Universiti Malaysia Terengganu Journal of Undergraduate Research
Volume 1 Number 4, October 2019: 77-82
eISSN: 2637-1138

STABILITY OF LACTIC ACID BACTERIA IN PASTEURIZED COW'S AND GOAT'S MILK

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Abstract: This study was done to determine the effect of pasteurization on the stability of lactic acid bacteria (LAB) and its enzyme in raw and pasteurized cow's and goat's milk. The total viable count for plate count of the bacterial growth concentration was higher in both pasteurized cow's and goat's milk at 2.48 log CFU/ml. This is followed by raw cow's milk (1.59 log CFU/ml) and raw goat's milk (0.65 log CFU/ml). Lactic acid bacteria (LAB) was found to be similar in both raw cow's and goat's milk ($p>0.05$), and pasteurized milk of both animals also contained the same amount of LAB ($p>0.05$). LAB was still detected in pasteurized milk ($p<0.05$), indicating the stability of LAB against the pasteurization temperature. Interestingly, based on API ZYM assay kit results, there were nine different enzymes detected in all samples, which were *leucinearylamidase, valinearylamidase,cystinearylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase*and *acid phosphatise*. The results revealed that different types of lactic acid bacteria were detected in treated and non-treated milk samples produced by different animals, indicating the different stability levels of LAB against pasteurization.

Keywords: Milk, cow, goat, pasteurization, lactic acid bacteria, stability

Introduction

Pasteurization is one of the essential methods to destroy any pathogens in milk (Abdul Elrahman*et al*., 2013). Different pasteurization methods such as Low Temperature Long Time (LTLT), High Temperature Short Time (HTST) and cross-contamination after pasteurization play an important role in killing bacteria growth in milk including beneficial bacteria (Dumalisile*et al*., 2005). Milk is one of the natural habitats rich in nutrients for lactic acid bacteria growth (Delavenne*et al*., 2012). Lactic acid bacteria (LAB) plays an important role in dairy products especially milk. It has an antimicrobial compounds that promote probiotic properties and can help to prolong milk shelf life and its nutritious components (Khedid*et al*., 2009; Teshome, 2015). However, the temperature of pasteurization may affect the existence of lactic acid bacteria (LAB). The growth rate of LAB is totally dependent on the available nutrients in the media (Hutkins& Nannen, 1993). As the nutrients are reduced due to the pasteurization temperature (Tesfay et al., 2015), the growth of LAB may be affected as well. LAB also helps in reducing the lactose intolerance (de Vrese *et al*., 2001). High temperature of pasteurization may reduce these effects too.

Lactic acid bacteria is classified to synthesize a diverse type of enzyme which may influence the composition in food (Patel *et al*., 2013). Enzymes found in milk are not easily present for digestibility of milk (Claeys*et al*. 2013). The activity of enzymes is mostly affected by the temperature, pH, presence of substrate, activators and inhibitors. Enzymes mostly are inactive at pasteurization condition due to long treatment with high temperature. However, not all enzymes will be destroyed during the pasteurization process. Many of these enzymes remain active even after the heat treatment of milk, which also may be related with the presence of lactic acid bacteria in pasteurized milk (Samarzija*et al*., 2012).

Materials and Methods

Sample collection

This experiment was conducted in Universiti Malaysia Terengganu. Milk samples were collected in Marang, Terengganu, and at Veterinary Laboratory, Kelantan. Samples were then analysed with microbiological viable count and lactic acid bacteria detection. The rest of the samples were kept in freezer at (-20°**C**) until required.

Microbiological analysis

Microbiological analysis was done based on a method by Tasci (2011) with minor modifications. A total of 25 g of milk sample was homogenized with 225 ml of buffered peptone water aseptically. A serial dilution was done up to 10^{-8} using buffered peptone water, and 0.1 ml of the dilution was spread on Plate Count Agar (PCA) plate and incubated at 37°C for 24 hours.

