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Evaluating the Effectiveness of Low Pressure Carbon Dioxide as a Hurdle in Raw Milk Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Author TSVR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the effectiveness of low pressure carbon dioxide as a hurdle in raw milk storage.
Study Design: Milk samples were stored at under low pressure carbon dioxide at 29°C for 6 hours and the microbial quality of milk was compared with control milk.
Place and Duration of Study: Department of Dairy Microbiology, Verghese Kurien Institute of Dairy and Food Technology (VKIDFT), Kerala Veterinary and Animal Sciences University, Mannuthy between January 2020 and December 2020.

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Methodology: Milk samples were collected from an organized farm. The initial microbial quality of milk was determined and samples were carbonated to a pressure of 20 psi and stored for six hours 29°C, uncarbonated milk sample kept at 29°C acted as the control. The microbial quality of the carbonated milk and uncarbonated milk was determined after storage in terms of total viable count, coliform count and gram negative organism count.
Results: Significant growth suppression (P=0.05) of bacteria was observed in the carbonated milk. Total Viable count showed a suppression of 1.05 log cfu/ml while coliforms showed a suppression of 1.3 log cfu/ml. The greatest log reduction was observed in gram negative organisms with a difference of 2.2 log cfu/ml and psychrotrophic organisms with 1.54 log cfu/ml.
Conclusion: Carbon dioxide was found to be an effective bacteriostatic agent which could be used for extending the keeping quality of raw milk. The bacteriostatic action could be due to anaerobic conditions developed by carbon dioxide and also due to the increased acidity of the medium.

Keywords: Milk; shelf life; carbon dioxide; hurdle technology; bacteriostatic action.

ABBREVIATIONS

°C : Degree celcius
Min : Minutes
CFU : Colony forming Units
CO_2 : Carbon dioxide
TVC : Total Viable Count

1. INTRODUCTION

Raw milk is an excellent source of nutrients and as a result also doubles as an excellent substrate for the growth of bacteria, consequently unprocessed milk has a very short shelf life. Many significant changes can happen in raw milk during transport and holding due to uncontrolled microbial growth. The most detrimental result from the release of lipolytic and proteolytic enzymes which breakdown the protein structure of the micelles and/or cause hydrolysis of lipids [1,2]. The quality of products made from milk thus also depends on the initial quality of raw milk used for production. Yield and quality of milk products, including cheeses, ice cream and yogurt mixes, cultured products, and related products, can also be affected by the condition of raw milk [3]. For these reasons the control of bacterial growth in raw milk has been an area of interest in the dairy sector.

Refrigeration is most widely implemented technology for the extension of shelf life of milk. Employment of refrigeration as the sole technique for shelf life extension can lead to the selective growth of psychrotrophic bacteria in milk. Psychrotrophic bacteria have been known to produce heat stable lipolytic and proteolytic enzymes which can have detrimental effect on the quality of the products made from such milk.

Several studies have been conducted over the past few decades which has shown that addition of CO_2 to the atmosphere surrounding a product reduces the rate of growth of many food spoilage and pathogenic microorganisms [4,5]. The largest inhibition occurs with gram-negative psychrotrophs, particularly Pseudomonas spp. and the least inhibition effect generally observed with gram-positive psychrotrophs, particularly *Lactobacillus* spp [6].

The current study was aimed to study the effectiveness of low pressure carbon dioxide on the growth of bacteria in raw milk and to evaluate whether low pressure carbon dioxide can be used as a hurdle in raw milk preservation.

2. MATERIALS AND METHODS

2.1 Carbonation System

Milk was carbonated using a carbonation system consisted of a compressed food grade carbon dioxide cylinder which was fitted with a pressure gauge, pressure regulator and an on-off valve, a 100 ml glass reagent bottle whose lid was fitted with a stainless steel ball valve and a pneumatic line connect the on-off valve of the carbon dioxide cylinder. This tube is connected to the outer end of the ball valve on the bottle lid to facilitate carbonation, another tube extending to the bottom of the bottle was connected to the inside end of the ball valve on the bottle lid to facilitate bubbling of carbon dioxide through the milk during pressurizing. The bottles were placed in a circulating water bath to maintain the samples at the required temperature. After carbonation samples were transferred to incubators maintained at the required temperature [7].

