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Survivability of *Salmonella* Pathogens and Physicochemical Characteristics of Powder Goat Milk Stored under Different Storage Treatment Regimens

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Abstract: Survivability of *Salmonella* pathogens in commercial powdered goat milk (PGM) under different storage treatments was investigated using three batches of PGM products stored at two temperatures (4 °C and 25 °C) and ten storage periods (0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 days). A cocktail of three *Salmonella* serotypes (*Salmonella agona*, *Salmonella enteritidis* and *Salmonella tennessee*) was inoculated to the PGM samples and then survival of *Salmonella* counts was enumerated in the inoculated and non-inoculated control groups. Results showed that the initial *Salmonella* counts were 7.103 Log CFU (colony forming unit)/g at both temperatures. At the first 3 days, the viable *Salmonella* counts were reduced about 0.94 and 1.40 Log CFU/g at 4 °C and 25 °C, respectively, where the same levels were sustained for 14 days. Further reductions continued and at the end of 180 days storage, *Salmonella* survivability was 1.15 Log CFU/g higher at 4 °C than at 25 °C under the same water activity condition. As the storage period advanced, viable pathogen counts were gradually decreased. The pH of samples stored at 4 °C for 0 and 4 month were higher than those stored at 25 °C except for 2 months, while no differences were found in water activity (a_w) between treatments of the PGM products. With regard to physicochemical characteristics, the samples stored at 25 °C showed higher POV (peroxide value) values than those stored at 4 °C for 2 and 4 month periods, indicating that the rate of lipid oxidation in the PGM was elevated by a higher storage temperature and a longer storage period. The basic nutrient compositions of the experimental PGM were similar to those reported in recent studies. Oleic acid (C18:1) was the highest, caprylic acid (C8:0) was the second highest, and behenic acid (C22:0) was the lowest concentration among all fatty acids identified in the PGM samples. Most of the fatty acid concentrations tended to decrease with advanced storage periods. This research indicates that the survivability of *Salmonella* pathogens in the PGM products stored at 4 °C for 180 days was higher than those stored at 25 °C under the same a_w condition.

Keywords: *Salmonella*; survivability; goat milk powder; storage; temperature; time; physicochemical indices; nutrients

1. Introduction

The advantages of powdered milk are the extension of shelf-life of the milk and convenient transport through reduced volume [1,2]. In terms of food safety, dehydrated milk has the advantage of limited growth of pathogenic and spoilage bacteria [3]. However, dry foods such as powdered milk can often pose potential food safety risks because certain foodborne pathogens such as *Salmonella* [4,5], *Escherichia coli* [6], *Listeria monocytogenes* [7] and *Cronobacter sakazakii* [8] can survive in an environment

of low water activity (a_w) in foods. In low a_w foods, water is in glassy and rubbery states, so it has limited mobility which helps water molecules contact bacterial cells during their interaction, and thus bacterial cells can survive for longer periods in dried foods such as dehydrated milk products [4].

Salmonella is one of the high-risk foodborne pathogens that cause salmonellosis, typhoid fever or paratyphoid fever. It is estimated that annually 1.35 million foodborne illnesses account for *Salmonella* infection, with 26,500 hospitalizations and 420 deaths in the U.S. every year [9]. In previous years, outbreaks of *Salmonella* serotypes (*Salmonella agona*, *Salmonella tennessee*) [10,11] were linked to dry milk products, which indicates the importance for specific study on the microbial safety of different species in milk powder. In addition to these overall outbreaks, incidents of foodborne illness linked to consumption of dry foods are largely caused by *Salmonella* as compared to other pathogens [12].

Previous studies have shown that the survival of *Salmonella* in dry milk has revealed higher survivability as compared to other dry foods at identical a_w and storage conditions [13]. In recent years, varieties of low a_w food products, including cheeses, apple chips, flax seed powder, dry whey powder, pistachios, flour, cereals, coconut flour, kratom powder, onion, papayas and peaches, have been recalled by the FDA [14]. This indicates a potential risk of *Salmonella* contamination in dry milk powder. It was found that high storage temperature could destroy *Salmonella* pathogens in powdered milk, but adversely affected the flavor of the products [15]. However, some strains of *Salmonella* in dried milk are found to be highly heat resistant, where viable cells could survive even after 10 h drying process at 76.6 °C [16].

Storage conditions are considered as the major factors affecting not only microbial survivability, but also the quality and stability of powdered milk products. The quality of powdered milk during storage is determined by the manufacturing and drying techniques of milk [2]. According to the standards of the U.S. Dairy Export Council (2005) [1], milk powder should be stored in a cool and dry environment at a temperature less than 27 °C and relative humidity less than 65%. In addition, milk powder needs to be stored in a light-, oxygen- and moisture-proof container, where these conditions can extend storage life up to 6–9 months.

Powdered milk is an important source of ingredients used for various food products such as bakery products, ice cream, chocolate, yogurt, infant formula, cheeses and reconstituted milk [17]. Due to lipid oxidation and Maillard reaction between reducing sugars and amino acids, dehydrated milk may have physical changes, caking and cohesion that occur during storage, which can deteriorate the sensory properties of powdered milk products [2,18].

The majority of previous studies on *Salmonella* survivability in dehydrated milk products have been conducted using bovine milk products. To our knowledge, there are almost no reports available on the survivability of *Salmonella* pathogens in dehydrated caprine milk in relation to physicochemical and nutritional characteristics of the powder products. Therefore, the objectives of this study were to: (1) investigate the survivability of *Salmonella* pathogens in powdered goat milk (PGM) during 6 months of storage at two different temperature (4 °C and 25 °C) treatments, and (2) evaluate the possible relationship between nutritional and physicochemical characteristics with the survivability of *Salmonella* pathogens in commercial PGM products.

