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STABILIZATION OF FRESH BUFFALO MILK BY ACTIVATING LACTOPEROXIDASE SYSTEM

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ABSTRACT

The aim of this study was preservation of buffalo raw milk by the activation of their natural lactoperoxidase system to extend shelf life at different temperatures. Milk samples treated with equal concentration of sodium thiocyanate ions and hydrogen peroxide i.e. 10, 20 and 30 ppm and stored at 35, 40 and 45 ^{0}C. A total of 30 fresh raw milk samples from buffalo were used for the present study. For each treatment group, 10 raw milk samples at different temperatures were used for acidity, pH, clot on boiling test (COB), alcohol test, methylene blue reduction and thiocyanate values. Each test was performed from 0 to 9 hours with an interval of three hours except thiocyanate values, which was recorded initially and at curdling. Acidity percentage was analyzed at 35^{0}C for 0 h, 3 h, 6 h and 9 h. At 6 h and 9 h; all the combinations were significantly ($P<0.01$) different, except 0 ppm versus 10 ppm at 9 h. At 40^{0}C for 0 h, 3 h, 6 h and 9 h. All the combinations were significantly different at 0 h and 3 h except 20 ppm versus 30 ppm. The remaining combinations were significantly ($P<0.01$) different pH were significantly ($P<0.05$) different at 0 h between 0 ppm versus 30 ppm, 10 ppm versus 20 ppm; at 3 h, 20 ppm versus 30 ppm; at 6 h and 9 h all the combinations. The remaining combinations were highly significant ($P<0.01$) from each other. Thiocyanate at 35^{0}C, 40^{0}C and 45^{0}C there was significant difference ($P<0.01$, $P<0.001$) at all the stages except at 10 ppm and 30 ppm at 35^{0}C. Clot on boiling test at 35^{0}C and 40^{0}C, 10 ppm and 30 ppm at 3 h, and 20 ppm COB started positive at 4 h. Alcohol test of the samples were positive for control and 10 ppm at 6 h and for 20 ppm at 7 h respectively. At 40^{0}C, for 10 ppm, 20 ppm and control the positive reaction started at 3 h. At 45^{0}C, the reaction was positive at 1 h for control, at 3 h for 10 ppm and at 6 h for 20 ppm and at 8 h for 30 ppm. Methylene blue reduction test of the control samples were positive at 3 h. At 35^{0}C, for 10 ppm, 20 ppm and 30 ppm positive reaction started at 5 h, 7 h and 7 h respectively. At 40^{0}C, for 10 ppm, 20 ppm and 30 ppm positive reaction started at 1 h, 2 h and 2 h respectively. And at 45^{0}C, for 10 ppm, 20 ppm and 30 ppm positive reaction started at 2 h, 5 h and 6 h respectively. Milk samples stabilized with 30 ppm were acceptable up to nine hours as compared to control which curdled within seven hours post milking. In Pakistan preservation of buffalo raw milk can be carried out by its lactoperoxidase enzyme system, which helps in collection of milk of high quality from widely scattered remote areas.
The study suggests that in stabilized samples, the changes in pH were slight up to 9 hours post-preservation, which might be due to the optimum activation of LP system. Milk samples stabilized with 30 ppm were acceptable up to nine hours as compared to control which curdled within seven hours post milking.

Key words: Milk, buffalo, dairy production, lactoperoxidase system, preservation.

INTRODUCTION

Despite a reasonable growth there are major concerns in the area of milk production faced by the dairy sector in Pakistan. Milk yield per animal is quite low as compared to developed and other developing countries. Milch animals are generally underfed and use of quality feed is limited especially in harsh months of summer and winter in different localities of the country. Only a small proportion of total milk production is marketed and the rural households traditionally process most of the produce to make “ghee” (butter oil) and other milk products. The raw milk marketed through a large chain of intermediaries often lack quality due to non-adoption of clean milk production practices at the farm level, lack of chilling facilities, use of substandard containers, and adulteration at different stages (Athar and Tariq, 1991; Karim and Kamkar, 2000)