LAB identification in milk

Lactic acid bacteria (LAB) identification in milk was done based on methods by Sengun *et al.* (2009) and Schillinger *et al.* (2010) with minor modifications. A total of 25 g of each milk sample was homogenized in 225 ml of buffered peptone water. Then, 1 ml of homogenized sample was added into 9 ml of Man Rogosa Sharpe (MRS broth). This step was repeated for every sample. These samples were incubated at 30°C for 24 to 48 hours in anaerobic condition. A serial dilution was done up to 10^{-8} using saline solution. 0.1 ml of dilution was pipetted and spread on MRS agar. The prepared petri dishes were incubated at anaerobic condition for 2 days.

API ZYM

The identification and confirmation of lactic acid bacteria (LAB) was done using API ZYM (BioMerieux, France). API ZYM is a system that is able to identify enzymes applied by 81 bacteria belonging to several species. The LAB presence in milk can be identified by the enzymes produced which are alkaline phosphatase, esterase, esterase lipase, lipase, leucinearylamidase, valinearylamidase, cystinearylamidase, trypsin, α-chymotrypsin, aidphosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase (Humble *et al.*, 1977). An incubation box was spread with 5 ml of distilled water into the wells of the tray to create humid atmosphere. The sample reference was analysed elongated flap of the tray. The strips were removed from the individual packaging and placed in the incubation box. 65µL was dispensed using micropipette of specimen into each cupule. After inoculation, the plastic lid was placed on the tray and incubated for 4 hours at 37°C. Then, a drop of ZYM A and ZYM B were added into each cupule and then kept in the dark for 5 minutes and under the light for about 10 seconds. This helped to eliminate any yellow colour which might appear in the cupules due to any excess of Fast Blue BB which had not reacted. The reactions were then recorded and graded depending on the intensity of the colour compared with the colour of representations.

Statistical Analysis

All the obtained data were analysed with two-way analysis of variance (ANOVA) using Minitab software to see the interaction between animals and pasteurization at ($p< 0.05$). Pearson correlation coefficient was also used to determine the linear relationship between all the variables.

Results and Discussion

Microbiological analysis

Total bacterial count was the highest in pasteurized milk for both ruminants (Table 1). Although raw milk contained lower bacteria ($p<0.05$), it had the possibility of having various bacterial population (Quigley et al. 2013). In contrast, the pasteurized milk microbiota was unable to appear due to low capability which increased the mesophilic bacteria in pasteurized milk.

Table 1: Total plate count and lactic acid bacteria in raw and pasteurized cow's and goat's milk.

Sample	RCM	RGM	PCM	PGM
Total plate count (cfu/ml)	1.59±0.28^{b}	0.65±0.06^{c}	2.48±0.00^{a}	2.48±0.00^{a}
Lactic acid bacteria (cfu/ml)	2.43±0.03^{a}	2.41±0.03^{a}	2.18±0.01^{b}	2.11±0.09^{b}

Note:*RCM = Raw cow's milk; RGM = Raw goat's milk; PCM = Pasteurized cow's milk; PGM = Pasteurized goat's milk.* Each value is presented as mean ± standard deviate on (n=3). Different letter indicates significant difference (p<0.05) between column

Tamime and Robinson (1999) stated that most of the bacteria that survived in pasteurized milk is known as thermoduric bacteria which may be in high number of colonies. Other probiotic bacteria known as LAB is also categorized as thermoduric bacteria and may increase its population. The amount of LAB in the milk may decrease the diversity of microorganisms (Widyastuti*et al*., 2014). This also known as synergistic effect, when LAB is able to overcome the population of microorganisms (Wedajo, 2015).

Higher LAB colony was detected in raw milk compared to pasteurized milk. This result was similar to a study by Bluma and Ciprovica (2015). Raw milk was reported to be rich in a variety of LAB (Wassie&Wassie 2016; Azhari Ali, 2011). The existence of LAB in pasteurized milk indicated the high stability of LAB against high temperature due to its thermoduric properties (Carminati *et al*., 2014). This property is important for the LAB to be developed as the starter culture for any fermented products.

