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Survival of *Lactobacillus acidophilus* in Fruit-Flavored Greek Yogurt Acid Whey

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Abstract

Greek yogurt has become much more popular within the last 15 to 20 years. The by-product of Greek yogurt manufacture is acid whey. Although acid whey has been considered a waste product, researchers are exploring various uses of this whey. Since the health benefits of consuming probiotics are widely known, one may propose adding probiotics to acid whey to form a probiotic beverage. Typically, probiotic bacteria do not thrive in acidic conditions. It would be beneficial to determine if the probiotic *Lactobacillus acidophilus* can survive in these acidic conditions. The objectives were to determine the growth of *L. acidophilus* in acid whey resulting from manufacturing Greek yogurt and to study any changes in apparent viscosity, pH, and titratable acidity over 4 weeks of refrigerated storage. Plain yogurt was manufactured, and whey was separated from plain yogurt to yield Greek yogurt and acid whey. Acid whey was batch pasteurized, cooled, sweetened, flavored with pineapple flavoring, inoculated with *L. acidophilus*, and stored at 4˚C for 4 weeks. The log *L. acidophilus* counts progressively decreased from 7.84 immediately after manufacture to 2.06 at week 4. There were no significant changes in pH and titratable acidity of the pineapple-flavored probiotic acid whey over 4 weeks of storage, indicating product stability over shelf life. Viscosity changed over the storage time with minimum values at week 2 and maximum values at week 4. Although the counts declined over 4 weeks of storage, some *L. acidophilus* survived in the pineapple-flavored acid whey.

Keywords

Acid Whey, Lactobacillus Acidophilus, Greek Yogurt, Whey Beverage, Probiotic

1. Introduction

Greek-style yogurt is a thick yogurt that has become much more popular within the last 15 to 20 years. Production of Greek yogurt has increased from 1% - 2% of the U.S. yogurt market in 2004 to 36% of the U.S. yogurt market in 2015, reaching 771,000 metric tons [1]. Lange *et al.* [2] reported that the per capita basis of yogurt consumption by Americans in 2017 was 13.7 liters, with 37.8% consisting of Greek-type yogurt. Greek yogurt can be manufactured using a cloth bag (traditional method), a mechanical separator, membrane filtration techniques, or by direct recombination as described by Lange *et al.* [2]. Greek yogurt has a healthy image and better nutritional properties than regular yogurt because of its approximately 2.5 times higher protein content and 1.5 times higher mineral content [2]. Because of the expelled acid whey, Greek-style yogurt has higher total solids content than conventional yogurt. Jovanovic *et al.* [3] analyzed Greek yogurts in Serbian markets and found a protein content of 8.01% and a total solids content of 13.54% for nonfat Greek yogurt and protein content of 7.90% and a total solids content of 15.69% for low-fat Greek yogurt.

One kilogram of Greek yogurt leads to two to three kilograms of expelled acid whey after straining or centrifuging [1]. Acid whey from commercial Greek yogurt was found to contain 6.0% - 6.2% total solids, 0.171% - 0.371% total protein, 0.64% - 0.75% ash, 3.33% - 3.5% lactose, and 0.56% - 0.65% galactose [4]. Titratable acidities ranged from 0.42% - 0.53%, and pH values ranged from 4.21 - 4.48. Chemical oxygen demand values ranged from 52,400 to 64,400 mg/L, and biochemical oxygen demand values ranged from 45,800 to 50,500 mg/L, indicating the difficulties in disposal [4]. Also, because of the low pH and the low true protein nitrogen content, it is difficult to spray dry for concentration and nutrient extraction [5] [6].

There are different ways to dispose or utilize Greek yogurt acid whey. Farmers can spread a limited amount of whey onto fields as fertilizer, but too much whey can lead to run-off to local waterways resulting in algal blooms, reduced dissolved oxygen, and death of fish. Some farmers and companies treat Greek yogurt acid whey in anaerobic digesters for production of methane for generating electricity [1]. Greek yogurt acid whey can also be used as animal feed when mixed with silage and [7] or used to produce industrial-grade ethanol [1].