2. Materials and Methods

2.1. Experimental Design

The microbial study was carried out in a $3 \times 2 \times 10$ factorial experiment. The storage stability and survivability of *Salmonella* of experimental powdered goat milk (PGM) samples were evaluated in the three batches at two storage temperatures (4 °C and 25 °C) and ten storage periods (0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 days).

Due to the fewer expected changes in nutrient and physicochemical parameters of powdered milk, a $3 \times 2 \times 3$ factorial experiment was conducted as three batches, with two temperatures (4 °C and 25 °C) and three storage periods (0, 2 and 4 months) for chemical indices. During the experiment,

chemical parameters (pH, water activity, fatty acid analysis, peroxide value) were evaluated at three storage periods, while the basic nutrient contents (protein, fat, moisture and ash) were analyzed one time at the beginning of the experiment.

2.2. Preparation of Experimental Powdered Goat Milk Products

Three lots of commercial powdered goat milk (PGM) (Meyenberg Whole Powder Goat Milk; Jackson-Mitchell, Inc., Turlock, CA, USA) were purchased from a local retail store at Warner Robins, Georgia. The experimental PGM samples were divided into two halves: non-inoculated control, and *Salmonella*-inoculated groups. For the pathogen-treated group, a cocktail of three *Salmonella* serotypes (*Salmonella agona*, *Salmonella enteritidis* and *Salmonella tennessee*) was inoculated into the PGM samples and the survival of *Salmonella* was determined. Non-inoculated control PGM samples were prepared by placing PGM samples into sterile 120 mL amber glass bottles (CG-820-02, Fisher Scientific, Hampton, NH, USA), then the samples were stored at refrigerated (4 °C) and room temperature (25 °C) treatments for different storage periods.

The control and pathogen-treated PGM sample groups in duplicates were placed in identical storage temperature and time treatments. All microbiological analyses were conducted in the biosafety level II laboratory at the Dept. of Food Science and Technology, the University of Georgia, Athens, GA, USA.

2.3. Salmonella Survivability Experiments

2.3.1. Preparation of Salmonella Serotypes and Their Inoculation in PGM Samples

Three serotypes of *Salmonella* pathogens (*Salmonella agona, Salmonella enteritidis and Salmonella tennessee*) were obtained from the University of Georgia, Culture Bank, Athens, GA, USA. Prior to inoculation, isolated colonies of strain had been stored in Microbank™ beads at −80 °C. At the time of the experiment, each *Salmonella* strain bead had been suspended individually in 9 mL tubes of Tryptic Soy Broth (TSB) (Difco, BD; Spark, MD, USA) and incubated for 24 h at 37 °C. After the growth period, the three tubes were transferred to new TSB tubes using sterile loops. The fresh culture was grown for about 18 h before inoculation. Ten g goat milk powder (PGM) samples were inoculated by using modified methods described by Hyeon et al. (2010) [19] and Koseki et al. (2015) [12] following this procedure: first, 5 g of PGM sample was placed in a sterile 120 mL amber glass bottle (CG-820-02, Fisher Scientific, Hampton, NH, USA) and 0.1 mL of fresh culture *Salmonella* was inoculated to milk powder sample, then additional 5 g of milk powder placed on top of the inoculated bottle, and the sample was sealed securely and shaken vigorously for two minutes in all directions to distribute the inoculum homogeneously. The 120 mL amber glass bottles with inoculated PGM were stored at 4 °C and 25 °C in duplicate of each batch for all storage conditions to examine the survivability of the pathogens at the 0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 days storage period.

2.3.2. Enumeration of Salmonella Cell Counts

The survived pathogen counts were enumerated at 0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 days after inoculation, using the plate count method. Then, 10 g of inoculated sample was combined with 90 mL of 0.1% peptone water and shaken vigorously for 1 min. to distribute the inoculum homogeneously. After homogenization, 0.9 mL homogenized sample was 10-fold serially diluted using sterile test tubes containing 9 mL 0.1% peptone water to obtain the appropriate dilution, and to obtain uniform distribution dilutions were vortexed before each serial dilution. Then, 0.1 mL of appropriate dilution was transferred onto a petri dish containing xylose lysine deoxycholate (XLD) agar (Difco BD™, Sparks, MD, USA) in duplicate, and spread manually over the plate for 15 sec. by sterile spreaders (RPI Lazy-L-Spreader, Mount prospect, IL, USA). Then, the petri dishes were inverted and incubated for 24 h at 37 °C. After the incubation period, *Salmonella* colonies were enumerated to determine the survival rate of the *Salmonella* in the experimental PGM samples. Microbial plate counts were

converted to colony forming units per gram of sample (CFU (colony forming unit)/gram) before statistical analysis.

2.4. Physicochemical Assay

2.4.1. pH and Water Activity

The experimental PGM samples were reconstituted with double deionized water by dissolving 12 g of the powdered milk sample in 88 mL deionized water. The pH of samples was determined at room temperature in triplicate using a pH meter (Accumet AR10 pH meter; Fischer Scientific, Fair Lawn, NJ, USA).

2.4.2. Water Activity

Water activity (aw) was determined at room temperature using the AquaLab water activity meter (cx-2; Decagon Devices, Pullman, WA, USA). Approximately 2 g of the PGM samples was loaded in the sample holding cup and measured for aw values.

