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Inactivation of Alkaline Phosphatase and Shelf Life Extension of Milk Processed by Hurdle Technology

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A B S T R A C T

This paper investigates the activity of alkaline phosphatase and shelf life of raw milk processed under hurdle technology (HT). In this study the hurdles given to process raw milk are pulsed electric field and mild heat. The raw milk was preheated to 40°C and 50°C and then it was subjected to different voltage gradients *viz.*,10KV/cm, 20KV/cm and 30KV/cm at two different treatment time (3 and 6 minutes). The processed samples were collected in sterile glass bottles. The HT processed milk was immediately subjected to alkaline phosphatase test to assess the inactivation level. The inactivation of alkaline phosphatase was observed in 10KV/cm at 40°C and 50°C for 3 and 6 minutes. It was observed that alkaline phosphatase was absent in the treated samples. Similar inactivation of alkaline phosphatase was also observed in 20KV/cm and 30KV/cm at 40°Cand 50°C for 3 and 6 minutes treatment. The collected samples were stored under refrigerated temperature to conduct storage studies. The treated samples were compared with the control (pasteurized) milk samples. It was observed that the shelf life of HT treated samples were highly significant ($p<0.01$) when compared to control samples. It was also observed that there was a significant difference ($p<0.01$) between treatments. The mean values of the shelf life of the HT treated milk samples ranged between 16.33 ± 0.21 to 30.83 ± 0.17 for various treatments.

Introduction

Consumers demand for fresh, safe food with favorable sensory properties and nutritional properties with an adequate shelf life of the food products. In recent years there is a considerable interest in hurdle technology for the processing of foods and beverages. The hurdle Technology (HT) is the combination of two or more preservation techniques (Khan *et al.*, 2017). The innovative technology of using a high voltage Pulsed electric field (PEF) for food preservation appears to be promising especially when combined with other preservation methods. The popularity of minimally processed foods has brought increased attention to preserve through the use of combined technique (Smith *et al.*, 2008).

Milk is a highly perishable food. The enzyme present in the milk plays a vital role in microbial growth. Milk contains high nutrient content and its rich composition serves ideal medium for the growth of micro organisms.

The proteins and lipids present in the milk are broken down by enzyme which leads to microbial growth. Thus the shelf life of milk is even limited at refrigerated conditions (Craven *et al.*2008). Proteases and lipase are the enzymes present in the milk that are produced by psychrotrophic bacteria. These enzymes hydrolyse milk proteins and leads to development of off flavours and rancidity (Zhang *et al.,* 2015).

Alkaline phosphatase is an enzyme that is naturally present in milk. Alkaline phosphatase test is the index for proper pasteurization. Proper pasteurization confirms the destruction of enzyme.

Alkaline phosphatase enzyme is more heat resistant than most pathogenic bacteria (Fadiloglu *et al.,* 2004).

Thermal treatments are used to inactivate the alkaline phosphatase. However, this method can affect the organoleptic and nutritional properties such as dissolved mineral, calcium, phosphorus and also damage the whey protein (Hadi *et al.,* 2016). Hence this study is envisaged to investigate the activity of alkaline phosphatase and shelf life extension of milk by hurdle technology treatment by combining the effects of PEF and mild heat.

Materials and Methods

The raw milk was obtained from the nearby local farms around Alamathi - Koduveli village. Platform test such as clot on boiling and sensory analysis were done initially to check the quality of the milk.

Hurdle processing of raw milk

Hurdle treatment

Raw milk samples were heated to 40°C and 50°C. The heated milk samples were filled in a lab model pulsed electric field (PEF) chamber comprising of the two stainless steel circular parallel plate electrodes. The lab model PEF system was used for processing the milk samples under hurdle technology. The PEF system produced square pulses of 2.5 µs pulse width with a maximum voltage gradient of 40KV with variable frequency. The milk was treated at different voltage levels (10KV/cm, 20KV/cm and 30KV/cm) with different time periods (3 minutes and 6 minutes). The treated samples were collected using sterile glass bottles and compared with the pasteurized milk to study the presence or absence of alkaline phosphatase and shelf life of milk stored at refrigerated temperature.