Utilization of Greek yogurt acid whey for isolating or producing valuable ingredients or for incorporating into human foods is an active research topic for many companies and universities. High-purity lactose and valuable minor components such as proteins and peptides can be isolated from Greek yogurt acid whey [7]. Greek yogurt acid whey can be used to produce galacto-oligosaccharides [7]. Acid whey from Greek yogurt production can be utilized in ranch dressing [5] and in pancakes and pizza crust [8]. Yoon and Rizvi [9] incorporated Greek yogurt acid whey into milk protein concentrate-based extruded snack products and improved quality of these products based upon increased radial expansion ratio, increased porosity, and decreased piece density.

Consumption of Greek yogurt acid whey could potentially provide some health benefits as there are reported health benefits provided by consumption of acid whey from cheese and from whey proteins. Wronkowska *et al.* [10] reported that concentrated acid whey obtained from Tvarog cheese fed to rats provided various beneficial effects within their cecum. Corrochano *et al.* [11] found that individual whey peptides have a bioactive effect on muscle and liver cell lines.

Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [12]. Various species of *Lactobacillus* and *Bifidobacteria* are the most common types of probiotics, but there are also other types of probiotics including *Enterococcus*, *Streptococcus*, *Pediococcus*, *Lactococcus*, *Saccharomyces*, *Leuconostoc*, *Bacillus*, and *Escherichia* [13]. A summary of health benefits provided by various types of probiotics has been given by Fijan [13]. Probiotics are commonly added to yogurt [14]. Sales of products containing probiotics are increasing because the health benefits that they provide are widely known.

L. acidophilus is a well-known probiotic. Some health benefits provided by *L. acidophilus* include improving lactose digestion [15], reducing serum cholesterol levels [16], aiding prevention of certain cancer types [17], stimulating the immune system [18], controlling female urogenital infections [19], preventing or controlling intestinal infections [20], improving bone health [21], alleviating type 2 diabetes [22], and protecting against skin aging [23]. *L. acidophilus*, depending upon the strain, do not grow well in acidic conditions as *L. acidophilus* counts drop substantially once pH of the dispersion falls below 2.5 [24].

Although much work has been conducted on the utilization of cheese whey and some work has been conducted on the utilization of Greek yogurt acid whey into certain products, it would be desirable to expand upon Greek yogurt acid whey utilization. Also, it would be desirable to incorporate probiotics into Greek yogurt acid whey to expand upon its likely health beneficial properties. Since Greek yogurt acid whey naturally contains some proteins and sugars needed for lactic acid bacterial growth, perhaps *L. acidophilus* in pineapple-flavored Greek yogurt acid whey could survive. The objectives of this research were to determine the growth of the probiotic *L. acidophilus* in pineapple-flavored Greek yogurt acid whey beverage and to study any changes in apparent viscosity, pH, and titratable acidity over 4 weeks of refrigerated storage.

2. Materials and Methods

2.1. Manufacture of Greek Yogurt Acid Whey

To prepare the yogurt base, pasteurized skim milk was warmed to 40˚C and inoculated with 0.2% (w/w) of starter culture in a 1:1 ratio of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5 (Chr. Hansen, Milwaukee, WI, USA). The inoculated skim milk was placed in an incubator at 40˚C until the pH dropped to 4.7. Curd was drained through a cheese cloth and acid whey was collected and batch pasteurized at 62.8˚C for 30 min before being placed into a cooler at 7.2˚C. After overnight storage, sugar was added at 10% (w/v) and pineapple flavor concentrate (Dippin Flavors, St. Louis, MO, USA) was incorporated at 3% (v/v). *L. acidophilus* LA-K (Chr. Hansen, Milwaukee, WI, USA) was added at 0.3% (v/v) into the sweetened and flavored Greek yogurt acid whey. Inoculated Greek yogurt acid whey was packaged into cups and stored until analyzed. Samples were analyzed immediately after inoculation with *L. acidophilus* and at weeks 1, 2, 3, and 4 for *L. acidophilus* counts, apparent viscosity, pH, and titratable acidity.