API ZYM Test

API ZYM is used to identify the enzyme profile of different lactic acid bacteria (LAB) in milk samples (Stoyanovski*et al*., 2013). The enzyme present had introduced the potential LAB in milk samples. Different enzymes had been detected in different strains of LAB in different samples (Table 2). This shows that there was interaction between conditions of the sample either due to the difference of available macronutrients or heating treatments. Higher macronutrients were found in pasteurized milk (Table 1) which might also help in the survival of LAB in pasteurized milk. Table 3 shows different types of enzyme profile by LAB isolated from different samples. The enzyme profile may be used to assume the potential type of LAB in the sample (Humble *et al.*, 1977).

Different enzyme activities were shown by different samples (Table 2). Most of the enzymes produced by the LAB strain were areleucinearylamidase, valinearylamidase,cystinearylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase,α-glucosidase,β-glucosidase and acid phosphatase. All of these enzymes have different characteristics, indicating the difference in stability between strains of LAB. More enzyme activities of LAB were shown by pasteurized milk. The difference was related to the types of animals and also heat treatments. Higher enzyme activity in pasteurized milk indicated the thermal stability by the LAB. In contrast, lower activity in raw milk could be due to the synergestic effect between other aerobic bacteria and LAB. The diverse types of enzymes by LAB may influence the composition and taste in food (Patel *et al*., 2013; Stoyanovski*et al.*, 2013).

Table 2: Enzyme activities of lactiç acid bacteria isolates grown on MRS agar as determined by the API ZYM reactions.

ENZYME ASSAY FOR	SAMPLE			
	RCM	**RGM**	**PCM**	**PGM**
control	+	+	+	+
Alkaline phosphatase	-	-	-	-
Esterase (C4)	-	-	-	+
Esterase Lipase (C8)	-	+	+	+
Lipase (C14)	-	-	-	-
Leucinearylamidase	-	+	+	+
Valinearylamidase	-	+	+	+
Cystinearylamidase	-	-	+	+
Trypsin	-	-	+	+
α-chymotrypsin	-	-	+	+
Acid phosphatase	-	+	+	+
Naphtol-AS-BI-phosphohydrolase	-	+	+	+
α-galactosidase	-	-	-	-
β-galactosidase	-	-	-	-
β-glucuronidase	-	-	-	-
α-glucosidase	-	-	+	+
β-glucosidase	-	-	+	+
N-acetyl-β-glucosaminidase	-	-	+	+
α-mannosidase	-	-	-	-
α-fucosidase	-	-	-	-

Note: *RCM = Raw cow's milk; RGM = Raw goat's milk; PCM = Pasteurized cow's milk; PGM = Pasteurized goat's milk*

Conclusion

The purpose of this study is to determine the effect of pasteurization on the stability of lactic acid bacteria (LAB) in milk of two ruminants—goats and cows. Goat's and cow's milk were tested to determine any interactions between different types of animals. Other general microorganisms were also tested to determine if their existence would create a synergestic condition towards the LAB growth. The results showed that raw milk of both animals contained slightly higher LAB compared to pasteurized milk. Lower count of total bacterial growth was also detected in the raw milk which may be due to the result of synergestic effect between both aerobic bacteria and LAB in the milk. By using API ZYM assay kit, all of the LAB strains showed different enzyme activities. Most of the enzymes produced by the LAB strains were areleucinearylamidase, valinearylamidase,cystinearylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase,α-glucosidase,β-glucosidase and acid phosphatase. All of these enzymes showed different characteristics, indicating the difference in stability between strains of LAB.

Acknowledgements

The authors would like to acknowledge the facilities and financial support provided by the School of Food Science and Technology, Universiti Malaysia Terengganu. The authors would also like to thank the owner of an abattoir in Marang, Terengganu, and Veterinanry Laboratory in Kelantan for the fresh samples.

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