Alkaline phosphate test

1.5g of sodium bicarbonate and 3.5g of anhydrous sodium carbonate was dissolved in water and made up to one litre. 0.15g of substrate (di sodium para nitrophenyl phosphate) was taken in 100ml measuring cylinder and made up to 100ml with buffer solution. 1 ml of milk was taken in a sterile test tube and 5 ml of para nitro phenyl phosphate buffer substrate was added to it and closed with a sterile rubber cork. The test tubes were kept in water bath at a temperature of 37°C for 2 hrs and the change in color was noted.

Results and Discussion

Inactivation of Alkaline Phosphate in hurdle technology processed raw milk at different voltage gradients, time and temperature in the Table 1.The raw milk treated at 10KV/cm at 40°C and 50°C for 6 minutes showed the

absence of alkaline phosphatase enzyme. The raw milk treated at 20KV/cm and 30KV/cm at 40°C and 50°C for 3 and 6 minutes showed the absence of alkaline phosphatase.

Shelf life extension of hurdle technology processed raw milk at different voltage gradients, time and temperature in the Table 2.The mean values of 16.33 ± 0.21, 21.67 ± 0.33, 16.83 ± 0.40, 21.67 ± 0.33, 21.33 ± 0.21, 21.83 ± 0.40, 21.33 ± 0.49, 26.33 ± 0.21, 27.00 ± 0.26, 31.50 ± 0.22, 26.50 ± 0.22, 30.83 ± 0.17, 10.50 ± 4.10, 4.00 ± 0.63 were obtained respectively for various treatments(T_1:T_{12}), control and raw milk. It was observed that the shelf life of HT treated samples were highly significant ($p<0.01$) when compared to control samples. It was also observed that there was a significant difference ($p<0.01$) between treatments.

Table.1

Treatment temperature / Voltage gradient (KV/cm)	40°C		50°C	
	Treatment time		**Treatment time**	
	3min	**6min**	**3min**	**6min**
10	-	-	-	-
20	-	-	-	-
30	-	-	-	-
Control milk	-			

Table.2

HT treatment parameters	Treatment	Mean values (shelf life in days)
10 KV 40° C 3 mins	T_1	16.33 ± 0.21^{c}
10 KV 40° C 6 mins	T_2	21.67 ± 0.33^{c}
10 KV 50° C 3 mins	T_3	16.83 ± 0.40^{d}
10 KV 50° C 6 mins	T_4	21.67 ± 0.33^{e}
20 KV 40° C 3 mins	T_5	21.33 ± 0.21^{d}
20 KV 40° C 6 mins	T_6	21.83 ± 0.40^{d}
20 KV 50° C 3mins	T_7	21.33 ± 0.49^{e}
20 KV 50° C 6 mins	T_8	26.33 ± 0.21^{e}
30 KV 40° C 3 mins	T_9	27.00 ± 0.26^{d}
30 KV 40° C 6 mins	T_{10}	31.50 ± 0.22^{d}
30 KV 50° C 3 mins	T_{11}	26.50 ± 0.22^{f}
30 KV 50° C 6 mins	T_{12}	31.83 ± 0.17^{f}
Control		10.50 ± 4.10^{b}
Raw milk		4.00 ± 0.63^{a}
F		43.441 **

@ average of 6 trails
** - Highly Significant (P<0.01)

The inactivation of alkaline phosphatase is observed in all the HT treated samples. This is due to the hurdles (PEF and mild heat) applied to the milk samples. The electric field strength, pulse duration of PEF treatment, mild heat influenced the inactivation of alkaline phosphatase and resulted in shelf life extension (Barsotti and Cheftel, 1999). The Duncan's analysis was carried out to find homogeneous subsets. The values revealed that the shelf life was highly significant ($p<0.01$) between the treatments. The mean values showed that the shelf life of raw milk subjected to treatment T_{12} (30 KV 50° C 6 mins) had greater shelf life compared to the other treatments. As the electric field intensity, PEF treatment time and temperature was greater than the other treatments, the HT treated samples (T_{12}) had a greater shelf life (Heinz *et al.*, 2003).

In conclusion, hurdle Technology treatment (PEF and mild heat) inactivated alkaline phosphatase and reduced the microbial load which increased the shelf life of the treated milk with acceptable sensory attributes. It was inferred that the effectiveness of the inactivation level was increased by increasing the treatment time and voltage gradient.

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