2.2. *Lactobacillus acidophilus* Enumeration

The method used for enumerating *L. acidophilus* was a modification of the procedure of Dave and Shah [25] and Tharmaraj and Shah [26]. MRS base medium without dextrose was prepared by weighing 10.0 g proteose peptone #3 (United States Biological, Swampscott, MA, USA), 10.0 g of beef extract (Becton, Dickinson and Co., Sparks, MD, USA), 5.0 g of yeast extract (Becton, Dickinson and Co., Sparks, MD, USA), 1.0 g of polysorbate 80 (Tween 80) (Sigma-Aldrich Inc., St. Louis, MO, USA), 2.0 g of ammonium citrate (Thermo Fisher Scientific, Instruments, Waltham, MA, USA), 5.0 g of sodium acetate, anhydrous (EMD Chemicals Inc., Gibbstown, NJ, USA), 0.1 g of magnesium sulfate, anhydrous (EMD Chemicals Inc., Gibbstown, NJ, USA), 0.05 g of manganese sulfate, monohydrate (Sigma-Aldrich Inc., St. Louis, MO, USA), 2.0 g of dipotassium phosphate (Fisher Scientific, Fair Lawn, NJ, USA), and 15.0 g of agar (EMD Chemicals Inc., Gibbstown, NJ, USA) and adding 1 L of distilled water. This mixture was heated to boiling with agitation before autoclaving at 121˚C for 15 min. A 10% (w/v) sorbitol (EMD Chemicals Inc., Gibbstown, NJ, USA) solution was prepared and filtered sterilized with Nalgene Membrane Filter Units (Nalge Co., Rochester, NY, USA), and the appropriate amount of this solution was aseptically added to the MRS base medium to form a 10% sorbitol solution (final sorbitol concentration of 1%) and 90% MRS base medium mixture immediately before pouring the plates. The appropriate dilutions of acid whey were made with 99 mL of sterilized 0.1% Bacto peptone (Becton, Dickinson and Co., Sparks, MD, USA). The pour plate method with this MRS-sorbitol agar was performed. Petri dishes were placed in BBL GasPaks (BBL, Becton, Dickinson and Co., Cockeysville, MD, USA). Anaerobic conditions were prepared with the use of a flame on a candle within the BBL GasPaks, and the petri dishes were incubated anaerobically at 37˚C for 48 h. A Quebec Darkfield Colony Counter (Leica Inc., Buffalo, NY, USA) was used to assist in enumerating the colonies.

2.3. The pH

The pH was determined using an Orion pH meter model 250 A/610 (Thermo Fisher Scientific, Instruments, Waltham, MA, USA) calibrated using commercial pH 4.00 and 7.00 buffers (Thermo Fisher Scientific, Instruments, Waltham, MA, USA).

2.4. Titratable Acidity

A 9 g sample of Greek yogurt acid whey was obtained to which 6 drops of phenolphthalein were added. The mixture was titrated against 0.1 N NaOH to a faint pink endpoint using a 1.89 L (1/2 gallon) Kimax NAFIS Automatic Acidity Test apparatus (Kimble Chase, Vineland, NJ, USA). The titratable acidity results were expressed as percent lactic acid.

2.5. Apparent Viscosity

The apparent viscosities of the Greek yogurt acid whey were determined at 7˚C using a Brookfield DV II+ viscometer (Brookfield Engineering Lab Inc., Stoughton, MA, USA) on a helipath stand. A disc spindle #1 was used at 50 rpm. The data were acquired using the Wingather® software (Brookfield Engineering Lab Inc., Stoughton, MA, USA). For each replication, 100 data points were obtained and averaged.

2.6. Statistical Analysis

The data for *L. acidophilus* counts, pH, titratable acidity, and apparent viscosity were each analyzed as a randomized block design using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). Differences of least squares means were used to denote significant differences between different weeks. Significance was set at $\alpha = 0.05$. Three replications were conducted. The data in the graphs are presented as mean of three replications ± standard error.

