RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 40 Issue 2: 129-135 (June 2021)

Processing Flavoured Milk by Thermal and Non Thermal Methods

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10.18805/ajdfr.DR-1561

ABSTRACT

Background: Research for new alternatives in food processing and preservation has become a priority of food scientists, not only to provide better quality products, but also to satisfy the needs and preferences of the consumer and further to fulfill regulations regarding food safety and to offer new varieties in the food market. These new alternatives include the use of other preservation factors, as opposed to heat, that are able to inactivate microorganisms and enzymes and provide better stability to the product with only minor changes in the overall quality of the food. Non-thermal processes have gained importance in recent years due to the increasing demand for milk with a high nutritional value and fresh-like characteristics, representing a substitute to conventional thermal treatments.

Methods: This study was aimed to compare and evaluate the effectiveness of thermal and non- thermal technologies in microbial inactivation and shelf life extension of flavored milks such as rose milk and chocolate milk. UV radiation and pulsed electric field (PEF) technology were selected for non-thermal treatment of flavoured milks. In the food laboratory, high voltage laboratory and UV treatment chamber the milk samples were analyzed for a week.

Result: Our findings in thermal and non- thermal (UV and PEF) methods of flavored milk showed that compare to thermal treatment of flavored milk non- thermal methods are good for preservation of flavored milk. Among the three methods, PEF treatment had an excellent effect on preserving the shelf life compared to thermal pasteurisation and UV radiation.

Key words: Flavoured milk, Pulsed electric field, Thermal pasteurization, Ultraviolet radiation.

INTRODUCTION

There are two methods for sterilization of foods, thermal and non-thermal. The thermal sterilization is now widely used for liquid food such as juices and milk. Compared to thermal methods, non-thermal methods gave better microbial inactivation and shelf life stability in milk preservation at lower temperatures and shorter treatment times. This study was aims to analysis the best method for the preservation of flavoured milk such as chocolate and rose milk. Flavoured milk is a healthy beverage targeted to children as a snack at home or at school. Milk is a nutrition powerhouse, both flavoured and plain milk contain nine essential nutrients including calcium, potassium, phosphorus, protein, vitamins A, D and B12, riboflavin and niacin (Jadhav and Pawar, 2016). Flavoured milk is becoming an integral part of market milk industry because it has good consumer acceptance as a refreshing and nourishing milk beverage. Infant and sick persons also cannot properly digest full fat milk. All these problems can be counteracted by manufacturing low fat flavoured milk (Charanjiv Singh *et al*., 2005). Half of the milk produced in the country is utilized at fluid milk and rest is concerted into tradional milk products, From these some part of milk is used for making special milk like flavoured milk, soft curd milk, fermented milk standardized milk and recombined milk are humanized milk (Repate *et al*., 2010). Pulsed electric field (PEF) is an emerging technology that has been extensively studied for non-thermal food processing, across a wide range of liquid foods (Maged *et al*., 2010).

Pulsed electric field (PEF) processing uses short burst of electricity for microbial inactivation and causes minimal or no detrimental effect on food quality attributes (Barbosa-Canovas *et al.,* 1999). Since it preserves foods without using heat, foods treated this way retain their fresh aroma, taste and appearance. PEF processing offers high quality fresh-like liquid and semi liquid foods with excellent flavour, nutritional value and shelf-life (Beatrice and Ahmed, 2002). Application of PEF technology has been successfully demonstrated for the pasteurization of foods such as juices, milk, yogurt, soups and liquid eggs (Bermúdez-Aguirre, *et al*., 2010) and is restricted to food products with no air bubbles and with low electrical conductivity. PEF pasteurization kills microorganisms and inactivates some enzymes. Unless the product is acidic, it requires refrigerated storage for safety (to prevent the growth of spores in low-acid foods) and in low-acid foods to preserve flavour quality for extended periods of times. For heat-sensitive liquid foods where

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How to cite this article: Kayalvizhi, V., Sowmiya, S., Priya, T.S.R. and Antony, U. (2021). Processing Flavoured Milk by Thermal and Non Thermal Methods. Asian Journal of Dairy and Food Research. 40(2): 129-135. DOI: 10.18805/ajdfr.DR-1561.