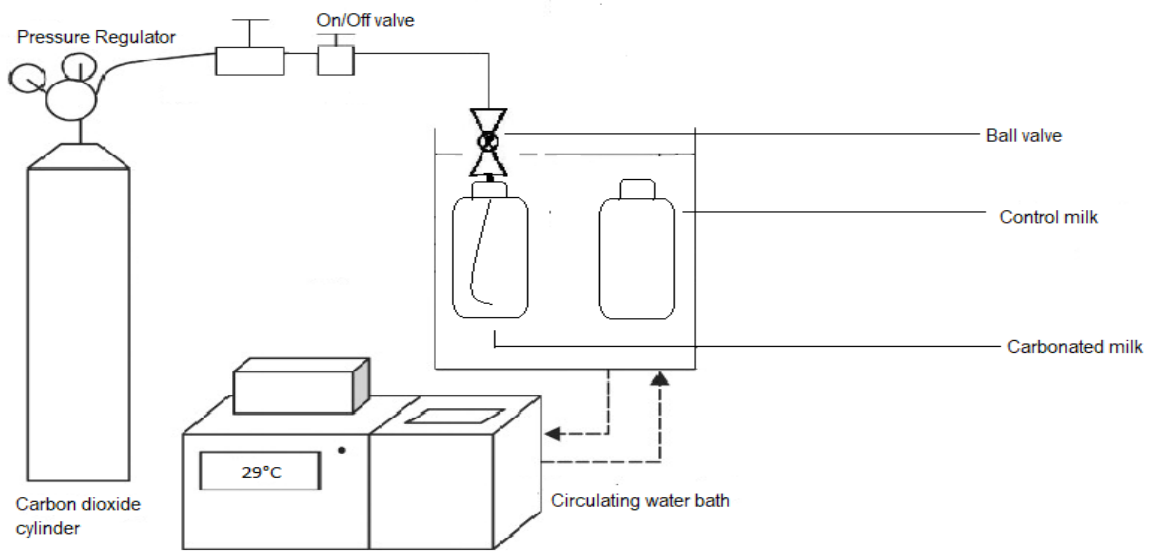

Fig. 1. Carbonation apparatus

After each treatment/ trial the apparatus was dismantled and the bottles, lids, silicon tubes inside the bottle and tube used to connect the lid to the on off valve were cleaned and sanitized as per the following protocol: fresh water rinse for 5 minutes followed by soaking in 1 per cent lysol (cationic surfactant) at 30°C for 20 minutes. After soaking, the items were rinsed using tap water at 50°C for 5 minutes and finally sterilized by autoclaving at 121°C/15 psi for 15 min. The sanitation protocol was validated by plating swab samples from the dismantled apparatus on nutrient agar and ensuring absence of any microbial growth on the plates. To ensure the complete removal of cleaning agent from the equipment, rinse water pH was measured after cleaning and affirmed that it was the same as the pH of the water before rinsing. A water bath was used to bring the samples to the required temperature. After carbonation samples were transferred to incubators maintained at the required temperature.

2.2 Carbonation Treatment

In this study raw milk was stored for six hours under a carbon dioxide pressure of 20psi at a temperature of 29°C. Raw milk without carbonation held at 29°C for six hours acted as the control sample. The effect of low pressure carbon dioxide on microbial quality of raw milk was assessed by comparing the specific plate count, gram negative count and coliform counts of treatment samples with that of control samples.

2.3 Sampling and Carbonation Procedure

Pooled raw milk was collected from an organized farm in Kerala within an hour of milking, following all the essential aseptic measures in a sanitized sample bottle to exclude any potential chance of external contamination. The milk was transferred to the lab within 10 minutes of collection.

