

Survivability of *Escherichia Coli* in Commercial Powder Goat Milk during Four Months Storage at Two Different Temperatures

Brittany I Davis, Aftab Siddique, Ajit K Mahapatra and Young W Park*

Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, USA

*Corresponding author: Young W Park, Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, GA 31030, USA, Tel: 478-827-3089; E-mail: parky@fvsu.edu

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Abstract

Bacteria in low moisture environments are not favorable for growth, but can survive and cause a possible food safety risk for consumers. A study was conducted to evaluate survivability of *Escherichia coli* and storage stability of commercial powdered goat milk (PGM) products. Three batches of whole milk PGM samples were purchased at a local outlet, and divided into two halves to assign non-inoculated control and *E. coli* inoculated groups, then stored at 4 and 22°C for 0, 2, and 4 months. Results showed that significant reduction ($P < 0.05$) in *E. coli* counts occurred at 22°C treatment group by more than 2 log CFU/g at 2 months storage, then further decreased by an additional 0.37 log CFU/g at 4 months storage. The survival of *E. coli* was significantly higher at 4°C, suggesting that *E. coli* could survive better at 4°C by extending a longer latent period than at higher temperature (22°C) under the low water activity condition. *E. coli* counts had negative correlations with water activity at both temperature treatments for all three storage periods except for 0 and 2 months at 22°C, indicating that the survivability of the *E. coli* would decrease in powdered whole goat milk for 4 months of storage because of decrease in water activity. It was concluded that *Escherichia coli* survival and storage stability of the commercial PGM products were significantly ($P < 0.05$) affected by storage temperature and time.

Keywords: Survivability; *Escherichia coli*; Powder goat milk; Storage; Stability

Introduction

Goat milk accounts for approximately 2.3% of the world's milk production [1]. Goat milk and its products have gained popularity for the past two to three decades among certain ethnic groups, health food lovers, goat producers, and especially those who have allergies to cow milk [2,3]. Various products have been made from goat milk, including cheese, yogurt, butter, ice cream, powder milk, infant formula, as well as cosmetic products.

Increase in demand for goat milk and its products necessitates the assurance of a safe food supply to consumers through verification of scientific studies on food safety of the products, including cheeses and powdered milk product. Reports have shown that several foodborne pathogens are present in raw milk of different dairy species, and these pathogenic microbes can also be present in manufactured products, because they can enter opportunistically into the manufactured products during processing, packaging, distribution and storage processes [4,5].

Outbreaks of food borne illness associated with the consumption of dry or low-water activity (a_w) foods and food ingredients have been reported [6]. *Escherichia coli* O157:H7 is an example of a known pathogen in milk. The bacterium *Escherichia coli* belongs to the family of Enterobacteriaceae [7,8], and these organisms are rod shaped, Gram negative, facultative anaerobic bacteria, and can use lactose and glucose for fermentation [8].

Escherichia coli O157:H7 is capable of causing a variety of diseases in humans, ranging from mild cases of diarrhoea to extreme cases of

hemorrhagic colitis and hemolytic uremic syndrome [9]. A previous study showed that viable cells of *Escherichia coli* O157:H7 are capable of surviving in infant formula powder for as long as one year at a storage temperature of 5°C [10]. *Escherichia coli* O157:H7 is characteristic of having a high level of virulence, with the capability of causing disease at a low dosage, ranging from 5-50 cells [11], making it a great concern for food safety.

Bacteria in low moisture environments are not favorable for growth, but can survive, posing as a possible food safety risk for consumers. The drying of milk into milk powder has two main benefits: extending shelf life and easier transport by volume reduction [12-14]. Dry foods, such as powdered milk, are often considered to be safe from microbial contamination since dry foods cannot sustain the growth of these organisms due to low moisture content and water activity [6,15]. However, dry foods can be contaminated, posing as vehicles for foodborne pathogens [6,15].

Goat milk powder has attracted increasing interest from the dairy industries, since dehydration is a major alternative for milk preservation and can extend the shelf-life of goat milk without changing its nutritional and sensory qualities [16,17]. Little research has been conducted on food safety and storage stability of dehydrated or powdered goat milk (PGM) products. Tehrany and Sonneveld [14] postulated that some physical changes, caking and cohesion, as the major problems that occur during the storage of milk powders, in addition to some chemical changes occur, such as Maillard reaction and oxidation of lipids.