Submitted: 29-07-2020 **Accepted:** 04-03-2021 **Online:** 07-04-2021

thermal pasteurization is not an option (due to flavour, texture, or colour changes), Incorporation of pulsed electric field (PEF) technology into food production was supported by the growing consumer interest in food of high nutritional value and the demand for fresh-like products.

Thus, the search for new alternatives in food processing and preservation has become a priority for food scientists, not only to provide a better quality product, but also to satisfy the needs and preferences of the consumer and further to fulfill regulations regarding food safety and to offer new alternatives in the food market. Non-thermal processes have gained importance in recent years due to the increasing demand for foods with a high nutritional value and fresh-like characteristics, representing an alternative to conventional thermal treatments (Dunn and Pearlman, 1987).

MATERIALS AND METHODS

Sample preparation

Rose milk

Fresh rose syrup was purchased from the local supermarket. Under sterile conditions 30mL of the syrup was thoroughly mixed with 1 litre of normal standardized milk. Fresh toned milk of 3% fat and 8.5% SNF obtained from the Aavin was used for this study.10 grams of sugar was added. The contents were transferred to sterile containers and taken for treatment and analysis.

Chocolate milk

Cadbury hot chocolate powder was purchased from the local supermarket and 30 grams of this powder was mixed with 1 litre of Aavin milk (Fresh toned milk of 3% fat and 8.5% SNF) and 10 grams of sugar. The contents were mixed and transferred to sterile containers and taken for treatment.

Statistical analysis

All treatments were carried out on two separate batches of prepared rose and chocolate milk on two different days and all chemical, nutritional and microbial analyses were done in duplicates. The data obtained in all the experiments were analyzed statistically by applying one way ANOVA in IBM SPSS® software (version 20.0) for windows as per the standard procedure of Snedecor and Cochran, 1994.

Equipment

For thermal pasteurization (Armfield FT75, Mumbai), For Ultraviolet (UV) processing, the UV treatment Chamber was used. Finally, the high electric field pulse equipment used in this study was designed and assembled as described in an earlier study (Kayal*viz*hi and Antony, 2015).

Processing methods

Thermal pasteurization

Thermal pasteurization method was carried out in the Department of Bio-Technology, Food Technology Laboratory Taramani, Anna University, Chennai at 2016. The equipment used for thermal pasteurization is shown in Fig 1. The flavoured milk samples were pasteurized in a laboratory scale pasteurizer (Armfield FT75, Mumbai) at 72°C for 15 seconds (Quass, 1997). The pasteurizer consisted of a heating section, a holding section and cooling section. The treated samples were finally collected in sterile containers and taken for analysis. Half of the treated samples were stored for a week at 4°C until analysis.

Ultraviolet treatment

Ultraviolet Treatment was carried out in the Department of Bio-Technology, Crystal Growth Laboratory, Anna University, Chennai at 2016. For UV treatment, the flavoured milk samples were spread on a plate or tray and placed inside the UV treatment chamber Fig 2. UV radiation was passed through the samples at 254 nm, 20 Jm^{-2} for 60 min. UV radiation at wavelengths between 220 nm and 280 nm have been proved to have germicidal action (James, 2003). The treated samples were immediately transferred to sterile containers and taken for analysis. Also half of the samples were stored at 4°C for one week and were then analyzed for bacterial count and physicochemical properties.

Pulsed electric field treatment

PEF treatment was carried out in the department of EEE, high voltage division, CEG, Anna university, Chennai at 2016. The specification of the equipment was as given in the earlier study Kayalvizhi and Antony U (2016). High electric field pulses were applied at 50 $kVcm^{-1}$ field strength (Fig 3). The flavoured milk samples were treated at 150 pulses for 110 s and stored for a week at 4°C. For all PEF experiments, 1000 mL of rose milk and chocolate milk were added inside the treatment chambers under sterile conditions. After treatment the samples were collected in sterilized glass bottles for analysis and 500 mL was immediately stored at 4°C until analysis.