3. Results

3.1. *Lactobacillus acidophilus* Enumeration

L. acidophilus log counts as a function of storage time are presented in Figure 1. Storage time (week) was significant ($P < 0.0001$). *L. acidophilus* log counts progressively decreased from 0 to 4 weeks, especially from the initial log counts

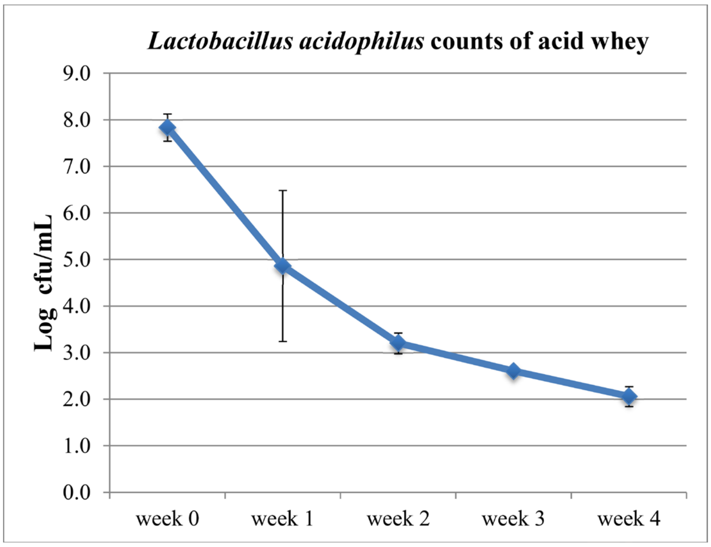

Figure 1. *Lactobacillus acidophilus* log counts of Greek yogurt acid whey as a function of storage time during 4 weeks of storage. The data in the graphs are presented as mean of three replications ± standard error.

(7.84) to week 1 (4.86) and then to week 2 (3.20). However, the decreases from week 2 (3.20) to week 3 (2.60) and from week 3 (2.60) to week 4 (2.06) were not significant.

3.2. The pH

The pH as a function of storage time is shown in **Figure 2**. Storage time (week) was not significant ($P > 0.05$). The pH values ranged from 4.35 at week 3 to 4.44 at week 4.

3.3. Titratable Acidity

The titratable acidity over 4 weeks of storage is presented in **Figure 3**. Similar to the pH, storage time (week) for titratable acidity was not significant ($P > 0.05$).

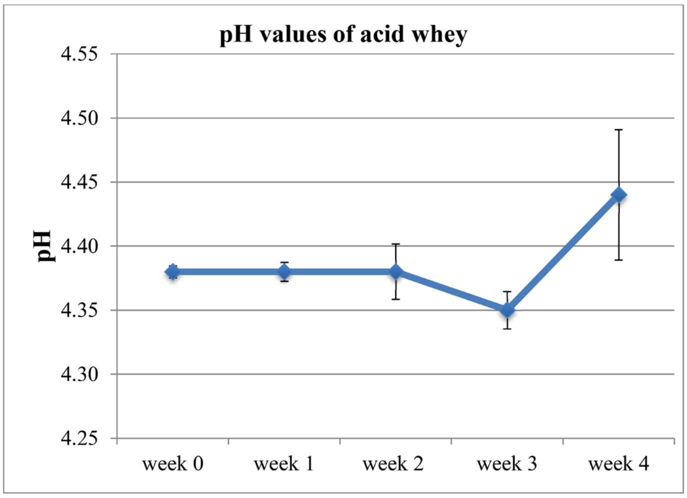

Figure 2. The pH of Greek yogurt acid whey as a function of storage time during 4 weeks of storage. The data in the graphs are presented as mean of three replications ± standard error.