2.4.3. Peroxide Value (POV)

Peroxide value was determined using the AOCS (American Oil Chemists' Society) (1975) [20] procedures. The lipids of all experimental GMP (goat milk powder) samples were extracted using Folch et al.'s (1957) [21] method. A total of 5 g of the extracted fat sample was weighed into a 250 mL Erlenmeyer flask. Thirty mL of acetic acid-chloroform solution (3:2 *v*/*v*) was added to sample flask, and the flask content was swirled for separation of fat and aqueous phases. Next, 0.5 mL of saturated potassium iodide was added and shaken for a minute. Thirty mL of deionized water was added to each sample, and again shaken vigorously for a minute. One mL starch solution (1%) was added to each sample. The sample was titrated with 0.01 N sodium thiosulfate until the bottom layer appeared milky, which indicated the end point of titration. At the time of titration, the sample was shaken repeatedly to ensure the release of iodide from the chloroform layer. A blank sample was also run together with actual milk samples. Reagents were prepared fresh and the saturated potassium iodide was kept in a light proof container. Peroxide value was calculated according to the following equation:

$$\begin{aligned} &\text{POV}\left(\text{milliequiv. of}\frac{\text{Peroxide}}{1000}\text{g sample}\right) \\ &= \frac{[\text{titration of sample (mL)} - \text{titration of blank (mL)}] \times 0.01\ \text{N} \times 1000}{\text{sample weight(g)}} \end{aligned} \quad (1)$$

2.5. Nutritional Analysis

2.5.1. Moisture and Ash

Moisture content of the goat milk powder (GMP) samples was determined using the AOAC (Association of Official Analytical Chemists) (1995) [22] procedure. The ash content of goat milk powder samples was determined by using a referenced method [21]. A total of 2 g of powdered milk sample was weighed into a chemically clean crucible and the crucibles were placed in a muffle furnace overnight at 550 °C for complete incineration.

2.5.2. Protein Content

Protein content of powdered goat milk was analyzed by the vario MAX cube nitrogen elemental analyzer (Elementar Americas, Mt. Laurel, NJ, USA) in CNS mode for analysis of nitrogen content. The blank and standard samples of 250 mg were prepared and 250 mg GMP samples were weighed into the ceramic crucibles. All samples were placed in the carousel and then the nitrogen contents of goat milk powder were analyzed by the automated software system of the analyzer. For protein calculation, % nitrogen (N) was multiplied by the factor of 6.38 to determine % protein of samples.

2.5.3. Fat Content

The fat content of the GMP samples was extracted using the Folch et al.'s (1957) [21] extraction method. After fat extraction, 10 mL of the chloroform fat extract was placed into a clean 25 mL Erlenmeyer flask, and the flask was placed under a fume hood overnight for complete solvent evaporation. The fat content of each sample was calculated by the following equation:

$$\text{Crude Fat (\%)} = \text{lipid weight (g)} \times (10 \text{ mL (sample extracted)})/(100 \text{ mL chloroform}) \times (100 \text{ \%})/(\text{sample weight (g)}) \quad (2)$$

2.6. Fatty Acid Analysis

2.6.1. Extraction of Fat

The crude fats of all PGM samples were extracted by Folch et al. (1957) [21] and AOAC (1995) [22] procedures. In a 50 mL glass beaker, 2 g PGM sample, 8 mL methanol and 18 mL chloroform were added, and then the contents were homogenized with Waring blender for 30 s. A total of 9 mL of chloroform was additionally added to each sample which was homogenized again for 30 s. Then, 9 mL aqueous solution of zinc acetate (0.115 g zinc acetate/5 mL of deionized water) was added to each sample and again homogenized for 30 s. After homogenization, each sample in the beaker was transferred to 125 mL separatory funnel, then the funnels containing homogenized samples were placed in a 4 °C refrigerator until separation of two distinct solvent layers appeared. The chloroform layer at the bottom was collected into a new 125 mL round bottom flask. The collected fat extracted samples were condensed using the Buchi Rotavapor (R-200: BUCHI Corporation, New Castle, DE, USA) with dry ice under nitrogen gas flushing. The condensed sample flasks were capped and stored in a freezer (−18 °C) until fat and fatty acid analysis.

2.6.2. Preparation of Fatty Acid Methyl Esters (FAME)

The FAME (fatty acid methyl esters) samples were prepared by the following procedure: a total of 5 mL methanolic sodium hydroxide (0.5 N) and boiling beads were added into 125-mL-size flasks containing previously extracted fats from each PGM sample. Each sample flask was, one at a time, attached to a cooling condenser assembly and the sample flask was heated on a hot plate for 10 min. Subsequently, 5 mL of boron trifluoride (BF3) methanol reagent (12%, 1.5 M, ACROS organics; Thermo Fisher Scientific, Waltham, MA, USA) was added to each sample flask and boiled for 2 min. Heating was removed from sample flasks, which continued to be attached to the condenser and cooled before the next step. Through the condenser, 7 mL hexane was added to each sample, which was heated again on a hot plate for 1 min. Hexane-extracted samples were cooled, and saturated sodium chloride solution was added to each sample flask in order to bring the hexane solution floating to the upper neck of the flask, and then the layer containing the derivatized fatty acid methyl esters was decanted. Approximately 6 mL of FAME layer was transferred into a new test tube containing a small amount of anhydrous sodium sulfate to remove moisture in the hexane extract sample. The hexane samples were stored in the freezer (−18 °C) until fatty acid analysis by a gas chromatograph.

2.6.3. GC (Gas Chromatograph) Analysis of Fatty Acids

Concentrations of fatty acids were quantified using the same method of Davis et al. [6]. A gas chromatograph (GC- 2010 Plus; Shimadzu Scientific Instruments, Canby, OR, USA) was used to analyze the fatty acid profiles of all samples. The GC was equipped with fused silica capillary column (100 m × 0.25 mm × 0.2 µm film thickness; SP- 2560, Supelco, Bellefonte, PA, USA), flame ionization detector (FID), AOC-20s auto sampler and AOC-20i auto injector. The temperature settings for the injector and detector were 250 °C and 270 °C, respectively. The optimal initial oven temperature was set at 40 °C to determine small molecular fatty acids. The 40 °C was held for 5 min, then increased to

240 °C at a rate of 4 °C/min. A total of 1 µL of the final extracted FAME sample was injected into the GC with a split ratio of 1:20 and the carrier gas used was helium.