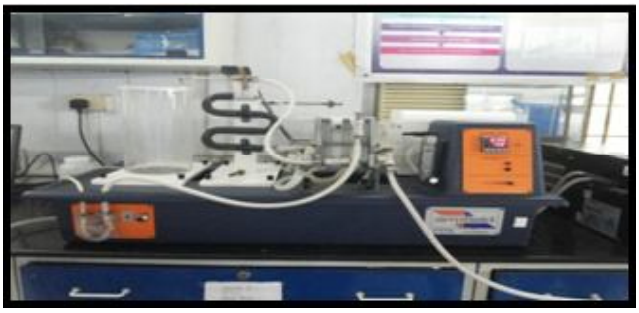

Fig 1: Thermal pasteurizer.



Fig 2: Ultraviolet radiation chamber.

Analysis of shelf life

Normally, Pasteurized milk could able to stable up to four hours at room temperature if unopened and also could retain the stability up to five to seven days if refrigerated below 4°C (40°F). For this reason Chemical and microbial tests on untreated and treated samples (stored at 4°C) were carried out on the first day and after 7 days in the Food Safety Laboratory of National Agro Foundation, Anna University Campus, Taramani. All chemical and reagents used were of analytical grade.

Chemical Analysis

Moisture

The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation. AOAC (2007) method is referred for this study (AOAC, 20007).

$$\% \text{ Moisture} = \frac{M_{INITLAL} - M_{DRIED}}{M_{INITLAL}} \times 100$$

pH ,Titratable Acidity (TA) and Protein

pH was measured using AP-1plus pH meter (Susima Chennai); acidity by titration with 0.1N sodium hydroxide to pale permanent pink end point with phenolphthalein and protein by kjeldahl method (Lynch and Barbano, 1999). The acidity in flavoured milk was calculated by titratable acidity method. Titratable Acidity (TA) is a measure of the amount of acid present in a solution. It is expressed as grams/liter (g/L) and is obtained by multiplying to percent TA by 10. So, a TA of 0.60% is expressed as 6g/L. This is the standard AOAC method 942.15 (AOAC, 2000).

Fat content

The fat content in milk was analyzed by Rose-Gotleib method, which is the standard AOAC official method 989.05, for the determination of fat content in milk. The fat % was calculated by

$$\text{Fat \% (w/w)} = \frac{\text{Weight of extracted fat}}{\text{Weight of the sample}} \times 100$$

Ash

The ash contents a measure of the total amount of minerals present within a food. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.



Fig 3: Pulsed electric field unit.

Principle

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600°C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, ***e.g.,*** iron, lead and mercury.

Calculation

The ash % was calculated by

$$\% \text{ ash (wet basis)} = \frac{M_{ASH}}{M_{WET}} \times 100$$

Where

M_{ASH} refers to the mass of the ashed sample and M_{WET} refer to the original mass of the wet samples.

Tss

The total soluble solids (TSS) content was determined at ambient temperature (28±2°C) by digital Refractometer (RX-7000α, Atago India Instruments Pvt. Ltd., India). The refract meter was calibrated with distilled water before measuring TSS of the sample. TSS was measured by placing a drop of flavoured milk on the prism of the refractrometer (Cavalcanti, 2006).

Calculation

The total solid content (%) was calculated by

Total solids (%) = 100 - Moisture

Microbial analysis

Samples were analyzed for the presence of microbes by serial dilution and pour plate method using standard plate count agar (Hi Media Mumbai). Plates were incubated at 37°C for 48 h. The colony counts were carried out using a digital colony counter (Lapiz). The flavoured milks were analyzed for bacterial count both on the 1st day and on the 7th day. The thermally pasteurized, UV treated and PEF treated flavoured milks were analyzed individually and based on the results, comparison of each method with the other two and finally identified the best method which extends the shelf life of milk without altering the nutritional properties.

RESULTS AND DISCUSSION

Physicochemical analysis

The physicochemical properties like pH, acidity, moisture, ash, TSS, fat and protein of flavoured milk samples, both treated and untreated by thermal pasteurization, ultraviolet radiation and pulsed electric field processing are given in Tables Table 1, Table 2 and Table 3.

pH

Cow's milk is slightly acidic and the pH ranges from 6.4 to

6.8. Milk from other bovines and non-bovine mammals varies in composition, but has a similar pH. The pH of milk changes over time. As milk goes sour, it becomes more acidic and the pH gets lower (USFDA, 2009). The pH of the thermally pasteurized, UV and PEF treated flavoured milk showed no significant change on storage for seven days at 4°C. The pH of untreated chocolate milk ranged between 6.6±0 and 6.84±0 and rose milk ranged between 6.27±0 and 6.61±0 which is the normal pH of milk. While thermal treatment of chocolate milk (6.71±0.07) and rose milk (6.64±0.04) caused no change, slight decrease was seen with treated PEF chocolate milk (6.13 ±0.04).