Milk contains many essential nutrients, such as carbohydrates, proteins, lipids, minerals and vitamins and therefore, acts as an ideal medium for rapid proliferation of harmful microorganisms (Saha *et al.,* 2003). Milk can usually be transported un-refrigerated for up to 20 km but after a certain period of time it will begin to deteriorate (Hirano *et al.,* 1998). Souring sets in and the milk quickly becomes useless. In Pakistan, generally in most of the cases milk is collected, handled and distributed in unsatisfactory and unhygienic conditions. Dairy farming, milk production and delivery systems are indigenous and very primitive. Farmers of smallholdings produce most of the milk but in small quantities. Non-availability of

cooling or chilling system and high ambient temperature are the main constrains in milk collection and distribution. These conditions decrease or shorten the shelf life of raw milk delivery to the consumer, especially during the summer, when the temperatures favor bacterial multiplication.

One specific system protecting the stomach and intestines of the newborn against bacterial infection is the lactoperoxidase/thiocyanate /hydrogen peroxide system– the LP-system (Marks *et al.,* 2001). Many studies has already been taken on buffalo *e.g*.; (Jandal, 1998; Girgis *et al.,* 2002) exploring the effect of naturally occurring lactoperoxidase system of fresh raw milk of buffalo by adding equal proportion of 10 ppm, 20 ppm, and 30 ppm of hydrogen peroxide and sodium thiocyanate. The result of the quality control that is total viable counts, total titrable acidity, pH, Clot on Boiling (COB) and Alcohol test indicated that the milk stabilized both 10 ppm, 20 ppm, 30 ppm of hydrogen peroxide and thiocyanate stored at 30 ^{0}C remained fresh/good from 8 to 12 hours, and more than 16 hours respectively as compared to control that curdled within 8 hours of milking. Rajesh Kumar and Bhatia (1999) analyzed thermostability of lactoperoxidase in buffalo milk and whey.

LPS activation mechanism catalyzes the hydrogen peroxide (H_2O_2) oxidation of several acceptor molecules: Acceptor + H_2O_2 oxidizes into + H_2O (de Wit and van Hooydonk, 1996) and then thiocyanate (SCN) converts into hypothiocyanite (OSCN; Wever *et al*, 1982)

The Lacto Peroxidase System naturally lasts in fresh milk for a short period of sometimes less than two hours, due to limitation of substrates (Martinez *et al,* 1998). The objective of present study i.e. addition of additives i.e. Sodium thiocyanate (NaSCN) and Hydrogen peroxide (H_2O_2) with different concentrations was to evaluate the longevity of preservation over natural LP system, as this is cheap, easy to use and readily applicable in developing countries with a minimum of training requirements.

MATERIALS AND METHODS

Buffalo milk: Samples of fresh Buffalo milk were collected from different dairy farms of Tandojam areas of District Hyderabad, Sindh. Fresh samples of buffalo milk were brought daily early in the morning to the laboratory of Dairy Technology Department, Sindh Agriculture University Tandojam for further processing. At the start, each milk sample was divided in to four portions (Three treatment and one control group) of 500 ml in a sterile conical flask. The lactoperoxidase system of the sample was then activated by the addition of equal proportion of Sodium thiocyanate (NaSCN) and Hydrogen peroxide (H_2O_2) at three different concentration 10 ppm, 20 ppm and 30 ppm. In the control sample of raw milk no any chemical or preservatives were added. After the addition of preservatives the samples were kept at three different temperatures 35 ^{0}C, 40 ^{0}C and 45 ^{0}C for 9 hours.

Preservation of buffalo raw milk: A total of 30 samples (500 ml) of fresh milk, 30 samples from Buffalo, 10 samples of each animal were preserved at three different temperatures i.e. 35 ^{0}C, 40 ^{0}C, and 45 ^{0}C. Parallel to treatment groups, a (500 ml) sample from the same milk was kept as control for comparison. Milk was preserved according to the method described by (FAO, 1991). Initially Alcohol test, Clot on Boiling test, Methylene blue reduction, pH values, Acidity % age and SCN values were recorded after every three hours. The entire tests were repeated till curdling occurred except SCN values were recorded initially and at the end.