2.7. Statistical Analysis

The general linear model (GLM) of SAS software program [23] and Steel and Torrie (1960) [24] methods were used for statistical analysis of all collected experimental data on the analysis of variance, least square means and Duncan's multiple mean comparison. Effects of main factors such as batch, storage temperature and storage time, as well as their interaction effects on physio-chemical indices for four months and survival of pathogens in different batches of GMP samples for the 6 month experimental storage periods, were evaluated.

Microsoft's Excel program was used to plot the survival graph of pathogens. The survival curves of Salmonella were fitted by the Weibull model using IPMP 2013 software system [25]. The Weibull survival model function is expressed as follows: Log CFU(t) = Log CFU0 − $(t/\delta)^{\alpha}$, where Log CFU(t) indicates the decimal log of the cell number per gram at time t, Log CFU(t)0 is the decimal log of the cell number per gram at day zero (after inoculation), δ is the scale parameter of the Weibull function in day/(Log CFU/g), α is the shape parameter and t is time in days. In the fitting function, the parameter Log CFU_0 is fixed at the average of the initial point called day zero.

3. Results and Discussion

3.1. Survivability of Salmonella in the Experimenatal Powdered Goat Milk

The survivability of *Salmonella* study revealed that the control (without pathogen inoculation) group of the commercial PGM samples had no pathogens for both 4 °C and 25 °C at the ten different storage periods (0, 3, 7, 14, 21, 30, 60, 90, 120 and 180 days). The water activity of the original experimental powdered milk samples was 0.21. However, the initial aw of the *Salmonella*-inoculated group samples was 0.33, because 0.1 mL of fresh culture *Salmonella* was inoculated to the pathogen-treated powdered milk samples.

The initial mean counts for the three batches of *Salmonella* (Figure 1) after inoculation in PGM samples at 0 day were 7.10 Log CFU/g for both storage temperatures at 4 °C and 25 °C. As the storage period advanced, viable counts of *Salmonella* cells were gradually decreased. At the first 3 days, the viable cell counts of *Salmonella* cells decreased to 0.94 and 1.40 Log CFU/g at 4 °C and 25 °C, respectively, and then were sustained at this level for the first two weeks (14 days). However, at 30 days storage, further reductions occurred by 0.32 Log CFU/g at 4 °C and 0.61 Log CFU/g at 25 °C, respectively. The reduction continued at 6 months by additional decreases by 0.29 Log CFU/g and 0.69 Log CFU/g at the 4 °C and 25 °C, respectively. At the end of 180 days storage, the survival of *Salmonella* pathogen in the PGM samples were 5.56 Log CFU/g 4 °C and 4.41 Log CFU/g at 25 °C. This end result showed survival of *Salmonella* at 4 °C was 1.15 Log CFU/g higher than those stored at 25 °C (Figure 1). Therefore, a distinct trend was found in the survivability of *Salmonella* of the commercial PGM products in this study, showing that the *Salmonella* survival rate was continuously reduced with the extended storage up to the 6 month period.

The mean values of three batches of survival curves showed a comparatively increased decline in initial pathogen population during the first three days, and then slower declines occurred at subsequent storage times in the samples at both 4 and 25 °C (Weibull model; Figures 2 and 3). Three different batches stored at 4 °C also showed a similar trend of survival curves, displaying declines in their pathogen populations. When *Salmonella* survival kinetics were compared among three batches at 4 °C, Batch 3 revealed a comparatively slower decline in the bacterial population than the other two batches at 3 days of storage.

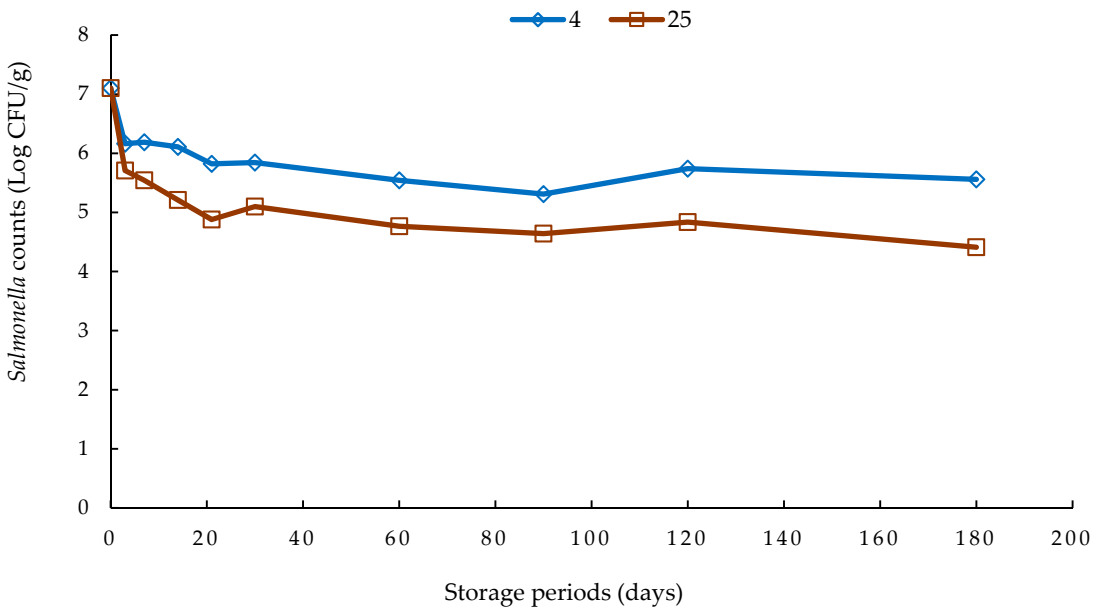

Figure 1. The trend of the survivability of Salmonella (Log CFU/g) (CFU = colony forming unit) in powdered goat milk stored at 4 and 25 °C for 180 days.