There has been a paucity of research reports available on the survivability of pathogens such as *E. coli* in powder goat milk during extended storage. Therefore, the objectives of this study were to: (1) assess the storage stability of commercial powdered goat milk (PGM)

samples stored at two different temperatures (4°C and 22°C) for three storage periods (0, 2, and 4 months), by evaluating basic nutrient contents, water activity and moisture contents, and (2) determine the survivability of *E. coli* in PGM samples stored at different temperatures and time storage treatments.

Materials and Methods

Experimental design

The proposed study was conducted in a 3 × 2 × 3 factorial experiment. Three batches of commercial whole GMP were purchased from a local retail outlet at Warner Robins, GA, USA and the experimental GMP samples were divided into two halves, as non-inoculated control and *E. coli* inoculated groups. For safety reasons, *E. coli* K12 was used for the study instead of the pathogenic *E. coli* O157:H7, since both bacteria have the same biological activities. Also, pathogen contaminated powders could spread by aerosol in the Biosafety laboratory. The evaluation of storage stability and survivability of *E. coli* K12 in the experimental powder milk samples were performed at two storage temperatures (4°C and 22°C) and three storage periods (0, 2, and 4 months).

Analysis of basic nutrient composition

The control (non-inoculated) GMP samples were prepared by putting 37 g of the commercial powdered milk into in 125 mL amber glass bottles, then basic nutrients analyses for all control samples were performed for fat, protein, moisture, ash and total solids contents using AOAC [18] and Davis et al. [19] methods. The basic nutrient compositions were determined only control samples at the beginning of the study, due to minimal changes would occur during the experimental storage periods. In addition, it was not practical and safe to take samples and analyze basic nutrient contents of the *E. coli* inoculated samples from the treated bottles due to the possibility of contamination of the pathogen in the laboratory and testing personnel.

Analysis of water activity

Water activity (a_w) was measured at room temperature using an AquaLab water activity meter (cx-2; Decagon Devices, Pullman, WA, USA). A small amount of dry sample (approximately 2 g) was placed into the measuring cup and loaded into the water activity meter. The water activity meter displayed the readout a_w values for the measurements of all experimental powder milk samples.

Microbiological Analysis

Escherichia coli treatment for the experimental PGM samples

For the bacterial treated group, *Escherichia coli* K12 was inoculated into milk powder and survival of the bacteria was determined using a modified version of the method described by Deng et al. [20]. The control and *E. coli* inoculated groups were treated with the same identical storage conditions for four months. The bacteria treated vials were carefully stored in the Biosafety Level II laboratory at the Stallworth Agricultural Research Station at Fort Valley State University, Fort Valley, Georgia, USA.

Enumeration and inoculation of *Escherichia coli* into milk powder

Prior to inoculation, enumeration of the initial concentration of inocula was performed by plating on 3M petrifilm (3M Center, St. Paul, MN, USA) in duplicate, along with serial dilution (1:10, Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA, USA)) and plating on 3M petrifilm according to the manufacturer's instruction. A 10 g milk powder sample was used for the inoculation procedure, in duplicate, using the procedure of Deng et al. [20]. Five grams of whole goat milk powder was placed in a sterile glass vial. Fifty microliters of the *E. coli* K12 was inoculated on top of the milk powder, and then an additional 5 g of milk powder was placed on top of the inoculated sample. Each sample was closed with a secure cap after inoculation. The glass vials with inoculated milk powder were stored at 4°C and 22°C until analysis was performed at the 0, 2, and 4 month storage periods. Three batches of whole goat milk powder were inoculated and stored into individual tubes for each storage period and each diluted sample was plated in duplicate.