Physical and chemical analysis of buffalo milk preserved by activation of its lactoperoxidase system: The milk samples were analyzed for qualitative examination by Alcohol test, Clot on boiling test, Titratable Acidity, pH Values after every three hours with the exception of thiocyanate values, which was recorded initially and finally at curdling.

Alcohol test: Alcohol test was performed according to the method previously described by Anon (1977). For rapid determination of elevated acidity of milk, 2 ml of milk sample was taken in a test tube and equal quantity of 68% alcohol was added in the sample and result was recorded as positive or negative. Positive result was confirmed when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostrum milk) and salt concentrates (mastitis) was considered as a positive result.

Clot on boiling test: Clot on boiling test was performed according to the method described by Anon (1977). For qualitative examination of milk, two ml of milk sample was taken in a test tube and heated over a flame or kept in boiling water for about five minutes and the result was recorded as positive or negative. Positive result was confirmed when the tested milk went through coagulation, clotting or precipitation, however, the first clotting due to acid development at 0.21-0.23% Lactic acid was not considered.

Methylene Blue: Methylene blue test was performed according to the method described by the Anon (1977). . 10 ml of milk sample was taken in a sterile test tube and one ml of Methylene blue was added in sample, tightly caped it and inverted 2-3 times and then kept in a water bath at 37 ^{0}C with duplicate, and result was recorded.

pH values: pH values were recorded by using the pH meter (Hanna Instrument, model HI 8471. Italy).

Titratable Acidity: Nine ml of milk sample was taken in a sterile conical flask and three drops of phenolphthalein indicator added in sample titrated with N/10 Sodium

hydro oxide NaOH solution till light pink color appeared. The results were calculated by the following formula.

$$\text{Acidity} = \frac{\text{No ml of N/10 NaOH used x 0.009}}{\text{Weight of sample used (Volume)}} \times 100$$

Thiocyanate values (SCN): Thiocyanate (SCN) values were determined by the method describe by the (FAO, 1991). Eight ml of milk sample was taken in test tube; four ml of 20 % Trichloro acetic acid solution was added in sample and kept for 30 minutes. After 30 minutes, filtered it through Watt man 40 No filter paper. 1.5 ml of the filtrate was taken in another test tube and 1.5 ml of the Ferric nitrate reagent was added in filtrate. Before recording the absorbance, the spectrophotometer meter was adjusted at 460 nm and the absorbance meter reading at 0 by using the blank sample, and then absorbance of the sample was recorded.

Statistical Analysis: The data for physico-chemical quality was analyzed by analysis of Variance (ANOVA), and Post Hoc Multiple comparison test and Paired samples T-Test using the SPSS release 7.5 Computer programmed (Copyright 1996, SPSS Inc).