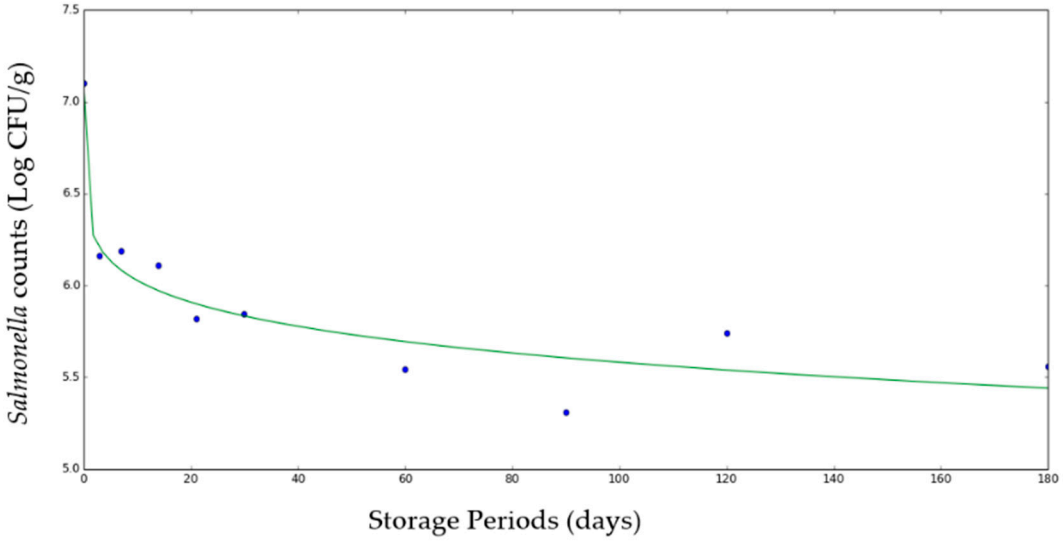

Figure 2. Weibull model curve showing the mean values of three batches of PGM (powdered goat milk) on the survivability of *Salmonella* at 4 °C for 180 days.

For the survival rate of *Salmonella* at 25 °C treatment for 180 days, the declining trend of the pathogen counts was similar to those counts observed at 4 °C (Table 1). However, the *Salmonella* bacterial counts Log CFU/g at 25 °C for each storage period were significantly ($p < 0.05$) lower than those of respective bacterial counts at 4 °C throughout the experimental period. Batch 3 showed a comparatively slower decline of *Salmonella* counts compared to the other two batches throughout the storage period. The survival rate of *Salmonella* cells in the PGM products in our study declined by 0.448 (Log CFU/g)/month at 25 °C storage temperature. In another study [26], walnut kernels with similar water activity showed a declining rate of 0.10 (Log CFU/g)/month at 23 °C, which appears to be a significantly lower reduction rate in *Salmonella* cell survival than our PGM samples. A number of researchers have observed that *Salmonella* pathogens in different low water activity foods declined at a fast rate in the initial phase, and then slowly declined after 3 month storage periods [27–29].

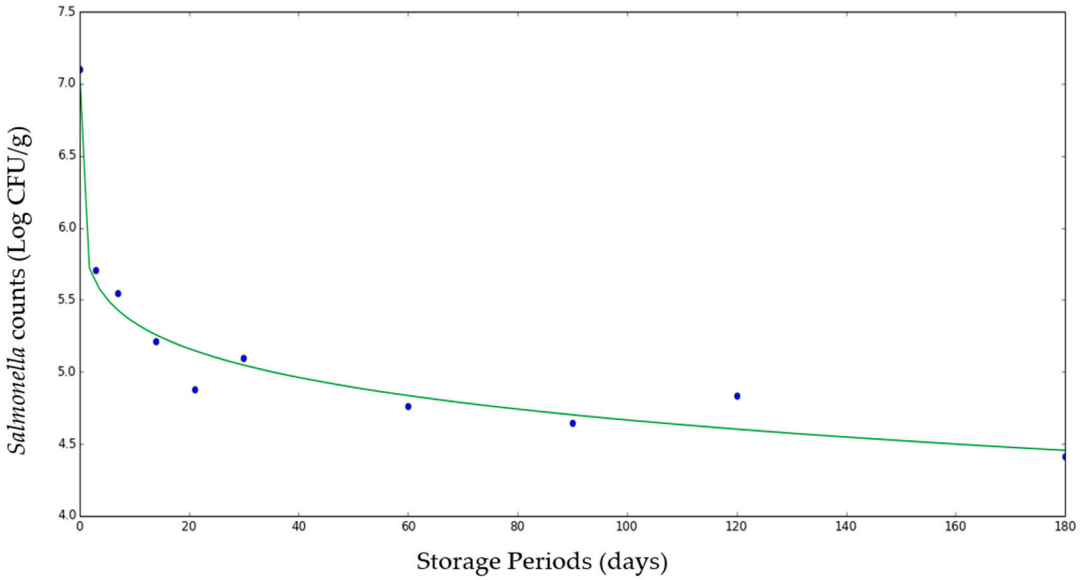

Figure 3. Weibull model curve showing the mean values of three batches of PGM on the survivability of *Salmonella* at 25 °C for 180 days.

Table 1. Analysis of variance (F value) on the effects of batch, storage period and temperature and their interactions on survivability of *Salmonella* for 180 days.

Parameter	DF	Mean Square	F-Value
B	2	0.91	8.83 *
SP	9	4.78	46.32 **
ST	1	15.49	150.15 **
B × SP	29	1.64	6.20 **
B × ST	5	3.47	7.33 **
SP × ST	19	3.22	30.17 **
B × SP × ST	59	1.13	15.39 **

B: batch, SP: storage period, ST: storage temperature, DF: degree of freedom; * $p < 0.05$, ** $p < 0.01$.