Microbial analysis

A 10 g powder milk sample was examined for background microbes from each control vial, at each storage period (0, 2, and 4 months). Aerobic plate count (APC), *E.coli*/coliform, and yeast/mold counts were determined by using 3M petrifilm (3M Center, St. Paul, MN, USA). A 10 g control sample was added to 90 mL Butterfield's phosphate buffer in a 400 mL blender bag (Fisherbrand; Thermo Fisher Scientific, Waltham, MA, USA), and pummeled in the Stomacher 400 circulator (Seaward, UK Brinkmann Instruments Inc., Westbury, NY) for 30 s at 230 rpm. One milliliter of the sample in duplicate was pipetted onto the three different types of 3M petrifilm for testing the different background microbes. Another 1 mL was used for serial dilution (1:10) in phosphate buffer. One milliliter of the 10-3 and 10-5 dilution, in duplicate, was pipetted onto each type of 3M petrifilm. Petrifilms were incubated at 37°C for 24-48 hrs, in order to examine for microbial contaminations. Petrifilms for yeast/mold count were incubated at room temperature for 3 and 5 days.

Analysis of *E. coli* K12 survival in whole goat milk powder was carried out through serial dilutions and plating, as well as enrichment of the remaining sample and plating. The 10 g inoculated sample was placed into a Fisherbrand blender bag, and combined with 90 mL of Butterfield's phosphate buffer. The sample was pummeled in a Stomacher 400 circulator for 30 s at 230 rpm. One milliliter of this sample was pipetted onto 3M petrifilm (*E.coli*/coliform) in duplicate, and a 1 mL portion of the remaining was serially diluted (1:10) in Butterfield's phosphate buffer. One milliliter of the serial dilutions in duplicate was pipetted onto 3M petrifilm, to check for the presence of viable *E. coli* cells. The petrifilms were incubated at 37°C for 24-48 hrs, and the colony count was used to enumerate the cell counts. The left over slurry of inoculated milk powder in Butterfield's phosphate buffer was incubated at 37°C for 18-24 hrs for enrichment or recovery of damaged cells [20]. One milliliter of this sample was pipetted onto 3M petrifilm for detection of *Escherichia coli* K12. The petrifilms were incubated at 37°C for 24 hrs, and then colonies were counted.

Statistical Analysis

All collected experimental data were statistically analyzed using the GLM procedure of SAS program version 9.4 [21] and Steel and Torrie [22] methods for analysis of variance, least square means, and

Duncan's multiple mean comparison. The differences between treatments were assessed for effects of batch, storage temperature, and storage time on survival counts of the *E. coli* bacteria in three batches of commercial whole goat milk powder. Microbial plate counts were converted to colony forming units per gram (CFU/gram) before being statistically analyzed.

Results and Discussions

Water activity

The average water activity for the experimental PGM samples stored at 4 and 22°C for 0, 2, and 4 months storage were 0.251, 0.224, 0.249;

Water Activity											
Month 0				Month 2				Month 4			
4°C		22°C		4°C		22°C		4°C		22°C	
Means	SD	Means	SD	Means	SD	Means	SD	Means	SD	Means	SD
0.25	0.01	0.26	0.04	0.22	0.05	0.22	0.06	0.24	0.02	0.22	0.02

Table 1: Comparison of average water activity values in powdered whole goat milk samples stored four months at 4 and 22°C. SD: Standard Deviation.

Variations and higher values of a_w have been known as direct contributors to the shelf life and spoilage of food products. Water activity is an important parameter to measure stability and shelf life of foods during storage. Several parameters associated with the production and storage of whole milk powder can affect the shelf life, which include: water activity, storage temperature, and preheat treatments of the fluid milk during processing [14].

Survivability of *Escherichia coli* in the commercial PGM

Prior to the *E. coli* survival study in the experimental commercial power goat milk, the original background microbial counts for *E. coli*,

Storage	0 month						2 month						4 month					
	<i>E. coli</i>		APC		Y/M		<i>E. coli</i>		APC		Y/M		<i>E. coli</i>		APC		Y/M	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4	0	0	0.73	0.63	1.1	0.17	0	0	0.76	0.39	1.18	0	0	0	0.63	0.59	0.93	0.4
22	0	0	1.03	0.58	1.16	0.28	0	0	0.57	0.51	0.47	0.4	0	0	0.63	0.59	0.86	0.28

Table 2: Naturally existing basal average microbial counts (log CFU/g) in the non-inoculated original commercial whole goat milk powder stored for four month at 4°C and 22°C.