Acidity

As the acidity has a major influence on the taste of the product, this parameter is used to test the quality of milk. Also, as the acidity of milk increases with the storage time, this parameter is also a means of checking storage conditions. The acidity of fresh milk has been commonly attributed to the presence of acid phosphates and casein. The acidity of milk from individual cow's ranges between 0.10 and 0.26%. In the thermally pasteurized chocolate milk the acidity % was between the normal levels at around 0.15 to 0.2. On storage, acidity of thermally pasteurized chocolate milk and rose milk was 0.14±0 and 0.15±0.01. Under UV treatment, the acidity of chocolate milk and rose milk was 0.21±0.09 and 0.19±0.04. From these results it was clearly seen that, there was no significant change in thermally pasteurized and UV treated samples, while PEF treated samples showed (0.5±0) a marginal increase (Zhang, 1995).

Table 1: Physicochemical analysis of thermally pasteurized chocolate milk and rose milk.

Tests	Chocolate milk			Rose milk		
	Control (untreated)	Treated sample		Control (untreated)	Treated sample	
	1st Day	1st Day	7th Day	1st Day	1st Day	7th Day
pH	6.60±0	6.61±0	6.71±0.07	6.61±0	6.68±0.06	6.64±0.04
Acidity	0.19±0.02	0.15±0.06	0.14±0	0.16±0.01	0.17±0.5	0.15±0.01
Ash	0.51±0.04	0.55±0.02	0.54±0.05	0.47±0	0.49±0.12	0.46±0.01
Moisture	82.42±4.60	85.51±4.12	84.46±3.71	82.89±4.35	83.31±3.35	84.68±0.95
Tss	17.52±7.53	14.64±3.89	12.53±3.70	17.66±3.55	12.69±3.35	14.26±0.53
Fat	3.45±0.35	3.45±0	3.2±0.07	3.8±0	3.7±0	3.6±0
Protein	3.93±0.04	2.85±0.19	2.59±0.04	3.85±0.08	3.35±0.09	2.88±0.01

Table 2: Physicochemical analysis of UV treated chocolate milk and rose milk.

Tests	Chocolate milk			Rose milk		
	Control (untreated)	Treated sample		Control (untreated)	Treated sample	
	1st Day	1st Day	7th Day	1st Day	1st Day	7th Day
pH	6.76±0.01	6.55±0.09	6.51±0.05	6.61±0.01	6.51±0.07	6.47±0.04
Acidity	0.19±0	0.209±0.07	0.21±0.09	0.17±0	0.18±0.07	0.19±0.04
Ash	0.67±0.03	0.72±0.09	0.823±0	0.76±0.09	0.72±0.03	0.74±0.08
Moisture	78.55±1.62	77.44±2.26	77.54±2.33	79.82±0	78.11±2.16	76.76±1.76
TSS	21.74±0.52	22.56±2.26	21.54±1.21	21.51±0.59	21.74±2.38	22.38±1.02
Fat	3.5±0.05	3.5±0.65	3.25±0.26	4.15±0	4.12±0.40	4.04±0.26
Protein	3.9±0.42	3.6±0.07	3.5±0.04	3.02±0	2.8±0.07	2.6±0.04

Table 3: Physicochemical analysis of PEF treated chocolate milk and rose milk.

Tests	Chocolate milk			Rose milk		
	Control (untreated)	Treated sample		Control (untreated)	Treated sample	
	1st Day	1st Day	7th Day	1st Day	1st Day	7th Day
pH	6.84±0	6.53±0	6.49±0.07	6.27±0	6.19±0.06	6.13±0.04
Acidity	0.349±0.02	0.4±0.06	0.5±0	0.327±0.01	0.4±0.5	0.5±0.01
Ash	0.55±0.04	0.54±0.02	0.53±0.05	0.704±0	0.704±0.12	0.704±0.03
Moisture	84.84±2.60	83.84±1.12	83.34±1.71	86.83±4.35	85.76±3.35	85.36±0.95
TSS	15.15±6.53	16.16±3.69	16.66±3.70	13.17±3.55	14.24±3.25	14.64±0.52
Fat	3.55±0.35	3.55±0.42	3.55±0.07	3.61±0	3.61±0	3.59±0
Protein	2.4±0.2	2.35±0.19	2.32±0.04	2.41±0.08	2.39±0.09	2.29±0.05

Both thermal and non-thermal treatments did not affect the acidity of the samples.