Many researchers have shown that survival of foodborne pathogens, such as *Salmonella* in milk powder, remain for a long period and resume growth when the milk is reconstituted and stored in favorable temperature and physical conditions [30,31]. McDonough and Hargrove (1968) [32] studied the heat resistance of *Salmonella* and the effect of storage on survival in dried milk, and reported that certain heat and moisture combinations lessened the chances of *Salmonella* survival during drying operation, but they did not find complete control with certain heat treatments. In the present study, the same phenomenon was observed, where higher temperature (25 °C) storage with the same moisture content showed lower survival of *Salmonella* bacteria than 4 °C refrigeration storage group of the powdered goat milk (Figures 1–3).

A study with bovine milk powder contaminated with *Salmonella* at different temperatures showed little reduction in the numbers of viable *Salmonella* at 15 weeks [32]. However, the results of our study revealed that caprine powdered milk stored at 25 °C had lower survivability of *Salmonella* than that stored at the lower temperature of 4 °C. Chances of survival of *Salmonella* increased as moisture and water activity (a_w), and heat resistance increased during the drying process. These phenomena suggest that *Salmonella* pathogens in dried milk powder are highly heat resistant, with viable cells detected after 10 h at 76.6 °C [16,30]. Keogh (1971) [15] found that high temperature storage destroyed *Salmonella* in powdered milk but would adversely affect the flavor of the dehydrated products.

Bacterial cells also normally survive longer periods in dried foods such as powdered milk. Dry foods often can be contaminated with pathogens which can survive an extended time in an

environment of low moisture and water activity, and can pose a possible food safety risk [6]. Beuchat et al. (2013) [3] showed that in dry food such as milk powder, survival of only few cells of some foodborne pathogens, e.g., *Salmonella* or *Escherichia coli O157:H7*, may be enough to cause diseases.

Recent reports have shown that a number of outbreaks have been associated with dry food products and survival of pathogens. In vegetative cells of foodborne pathogens, bacterial and fungal spores may survive in foods and food ingredients with low water activity less than 0.85 for long periods [3]. However, food products with a water activity value of below 0.80 do not support the growth of microorganisms [24]. Lian et al. (2015) [4] demonstrated that aw significantly influenced the survival of *Salmonella* at all temperatures, and the survival of microorganisms in dried food increased with lower a_w. They also reported that water in low a_w foods is in glassy and rubbery states, so that water has limited mobility which helps water molecules contact bacterial cells during their interaction.

As far as effects of main factors and their interactions on the survivability of *Salmonella* in the PGM go, all the main factors (batch (B), storage temperature (ST) and storage period (SP)) significantly ($p < 0.05$, $p < 0.01$) influenced the *Salmonella* survival counts, and 2-way and 3-way interaction effects also significantly ($p < 0.01$) affected the survivability of *Salmonella* in the PGM samples at both 4 and 25 °C for 180 days storage (Table 1). These data suggest that the survivability of *Salmonella* in the commercial goat milk powder can be affected by batch, storage temperature and time, and their interaction effects. The significant interaction effects among the main factors may be attributable to the variations of pathogen survivability between batches.

3.2. Physicochemical Characteristics of the PGM

3.2.1. pH and Water activity

There were no differences in pH of the PGM samples between batches as well as batch × storage temperature interaction effects (Table 2; Figure 4A). The pH of the powdered goat milk samples stored at 4 and 25 °C for 0, 2 and 4 months were: 6.33, 6.32; 6.40, 6.41; 6.43, 6.34, respectively. The samples stored at 4 °C for 0 and 4 months showed higher pH values than those stored at 25 °C except for a 2 month period. The exact reason for the lower pH in PGM samples stored at 25 °C compared to those at 4 °C is not known; more proteolysis might have occurred and/or organic acids were generated at higher storage temperature treated samples. Significant differences in pH were found between storage period ($p < 0.01$), storage temperatures ($p < 0.05$) and between 2-way interaction effects of B × SP ($p < 0.01$) and SP × ST ($p < 0.01$) (Table 2).

Table 2. Analysis of variance (F value) on the effect of batch, storage period and temperature and their interaction on physicochemical characteristics of PGM samples during 4 month storage at 4 and 25 °C.

Parameter	pH	Water Activity	Moisture	Peroxide Value
B	0.83	3.08	0.38	2.13
SP	19.21 **	0.06	3.54 *	28.25 **
ST	8.20 *	1.01	17.14 **	3.59
B × ST	1.11	1.65	4.64 **	1.4
B × SP	4.12 **	1.26	1.56	6.68 **
SP × ST	25.36 **	0.48	5.57 **	16.19 **
B × ST × SP	13.72 **	0.83	60.8 **	11.84 **

B: batch, SP: Storage period, ST: storage temperature. * Significant: $p < 0.05$, ** Significant: $p < 0.01$.

The a_w prior to inoculation was 0.22 and after inoculation 0.33 for microbial experimental PGM sample. Figure 4B shows the a_w values of the powdered goat milk samples stored at 4 and 25 °C for 0, 2 and 4 months, where a_w values were: 0.23, 0.22; 0.22, 0.22; 0.23, 0.22, respectively. No significant differences were observed in a_w between main factors, nor between their interaction effects in the tested commercial powdered goat milk products (Figure 4B). Food stability, safety and other properties can be predicated for more reliability from a_w than from water content [33]. Powdered milk has the

advantages of controlling the growth of pathogenic and spoilage microorganisms due to its low water activity and moisture content [3,4].