RESULTS AND DISCUSSION

The result of this study revealed that initial total titrable acidity and pH of the control and stabilized milk sample were the same. In control milk samples after nine hours, a considerable increase in the acidity and decrease in pH was noted, whereas in stabilized samples, the changes were slight. This might be due to the optimum activation of LP system. Milk samples stabilized with 30 ppm were acceptable up to nine hours as compared to control which curdled within seven hours post milking. Acidity percentage was analyzed at 35^0C for 0 h, 3 h, 6 h and 9 h. At 6 h and 9 h, all the combinations were significantly (P<0.01) different, except 0 ppm versus 10 ppm at 9 h. At 40^0C for 0 h, 3 h, 6 h and 9 h, all the combinations were significantly different at 0 h and 3 h except 20 ppm versus 30 ppm. The remaining combinations were significantly (P<0.01) different. pH, were significantly (P<0.05) different at 0 h between 0 ppm versus 30 ppm, 10 ppm versus 20 ppm; at 3 h, 20 ppm versus 30 ppm; at 6 h and 9 h all the combinations. The remaining combinations were different from each other (P<0.01). Thiocyanate at 35^0C, $4^0$0C and 45^0C there was significant difference (P<0.01, P<0.001) at all the stages except at 10 ppm and 30 ppm at 35^0C. Clot on boiling test at 35^0C and 40^0C, 10 ppm and 30 ppm at 3 h, and 20 ppm COB started positive at 4 h. Alcohol tests of the samples were positive for control and 10 ppm at 6 h and for 20 ppm at 7 h respectively. At 40^0C, for 10 ppm, 20 ppm and control the positive reaction started at 3 h. At 45^0C, the reactions were positive at 1 h for control, at 3 h for 10 ppm and at 6 h for 20 ppm and at 8 h for 30 ppm. Methylene blue reduction test of the control samples were positive at 3 h. At 35^0C, for 10 ppm, 20 ppm and 30 ppm positive reaction started at 5 h, 7 h and 7 h respectively. At 40^0C, for 10 ppm, 20 ppm and 30 ppm positive reaction started at 1 h, 2 h and 2 h respectively. And at 45^0C, for 10 ppm, 20 ppm and 30 ppm positive reaction started at 2 h, 5 h and 6 h respectively. Milk samples stabilized with 30 ppm were acceptable up to nine hours as compared to control which curdled within seven hours post milking. In Pakistan preservation of buffalo raw milk can be carried out by its lactoperoxidase enzyme system, which helps in collection of buffalo milk of high quality from widely scattered remote areas.

REFERENCES

Anonymous (1977). Milk processing FAO Regional dairy development and training team for Asia and the pacific. Government of Philippines and Denmark.

Athar, I. H. and M. Tariq (1991). Practical problems of milk collection and research approach for their solution. Progressive Farming. 11: 132-136.

FAD/WHO. (1991). Codex Alimentarius commission during its 19th session held in July 1991 in Rome, Italy.

Girgis, E. S., A.A. Ismail, S.M. El-Dieb. and W.M Zaky (2001). Isolation, purification and characterisation of buffaloes' and cows' milk lactoperoxidase. Egyptian J. Food Science, 29: 215.

Hirano, R., M. Hirano, M. Oooka and K. Hatanaka (1998). Effects of lactoperoxidase on gelation properties of yogurt. Food Research International, 31: 1-6.

Jandal, J. M. (1998). Lactoperoxidase/garlic extract/ethanol system in the preservation of buffalo milk Buffalo J. 14: 95-101.

Karim, G. and A. Kamkar (2000). A study on the effect of lactoperoxidase system (LPS) and LPS plus riboflavin on the aflatoxin M1 in milk. J. Faculty of Vet. Med., University of Tehran, 55: 5-7.

Marks, N. E.; A. S. Grandison and M. J. Lewis (2001). Challenge testing of the lactoperoxidase system in pasteurized milk. J. Applied Microbiology, 91: 735-741.

Martinez C. E.; P. G. Mendoza, J. F. Alacon and H. S. Garcia (1988). HS Reactivation of the Lactoperoxidase system during raw milk storage and its effect on the characteristics of pasteurized milk. J. Food Protection, 51(7) 558-561.

Rajesh Kumar and K. L. Bhatia (1999). Thermostability of lactoperoxidase in buffalo milk and whey. Indian J. Dairy Sci., 52,(3),142-148.

Saha, B. K., M. Y. Ali, M. Chakraborty, Z. Islam and A. K. Hira (2003). Study on the preservation of raw milk with hydrogen peroxide for rural dairy farmers. Pakistan Journal of Nutrition, 2 (1) 36-42.

Wever, R., W. M. Kast, J. H. Kasinoedin, R. Boelens, (December 1982). "The peroxidation of thiocyanate catalysed by myeloperoxidase and lactoperoxidase". *Biochim. Biophys. Acta* 709 (2): 212–9.

de Wit J. N., and van A. C. M. Hooydonk (1996). Structure, functions and applications of lactoperoxidase in natural antimicrobial systems. Netherlands Milk & Dairy Journal 50: 227–244.