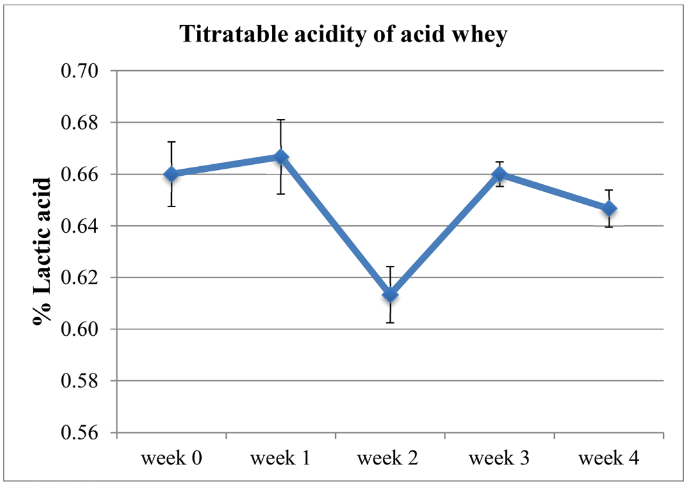

Figure 3. The titratable acidity (expressed as % lactic acid) of Greek yogurt acid whey as a function of storage time during 4 weeks of storage. The data in the graphs are presented as mean of three replications ± standard error.

The titratable acidity values ranged from 0.61% at week 2 to 0.67% at week 1.

3.4. Apparent Viscosity

The apparent viscosity over 4 weeks of storage is shown in **Figure 4**. Storage time (week) was significant ($P < 0.038$). The apparent viscosity at week 4 (14.46 cP) was significantly higher than the apparent viscosity at week 2 (13.09 cP).

4. Discussion

L. acidophilus was first identified by Ernst Moro in 1900 [27]. Some early papers described the development of suitable media [27] and nutritional requirements for *L. acidophilus* [28]. Kulp and Rettger [27] reported that casein digest and tryptic digest of powdered skim milk and galactose were beneficial for *L. acidophilus* growth. Kitay and Snell [28] reported that all 10 of their *L. acidophilus* cultures required oleic acid and 9 out of 10 required thymidine, other desoxyribosides, and vitamin B_{12}, but none of these 10 cultures required enzymatic casein digest. More recently, Lv *et al.* [29] measured nutrition consumption patterns of *L. acidophilus* grown in a chemically defined media to assist with the design of the optimum cultivation media for *L. acidophilus*. They found high consumption rates for aspartate, arginine, folic acid, pyridoxine, Fe^{2+}, Mn^{2+}, uracil, and thymine. The detailed composition including individual proteins, amino acids, minerals, sugars, fatty acids, vitamins, and organic acids of commercial Greek yogurt acid whey was provided by Menchik *et al.* [4]. Addition of required nutrients for growth of a particular strain of *L. acidophilus* that are not present or not present at adequate levels in Greek yogurt acid whey would likely increase *L. acidophilus* counts.

Various types of probiotics and prebiotics have been added to whey-based beverages. Drgalić *et al.* [30] were able to grow *L. acidophilus*, *L. casei*, and *Bifidobacterium bifidum* in a reconstituted beverage of whey, presumably from

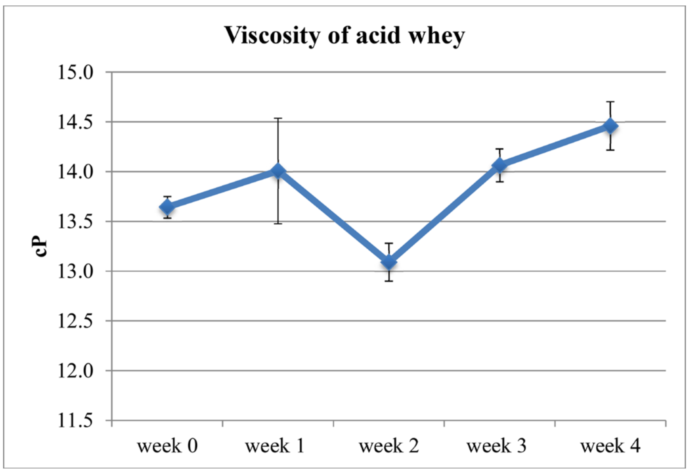

Figure 4. The apparent viscosity of Greek yogurt acid whey as a function of storage time during 4 weeks of storage. The data in the graphs are presented as mean of three replications ± standard error.