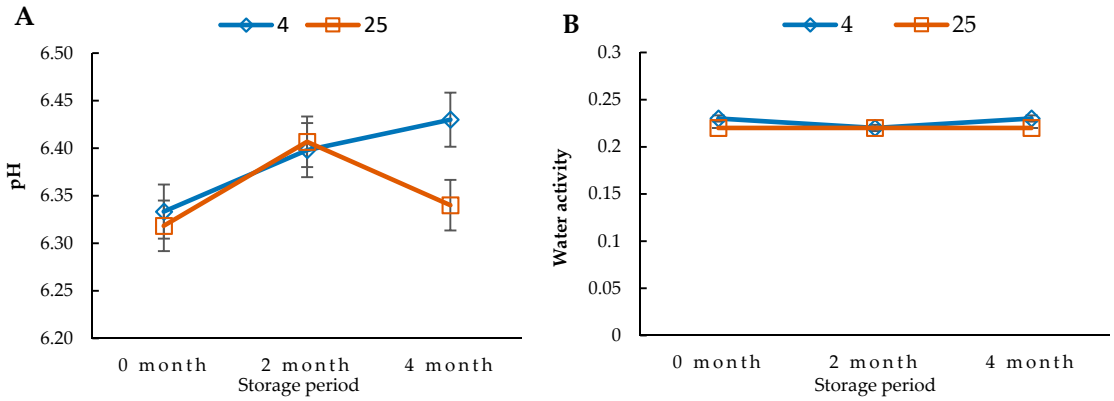

Figure 4. (**A**) Comparison of pH changes in PGM samples between two temperature treatments (4 and 25 °C) during the 4 month storage. (**B**) Comparison of water activity (a_w) changes in PGM samples between two temperature treatments (4 and 25 °C) during the 4 month storage.

3.2.2. Peroxide Value (POV)

The average peroxide values of the commercial PGM samples stored at 4 and 25 °C for 0, 2 and 4 months were: 0.13, 0.11; 0.14, 0.17; 0.17, 0.20, respectively (Figure 5). There were significant ($p < 0.01$) differences in the POV for effects of storage period (SP), batch × storage period (B × SP), SP × ST (storage temperature) and B × ST × SP. The powdered goat milk samples stored at 25 °C showed higher POV values than those stored at 4 °C for 2 and 4 months of storage (Table 2). These results reflect that the rate of lipid oxidation of the PGM samples was elevated by higher storage temperature with extended storage periods.

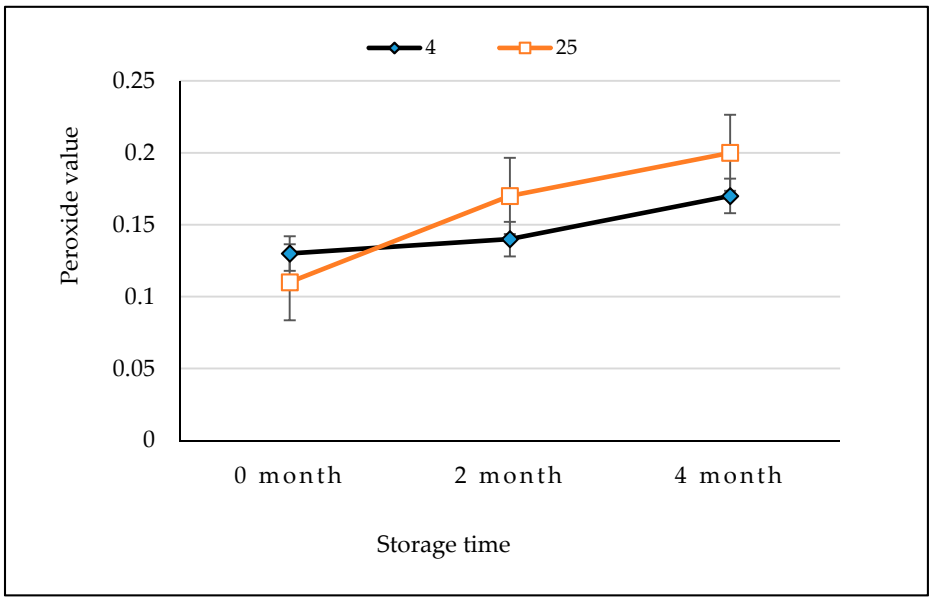

Figure 5. Comparison of peroxide values (POV) of PGM samples between 4 and 25 °C temperature treatments for 0, 2 and 4 months. Differences in POV are significant ($p < 0.05$) at the 0 and 4 month storage.

Peroxide value is one of the indices to determine lipid oxidation in food samples, where lipid molecules undergo oxidative degradation and give rise to hydroperoxides [34]. Lipid oxidation has been a great influence on the quality of powdered milk products due to deterioration of lipids in the

products, which causes human health problems. Furthermore, lipid oxidation is detrimental to the dairy industries owing to negative effects on the shelf life of powdered milk products [2,34,35]. The rate of lipid oxidation in dehydrated milk products reported in previous studies [2,36] is in agreement with the present study, where lipid oxidation in powdered milk products was influenced by several factors including oxygen, exposure to UV light, temperature, moisture, water activity and the composition of unsaturated fatty acids.

3.3. Nutritional Characteristics of the PGM

3.3.1. Basic Nutrients

The basic nutrient composition of the experimental GMP in this study was similar to those values reported in a previous study [6]. There were no significant differences in basic nutrient composition between batches and between temperature treatments. The mean (%) of nutrient contents of the GMP stored at 4 °C and 25 °C were: 27.09, 26.78, fat; 28.53, 28.36, protein; 2.32, 2.24, moisture; and 6.45, 6.39, ash, respectively. This indicates that protein content was somewhat higher than that of the USDEC (2005) [1] and previous reports [3,6]. However, the basic nutrient composition reportedly varies with diet, breed and animals within breed, feeding, environmental conditions, seasons and stage of lactation [37,38].