Moisture content

Milk is approximately 87% water, so it is a good source of water in the diet. Water does not provide a nutritional benefit in the same manner as proteins or vitamins. However, water is extremely important in human metabolism. Water content of milk is dependent upon the synthesis of lactose. Water provides the aqueous medium for suspension of organic components of milk. The moisture % in the treated flavoured milk samples are shown in the Fig 4. In the chocolate milk, the moisture content of the untreated sample was maintained for 7 days even after treatment. Only a slight change in moisture content was noticed. PEF treated sample showed a higher amount of moisture than the other methods. UV treated milk typically showed no change in moisture content. In the rose milk sample, the UV and PEF treated sample showed gradual decrease in moisture content over a period of 7 days. PEF treated sample showed a higher amount of moisture than the other methods. Thus on comparing the results obtained, PEF was found to be more effective than the other two methods.

Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed. It provides a measure of the total amount of minerals within a food. The normal range of ash content present in milk is between 0.5 and 0.79%. The ash content in the untreated chocolate milk is maintained even after treatment. The content remained unaltered after 7 days. The UV treated chocolate milk (0.823±0) showed an slight increase in ash content of on the 7th day. The ash content in the pasteurized and PEF treated sample did not show any alteration. The ash content in rose milk did not vary widely by the three treatment methods and was maintained.

TSS

Total soluble solids are primarily sugars. The acids and minerals in the food also contribute to the soluble solids. The normal TSS content ranges from 13.26 to 26.30 for milk drinks. The TSS% remained unaltered for 7 days after treatment in the UV and PEF treated milk. The thermally pasteurized sample showed a decrease in TSS%. But the overall TSS% remained between the normal ranges. The TSS% in the PEF treated rose milk sample showed minimum changes while those in the UV and TP treated samples showed some changes. Thus PEF was found the better method among the three methods.

Fat content

Fats are a structural component of cell membranes and hormones. The fatty acids in milk fat are approximately 65% saturated, 29% monounsaturated and 6% polyunsaturated. Milk is approximately 3.8% fat. The fat content in the treated flavoured milk samples are compared in Fig 5. The fat content in the thermally pasteurized and PEF treated chocolate milk was found to lower after 7 days. The fat content in the UV treated chocolate milk remained unaltered at the end of 7th day. The PEF treated chocolate milk remained high when compared with the other two methods. The fat content was high in UV treated milk and remained constant with minimal changes. Also the fat content in PEF treated milk was excellently maintained for 7 days. Thus on comparing the results obtained PEF was found to be a better method for milk processing.

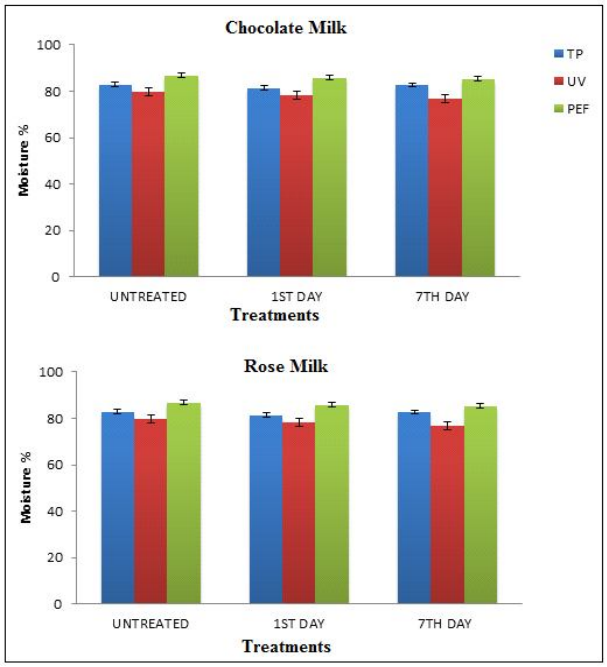

Fig 4: Moisture content of chocolate milk and rose milk.