Milk sample was then uniformly mixed and 75 ml milk was added into the treatment and control bottles. The treatment bottle was closed air tight using the modified lid (with the ball valve) and the control bottle was closed using a normal lid.

Both bottles were then placed in a water bath set at the 29°C temperature. When the desired temperatures were attained in both treatment and control bottles, the treatment bottle was attached to the carbonating apparatus. CO_2 was introduced into treatment bottle until the set pressure was reached. When the set pressure was achieved in the bottle the ball valve on the lid was closed followed by the on off valve of the carbon dioxide cylinder, the bottle was then carefully detached from the apparatus. The treatment and control bottles were then kept for storage in an incubator set at the desired temperature. After storage for the stipulated time, the treatment and control bottles were removed from the incubator and the bottles were depressurized and the contents transferred into sterile containers for microbial analysis.

Table 1. Effect of 20 psi carbon dioxide pressure at 29°C for 6h on raw milk quality

Parameter	Initial quality	Control (A)	Carbonated milk (B)	Suppression (%)	t value
	0 h	29°C/6h	20psi CO2/29°C/6h		
TVC(log cfu/ml)	1.96	10.36	9.31	1.05	25.815*
Coliform count (log cfu/ml)	7.52	6.36	5.08	1.3	68.578*
Gram negative count (cfu/ml)	3.43	8.52	6.32	2.2	3.974*
Psychrotrophic count (cfu/ml)	4.62	7.64	6.1	1.54	2.821*

** there is significant difference between the control and carbonated milk at P=0.05*

2.4 Microbiological Methods

For all microbiological assays, milk sample aliquots of 1 ml were used in dilution series. Standard plate counts were performed as per the method described in IS:1479,1960.

Gram-negative bacteria were enumerated on Crystal Violet Tetrazolium agar after pour plating and incubation at 37°C for 24 h. Crystal Violet Tetrazolium agar is a selective media which can be used for selectively enumerating gram-negative bacteria. Coliform bacteria were enumerated using Violet Red Bile Agar (VRBA) after pour plating and incubation at 37°C for 24 h.

Initial total, coliform, and gram negative counts were each determined for control and treated samples.

Inferences were drawn by comparing the results of control and treatment samples.

2.5 Statistical Analysis

For statistical analysis, all the counts were analysed using SPSS, version 26 (IBM, New York, US). The TVC, coliform counts and gram negative counts the control and treatment samples were subjected to paired comparison t test.

3. RESULTS AND DISCUSSION

In a local study conducted it was found that on an average at least four hours lapse between the production and processing of raw milk during which milk is subjected to storage at room temperature. This storage at ambient temperature can cause an explosion of bacterial growth in raw milk. A growth suppression of 91.16% was observed at 29°C when using 20psi Carbon dioxide which is in line with the observations of Rajagopal et al in [7] who studied bacterial growth suppression due to carbon dioxide at 25psi pressure. In the present study Gram negative organisms were found to be the most affected by carbon dioxide meanwhile coliforms were the least affected showing only 94 % suppression. It can be inferred from the result that action of carbon dioxide is more pronounced in gram negative non coliform bacteria. Suppression of psychrotrophic bacteria by levels of 97% substantiates this finding. It has been reported by Amigo et al. [8] that carbon dioxide effectively suppresses the growth of multiple species of Pseudomonas group one of the most prominent group of non coliform gram negative psychrotrophs by increasing the lag as well as affecting its log phase. Since refrigeration is the most sort after method for extending shelf life of food, psychrotrophic organisms plays a central role in keeping quality of food. Ma et al. [3] has also reported significant suppression of psychrotrophic bacterial growth under carbon dioxide environments.

4. CONCLUSION

Carbon dioxide was found to be able to successfully suppress the growth of bacteria in raw milk. Carbon dioxide was found to be extremely effective in suppressing gram negative psychrotrophs and as refrigerated storage is the most popular method of raw milk storage carbonation can be used as an effective hurdle to control the growth gram negative psychrotrophic organisms in raw milk.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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