3.3.2. Fatty Acid Profiles

Fatty acid profiles of the experimental PGM (control group without *Salmonella* inoculation) samples stored at two storage temperatures (4 and 25 °C) for 4 months are shown in Table 3. Results of statistical analyses revealed that the concentration of oleic acid (C18:1) was the highest, caprylic acid (C8:0) was the second highest, and behenic acid (C22:0) was the lowest concentration among all fatty acids identified in the PGM samples. These results indicate similar outcomes from our previous study in commercial powdered goat milk products [6]. Most of the fatty acid concentration decreased with advanced storage periods, while some of fatty acids (C4:0, C10:0, C14:1) had higher concentration at 4 °C in 4 months of storage. It was noticed that the levels of most of fatty acids stored at 4 °C had slightly higher values than those stored at 25 °C except C18:0 for 2 and 4 months storage, and C18:1 for 4 month storage (Table 3).

The analysis of variance on main factors and their interaction effects on short chain fatty acids of the PGM samples are shown in Table 4 as an example, while other fatty acid data have been omitted. The data in Table 4 demonstrate that many of main factors and their interaction effects are significant ($p < 0.05$, $p < 0.01$, or $p < 0.001$) for short chain and long chain fatty acids (data omitted). On the other hand, the effect of storage temperature was not significant for concentrations of C8:0, C14:0, C16:0, C18:1, C18:3, C:20, C:22, C24:0. Two-way interaction effects of B × ST, B × SP, and ST × SP were also significant ($p < 0.05$, $p < 0.01$ or $p < 0.001$) for levels of the majority of fatty acids tested. It is unknown whether these fatty acids compositions are related to or have any impact on the survivability of the inoculated *Salmonella* pathogens in the experimental samples.

Table 3. Comparison of mean fatty acid (mg/g) concentrations of experimental PGM samples stored at 4 and 25 °C for four months.

Fatty Acid	Storage Temperature (°C)	0 Month		2 Months		4 Months	
		Mean	SD	Mean	SD	Mean	SD
C4:0	4	0.23	0.03	0.22	0.03	0.24	0.06
	25	0.23	0.03	0.20	0.01	0.18	0.01
C6:0	4	0.54	0.02	0.53	0.02	0.49	0.01
	25	0.54	0.02	0.49	0.01	0.45	0.01
C8:0	4	6.68	0.29	6.59	0.34	6.48	0.36
	25	6.68	0.29	6.49	0.05	6.42	0.01
C10:0	4	0.15	0.03	0.27	0.33	0.42	0.47
	25	0.15	0.03	0.08	0.03	0.06	0.01
C12:0	4	0.07	0.00	0.06	0.01	0.05	0.01
	25	0.07	0.00	0.04	0.02	0.03	0.02
C14:0	4	0.34	0.04	0.31	0.07	0.27	0.04
	25	0.34	0.04	0.31	0.02	0.25	0.01
C14:1	4	0.23	0.03	0.34	0.14	0.31	0.14
	25	0.23	0.03	0.23	0.02	0.19	0.02
C16:0	4	1.52	0.05	1.49	0.08	1.41	0.08
	25	1.52	0.05	1.45	0.03	1.37	0.02
C16:1	4	0.75	0.05	0.73	0.05	0.69	0.07
	25	0.75	0.05	0.70	0.03	0.62	0.05
C18:0	4	1.84	0.06	1.55	0.37	1.38	0.29
	25	1.84	0.06	1.72	0.02	1.67	0.03
C18:1	4	7.88	0.13	7.75	0.21	7.58	0.30
	25	7.88	0.13	7.73	0.03	7.62	0.02
C18:2	4	2.22	0.12	2.17	0.13	2.11	0.14
	25	2.22	0.12	2.12	0.11	1.91	0.05
C18:3	4	0.38	0.03	0.34	0.06	0.32	0.04
	25	0.38	0.03	0.32	0.02	0.28	0.03
C20:0	4	0.07	0.00	0.06	0.01	0.15	0.22
	25	0.07	0.00	0.05	0.01	0.04	0.01
C22:0	4	0.01	0.00	0.02	0.01	0.02	0.01
	25	0.01	0.00	0.01	0.00	0.02	0.02
C24:0	4	0.05	0.00	0.05	0.01	0.03	0.01
	25	0.05	0.00	0.04	0.02	0.01	0.01

SD: Standard deviation.

Table 4. An example summary of analysis of variance (F-value) on the effects of batch, storage periods and temperatures on levels of short chain fatty acids.

Parameter	DF	C4:0	C6:0	C8:0	C10:0
B	2	2.15	2.56	27.82 ***	3.04
SP	2	1.88	37.34 ***	6.82 **	0.52
ST	1	4.98 *	21.24 ***	1.09	6.15 *
B × SP	8	3.49 *	7.82 **	9.28 **	1.58
B × ST	5	2.52	1.56	15.86 ***	4.82 *
SP × ST	5	2.5	26.23 ***	1.09	2.08
B × SP × ST	17	69.28 ***	23.33 ***	124.63 ***	5.38 **

B: batch, SP: storage period, ST: storage temperature, DF: degree of freedom. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Conclusions

In terms of survivability of *Salmonella* pathogens in the powdered goat milk during the 6 month storage, the general declining trend of the pathogen counts was similar for both temperature treatments at 4 °C and 25 °C. However, the *Salmonella* pathogen counts (Log CFU/g) at 25 °C for each storage period were significantly ($p < 0.05$) lower than those of pathogen counts at 4 °C throughout the storage period. This indicates that the survivability of *Salmonella* pathogens in the PGM products stored at 4 °C for 180 days was higher than those stored at 25 °C under the same a_w condition.

With regard to the physicochemical stability of the goat milk powder products during 6 months of storage, the powdered goat milk samples stored at 25 °C showed higher POV values than those stored at 4 °C for 2 and 4 month periods, indicating that the rate of lipid oxidation in the PGM samples was elevated by higher temperature with the advanced storage period. No significant differences were found in water activity (a_w) of the PGM products between main factors, nor between their interaction effects. Further studies may be necessary to examine the survivability of *Salmonella* pathogens in dehydrated goat milk products stored under more storage periods and storage temperatures for the food safety of general consumers.

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