cheese. *L. acidophilus* counts in their beverage also tended to decline during storage, especially after 14 days of storage. Unlike the present study, the pH of the fermented whey beverage in their study decreased by about 0.3 pH units. Pescuma *et al.* [31] formulated a fermented whey beverage with *L. acidophilus*, *L. bulgaricus*, or *S. thermophilus* and found that the beverage with *L. acidophilus*, unlike the other beverages, only had a slight decrease in pH. They also found that *L. acidophilus* grew slower than the other cultures. Skryplonek *et al.* [32] prepared various probiotic beverages based on acid whey originating from semi-fat quark cheese production, milk, and skim milk powder fermented with either *L. acidophilus* or *Bifidobacterium animalis* ssp. *lactis* and was able to maintain counts greater than 10^8 cfu/mL during 21 days of storage. Castro *et al.* [33] prepared various probiotic beverages formulated with various ratios of pasteurized milk and Minas Frescal cheese whey ranging from 100% pasteurized milk to 20% pasteurized milk and 80% whey. Their *L. acidophilus* counts after 7 days of storage at 5˚C ranged from 8.69 to 8.83, indicating that the percent of Minas Frescal cheese whey had only minor effects on *L. acidophilus* counts. Matijević *et al.* [34] inoculated reconstituted sweet whey with *L. acidophilus* La-5 and found counts decreased from almost 8.0 log cfu/mL at day 1 of cool storage to about 5.0 log cfu/mL at day 28. Hernandez-Mendoza *et al.* [35] prepared a reconstituted whey beverage inoculated with *L. reuteri* and *B. bifidum* and found levels of both probiotics remained above 10^6 cfu/mL throughout the 30 day storage period. Sharma *et al.* [36] developed a whey protein concentrate product containing oats as a prebiotic and fermented this product with *L. acidophilus* and *L. bulgaricus*. They found the total viable counts decreased from 9.1×10^{10} cfu/mL at day 0 to 8.8×10^9 cfu/mL at day 15 of storage, but the viable counts of each type of *Lactobacillus* were not specified. Skryplonek and Jasińska [37] developed various acid whey fermented beverages by combining acid whey obtained from manufacture of semi-fat tvorog cheese with various combinations of UHT milk (full fat and skim), unsweetened condensed milk, skim milk powder, whey protein concentrate (WPC 35 and WPC 80), inulin, and oligofructose.

Since whey does not have an appealing taste, a pineapple flavor concentrate was added in the present study. Many different flavors, including orange and citrus, fruits including tropical fruits, berries, vanilla, chocolate, and various grains, of whey-based beverages have been developed in other studies [38]. Djurić *et al.* [38] flavored whey beverages with orange, pear, peach, and apple flavorings and concluded that the peach-whey blend was the most desirable.

Further research could be performed with the goal of increasing the survival of *L. acidophilus*. Approaches could include use of different strains of *L. acidophilus*, increasing the inoculation level of *L. acidophilus*, and addition of prebiotics or other nutrients to the whey.

Possible uses of Greek yogurt acid whey inoculated with *L. acidophilus* include development of a probiotic-containing flavored acid whey beverage and a probiotic-containing ingredient for incorporation into other foods. The utilization of probiotic-containing acid whey from Greek yogurt would help to reduce the need for disposal of an environmentally hazardous by-product by developing healthy flavored beverages and formulated functional foods.

5. Conclusion

This project was performed to determine if *L. acidophilus* can survive during storage in pineapple-flavored acid whey prepared by manufacturing Greek yogurt. Although *L. acidophilus* survived in the flavored acid whey, their counts declined over 4 weeks. No significant changes in pH and titratable acidity and only minor changes in viscosity were found during this storage. The data from this project may be beneficial in reducing the disposal of an environmentally hazardous by-product by developing a healthy functional and flavored beverage and ingredients. Some opportunities for future experimentation may even exist to further increase *L. acidophilus* counts in this type of beverage or an ingredient made from this beverage that could possibly provide improved health benefits.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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