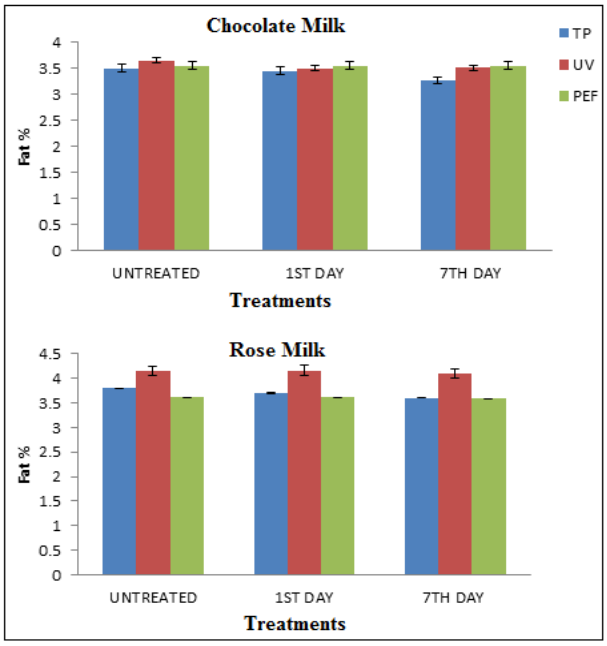

Fig 5: Fat content of chocolate milk and rose milk.

Protein content

Proteins are the fundamental building blocks of muscles, skin, hair and cellular components. They play a critical role in many body functions as enzymes, hormones and antibodies. Proteins may also be used as an energy source by the body. Milk is approximately 3.3% protein and contains all of the essential amino acids. Milk protein consists of approximately 82% casein and 18% whey (serum) proteins. The standard graph for protein estimation is shown below in Fig 6. The protein content in the treated flavoured milk is compared. The protein content in TP and UV treated chocolate milk showed greater variation while that of the PEF treated sample remained constant. PEF was found to be better method with minimum alteration in protein content. But there is some change in the untreated milk during PEF processing which accounts for the low level of protein. This property which causes the change should be looked into by researchers. The protein content of PEF treated rose milk sample showed a slight decrease whereas TP and UV treated sample showed greater reduction. Thus, on comparing the results above, PEF was found to be a better method in preserving the shelf life of flavoured milk.

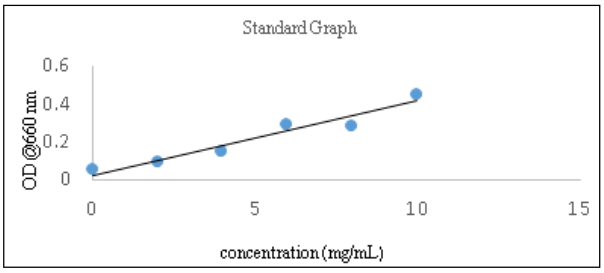

Fig 6: Standard graph for protein estimation.

Microbial analysis

The flavoured milk (rose milk and chocolate milk) were treated by thermal pasteurization, ultraviolet radiation and pulsed electric field processing. The treated samples were immediately plated for bacterial count by serial dilution and the colonies were counted. Similarly the samples stored at 4°C for a week were also plated and counted. From the data obtained, the microbial log reduction was calculated for each processing method and is presented in Table 4,5 and 6. Thus, thermal pasteurization led to significant log

Table 4: Bacterial log reduction in thermal pasteurization.

Milk sample	Microbial count 1st Day log cfu mL^{-1}	Microbial log reduction cfu mL^{-1}	Microbial count 7th Day log cfu mL^{-1}	Microbial log reduction cfu mL^{-1}
CMC	6.14±0.11	Nil	6.97±0.04	Nil
CMS	5.85±0.28	5.97	6.44±0.07	5.94
RMC	6.45±0.36	Nil	6.82±0.88	Nil
RMS	5.85±0.05	6.76	6.04±0.22	5.98

CMC - Chocolate Milk Control (untreated), CMS- Chocolate Milk Sample (treated).
RMC - (untreated), RMS - Rose Milk Sample (treated).