The survivability of *E. coli* in the *E. coli* inoculated experimental PGM samples at two temperatures for four months storage is displayed in Figures 1 and 2. A significant ($P < 0.05$) decrease in the survival of *E. coli* was found in the samples stored at room temperature (22°C). *Escherichia coli* in samples stored at room temperature (22°C) were reduced by more than 2 log CFU/g in the first two months of storage, and at four months storage were reduced further by an additional 0.37 log CFU/g. Samples stored at 4°C also had a significant reduction in the survivability of *E. coli* during the four months storage, while

0.268, 0.229, 0.221, respectively (Table 1), suggesting that no differences were found in water activity (a_w) between the two temperature treatments, nor among the three storage periods. On the other hand, slight differences were found in a_w among the three batches of the PGM. Similarly, all previous studies have shown that a_w values remained around 0.2, which are similar to the values found in the current study of the powder milks [23-25]. The level of a_w as 0.2 normally implies that there was little chance of microbial growth for the experimental PGM samples for the storage conditions of our experiment.

aerobic plate count (APC), and yeast/mold (Y/M) counts were determined in the non-inoculated control PGM samples. The results of these microbial enumerations (Table 2) revealed that average levels of these microbial counts in the control samples contained no *E. coli* and also APC and Y/M counts were well below the counts described in the typical microbiological analysis by the USDEC [12].

survival levels of the bacteria at 4°C were significantly higher than those of the samples stored at 22°C. These results may imply that the *E. coli* could survive better at lower temperature of refrigeration (4°C) than at higher room temperature (22°C) at the low level of water activity ($a_w=0.2$) in this study. On the other hand, although the refrigeration temperature (4°C) storage showed higher survival rate of *E. coli* than the room temperature (22°C) in the experimental PGM samples, the microbial counts of both treatment groups declined continuously due to low water activity condition.

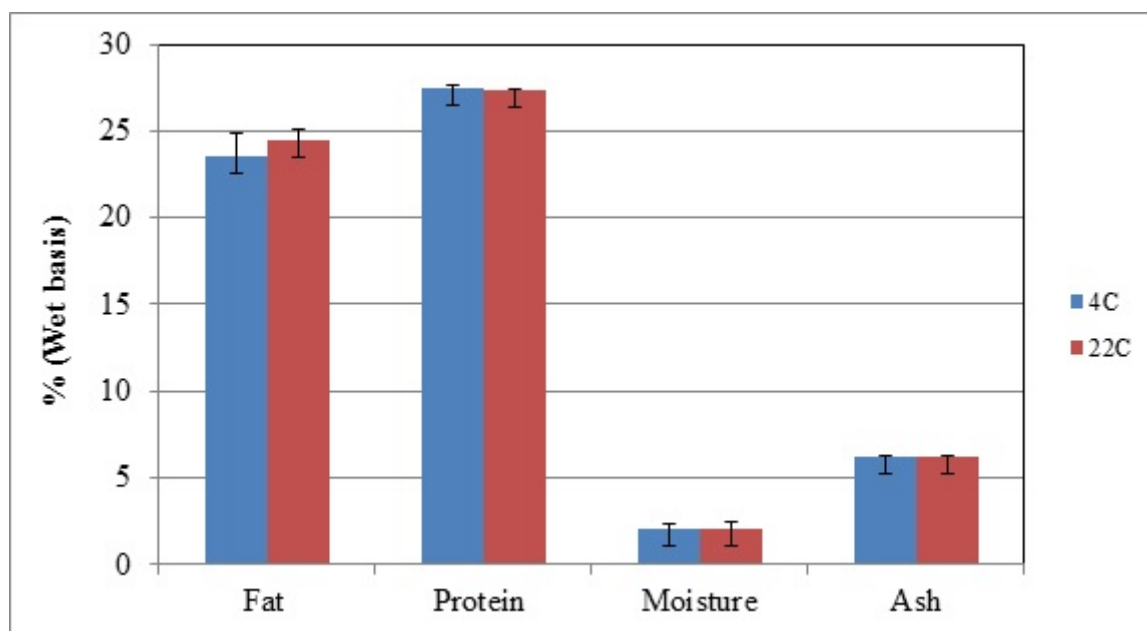


Figure 1: Comparison of basic nutrient compositions of experimental powder goat milk samples stored at 4°C and 22°C.

