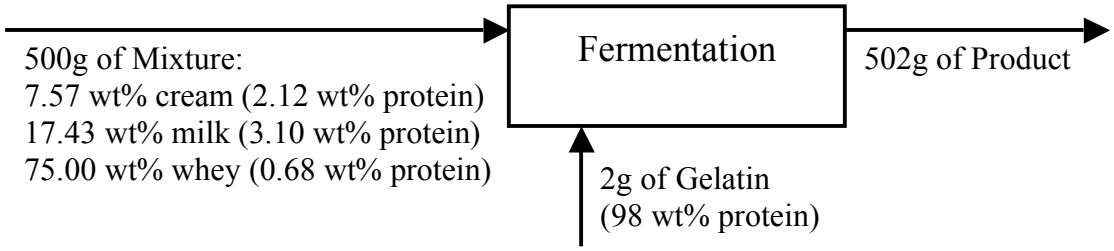
2. Percent yield

$$\frac{Experimental\ yield\ of\ solids}{Theoretical\ yield\ of\ solids}\ x\ 100\% \qquad \frac{9.56}{11.5}\ x\ 100\% = 83.1\%$$

Apendix 16: Mass Balance for Protein Content

A. 75/25 mixture:

Gelatin



Mass Balance of Protein:

$$M_{\text{protein in final product}} = M_{\text{protein in cream}} + M_{\text{protein in milk}} + M_{\text{protein in whey}} + M_{\text{protein in gelatin}}$$

$$M_{\text{protein in final product}} = y_{\text{cream}} x_{\text{cream}} M_{\text{mixture}} + y_{\text{milk}} x_{\text{milk}} M_{\text{mixture}} + y_{\text{whey}} x_{\text{whey}} M_{\text{mixture}} + y_{\text{gelatin}} M_{\text{protein in gelatin}}$$

$$M_{\text{protein in final product}} = \left(y_{\text{cream}} x_{\text{cream}} + y_{\text{milk}} x_{\text{milk}} + y_{\text{whey}} x_{\text{whey}}\right) M_{\text{mixture}} + y_{\text{gelatin}} M_{\text{protein in gelatin}}$$

1. Where: M is the mass of the original mixture, x_{cream}, x_{milk}, and x_{whey} are the weight percents of cream, milk and whey in the original mixture, and y_{cream}, y_{milk}, y_{whey}, and y_{gelatin} are the theoretical weight percents of protein in cream, milk, whey, and gelatin, respectively.

2. Thus, substitution of given values yields:

$$M_{\text{protein in final product}} = \left(0.0212 \times 0.0757 + 0.0310 \times 0.1743 + 0.0068 \times 0.75\right) 500 \text{ g} + 0.98 \times 2 \text{ g}$$

$$M_{\text{protein in final product}} = 8.01 \text{ g}$$

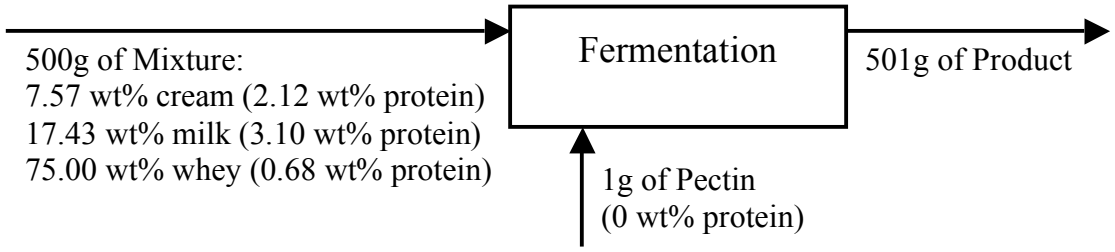
3. And the theoretical weight percent of protein in the final product is:

$$\frac{8.01 \text{ g}}{502 \text{ g}} \times 100\% = 1.60\%$$

4. Percent yield

$$\frac{Experimental\ yield\ of\ solids}{Theoretical\ yield\ of\ solids} x\, 100\% \qquad \frac{1.07}{1.60} x\, 100\% = 66.9\%$$