Table 5: Bacterial log reduction in UV radiation.

Milk sample	Microbial count 1st Day log cfu mL^{-1}	Microbial log reduction cfu mL^{-1}	Microbial count 7th Day log cfu mL^{-1}	Microbial log reduction cfu mL^{-1}
CMC	6.65±0.06	Nil	6.77±0.04	Nil
CMS	6.08±0.46	6.25	6.15±0.51	6.3
RMC	6.84±0.18	Nil	6.72±0.06	Nil
RMS	6.09±0.59	6.43	6.20±0.53	6.25

CMC - Chocolate Milk Control (untreated), CMS- Chocolate Milk Sample (treated), RMC- Rose Milk Control (untreated), RMS- Rose Milk Sample (treated).

Table 6: Bacterial log reduction in pulsed electric field.

Milk sample	Microbial count 1st Day log cfu mL^{-1}	Microbial log reduction cfu mL^{-1}	Microbial count 7th Day log cfu mL^{-1}	Microbial log reductionc fu mL^{-1}
CMC	6.85±0.13	Nil	7.95±0.31	Nil
CMS	5.49±0.22	6.83	6.00±0.15	7.94
RMC	6.34±0.19	Nil	7.30±0.17	Nil
RMS	5.11±0.23	6.32	7.00±0.34	7.00

CMC - Chocolate Milk Control (untreated), CMS- Chocolate Milk Sample (treated), RMC - Rose Milk Control (untreated), RMS- Rose Milk Sample (treated).

reduction both in chocolate milk (5.97 cfu mL^{-1}) and rose milk (6.76 cfu mL-1). This log reduction was maintained for a period of 7 days when stored at 4°C resulting in 5.94 and 5.98 cfu mL^{-1} log reduction in chocolate milk and rose milk, respectively. This shows that thermal pasteurization preserves the shelf life of milk with significant changes in the microbial load. Ultraviolet radiation led to a 6.25 and 6.43 cfu mL^{-1} log reduction in the treated chocolate milk and rose milk samples, respectively. This was also maintained for a period of a one week. Ultraviolet radiation proves to possess germicidal action at a wavelength of 254 nm and restricts the growth of microbes even after treatment. Thus on comparing the above results it is clear that non thermal methods led to a higher log reduction than the thermal method. While comparing the non-thermal methods, Pulsed Electric field showed excellent effects in reducing the bacterial load than the UV radiation. Pulsed electric field led to a higher log reduction of 6.83 and 6.32 cfu mL^{-1} in treated samples. This was found to increase on the 7th day with 7.94 and 7.00 cfu mL^{-1} log reductions in microbial load. Pulsed electric field had damaged the microbial cells during treatment, restricted their growth and because of this the reduction was found higher after 7 days. In another trial, PEF processing of milk was combined with heat treatment up to 55-60°C and a significant reduction was observed in the microbial load.

CONCLUSION

Thermal pasteurization extends the shelf life of flavoured milk for up to a week when refrigerated at 4°C. But it showed some minor variations in the nutritional content of flavoured milk. In thermal pasteurisation and UV radiation, bacterial count log reduction of 6.43, 6.00 cfu mL^{-1} and 5.88 and 5.60 cfu mL^{-1} were obtained in chocolate milk and rose milk. Similarly, by the application of pulsed electric field method, there was a log reduction of 7.94 and 7.00 cfu mL^{-1} in chocolate milk and rose milk respectively. Ultraviolet radiation reduces the microbial count drastically but it has minimal effect on the physicochemical properties of milk. Pulsed electric field treatment had an excellent effect on preserving the shelf life of flavoured milk for up to a week without altering the physicochemical properties. From the above data, concluded that Non-thermal method of food preservation is better than the thermal method used. Also, on comparing the results of the thermally treated, UV treated and PEF treated samples; Pulsed Electric Field is the best method in extending the shelf life of chocolate and rose flavoured milk.

ACKNOWLEDGEMENT

We gratefully acknowledge Division of High Voltage Engnieering, Anna University, Chennai http://www.annauniv.edu/HighVoltage/index.php and Food Safety and Standards Division, “National Agro Foundation”, Centre for Biotechnology, Taramani, Chennai for the provision of laboratory facilities.

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