This higher survival rate among *E. coli* stored at the lower temperature found in this study is in the agreement with previous reports involving other low water activity foods. Deng et al. [20] reported higher survival rates of *Escherichia coli* O157:H7 in various low water activity foods including buttermilk powder, almond paste, powdered chicken, and sour cream powder when stored for 19 weeks at 5°C. *Escherichia coli* O157:H7 has also been reported to survive in powdered infant formula during 1 year storage at 5°C [10].

both temperature treatments for all three storage periods (0, 2, and 4 months) except for 0 and 2 months at 22°C (Table 3). For higher temperature (22°C), the *r* value during 4 months was negative and significant ($P < 0.05$), while the *r* values at 0 and 2 week storage were not significant. These correlation data may indicate that the survivability of the *E. coli* would decrease in the commercial powdered whole goat milk during 4 months of storage, as the water activity (water content) decreased for the prolonged storage period.

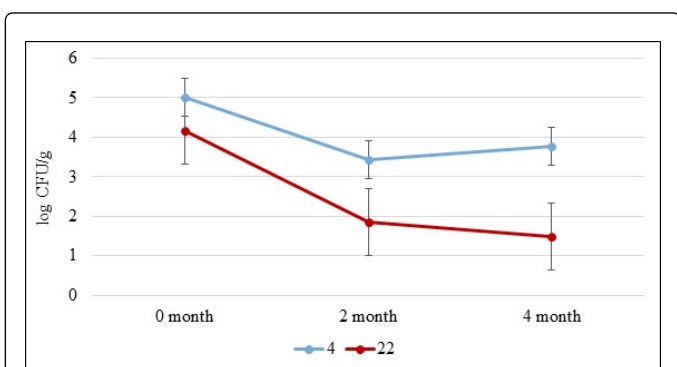


Figure 2: Comparison of the survival of *Escherichia coli* (log CFU/g) counts in powder whole goat milk stored for four months at 4°C and 22°C.

Correlations between *E. coli* counts and water activity

There were significant gradual decreases in *E. coli* counts during 0, 2 and 4 months storage of the commercial PGM samples. On the other hand, there were slight decreases in water activity for both temperature treatments except at 4°C for 4 month period. These data resulted in negative correlations (*r*) between *E. coli* counts and water activity at

Water activity	<i>E. coli</i> count		<i>E. coli</i> count		<i>E. coli</i> count	
	Month 0		Month 2		Month 4	
	4°C	22°C	4°C	22°C	4°C	22°C
	-0.574	0.404	-0.644	0.346	-0.345	-0.854*

Table 3: Correlation coefficients between *E. coli* count and water activity. *Significant at $P < 0.05$.

Conclusion

The results of this study revealed that the survivability of *Escherichia coli* in the commercial whole goat milk powder was affected by the storage temperature and time treatments. The survival of *E. coli* was significantly higher at 4°C than at 22°C, indicating that *E. coli* survived better at refrigeration temperature than at room temperature due to a longer latent period in the low water activity ($a_w = 0.2$) environment of the dried milk products. The reduction of *E. coli* at 22°C was by more than 2 log CFU/g at 2 months storage and decreased by additional 0.37 log CFU/g at 4 months storage period.

With regard to relationship between water activity and survivability of *Escherichia coli* in the commercial whole goat milk powder products, negative correlations (*r*) were found between *E. coli* counts and water activity at both temperature treatments for all three storage

periods except for 0 and 2 months at 22°C. These correlations suggest that the survivability of the *E. coli* would decrease in powdered whole goat milk after 4 months of storage due to decrease in water activity. Further studies are desired to assess storage stability and survivability of *Escherichia coli* in powdered goat milk products by examining longer storage time and more temperature treatments.

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