Pectin



1. Thus, substitution of given values yields:

$$M_{\text{protein in final product}} = \left(0.0212 \times 0.0757 + 0.0310 \times 0.1743 + 0.0068 \times 0.75\right)500\ \text{g} + 0.00 \times 1\ \text{g}$$

$$M_{\text{protein in final product}} = 6.05\ \text{g}$$

2. And the theoretical weight percent of protein in the final product is:

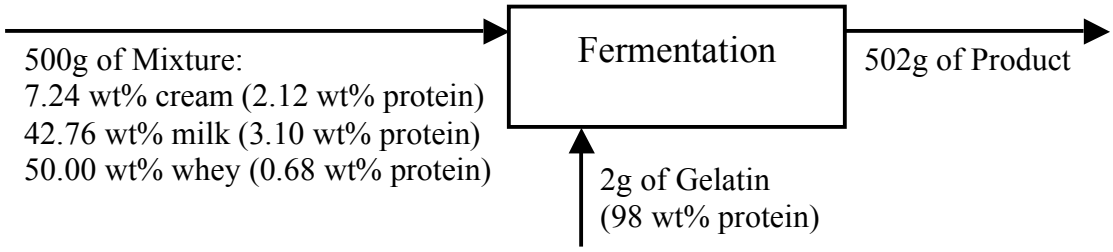
$$\frac{6.05\ \text{g}}{501\ \text{g}} \times 100\% = 1.21\%$$

3. Percent yield

$$\frac{\textit{Experimental yield of solids}}{\textit{Theoretical yield of solids}}\, x\, 100\% \qquad \frac{0.73}{1.21}\, x\, 100\% = 60.3\%$$

B. 50/50 Mixture:

Gelatin



Mass Balance of Protein:

$$M_{\text{protein in final product}} = M_{\text{protein in cream}} + M_{\text{protein in milk}} + M_{\text{protein in whey}} + M_{\text{protein in gelatin}}$$

$$M_{\text{protein in final product}} = y_{\text{cream}} x_{\text{cream}} M_{\text{mixture}} + y_{\text{milk}} x_{\text{milk}} M_{\text{mixture}} + y_{\text{whey}} x_{\text{whey}} M_{\text{mixture}} + y_{\text{gelatin}} M_{\text{protein in gelatin}}$$

$$M_{\text{protein in final product}} = \left(y_{\text{cream}} x_{\text{cream}} + y_{\text{milk}} x_{\text{milk}} + y_{\text{whey}} x_{\text{whey}}\right) M_{\text{mixture}} + y_{\text{gelatin}} M_{\text{protein in gelatin}}$$

1. Where: M is the mass of the original mixture, x_{cream}, x_{milk}, and x_{whey} are the weight percents of cream, milk and whey in the original mixture, and y_{cream}, y_{milk}, y_{whey}, and y_{gelatin} are the theoretical weight percents of protein in cream, milk, whey, and gelatin, respectively.

2. Thus, substitution of given values yields:

$$M_{\text{protein in final product}} = \left(0.0212 \times 0.0724 + 0.0310 \times 0.4276 + 0.0068 \times 0.50\right)500\ \text{g} + 0.98 \times 2\ \text{g}$$

$$M_{\text{protein in final product}} = 11.06\ \text{g}$$

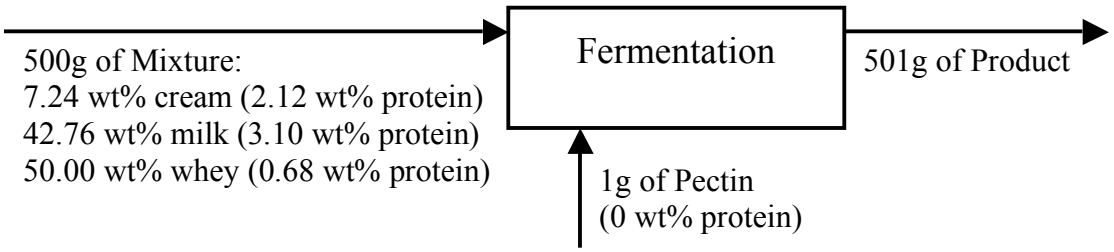
3. And the theoretical weight percent of protein in the final product is:

$$\frac{11.06\ \text{g}}{502\ \text{g}} \times 100\% = 2.20\%$$

4. Percent yield

$$\frac{\textit{Experimental yield of solids}}{\textit{Theoretical yield of solids}}\, x\, 100\% \qquad \frac{1.93}{2.20}\, x\, 100\% = 87.7\%$$

Pectin



1. Thus, substitution of given values yields:

$$M_{\text{protein in final product}} = \left(0.0212 \times 0.0724 + 0.0310 \times 0.4276 + 0.0068 \times 0.50\right)500\ \text{g} + 0.0 \times 1\ \text{g}$$

$$M_{\text{protein in final product}} = 9.10\ \text{g}$$

2. And the theoretical weight percent of protein in the final product is:

$$\frac{9.10\ \text{g}}{501\ \text{g}} \times 100\% = 1.82\%$$

3. Percent yield

$$\frac{\textit{Experimental yield of solids}}{\textit{Theoretical yield of solids}}\, x\, 100\% \qquad \frac{1.41}{1.82}\, x\, 100\% = 77.5\%$$