Evaluation of Thermal Processing of Commercially Available Milk Brands in Lahore

Ayesha[1], Raiha Fatima[2], Saddam Hussain[3], Ali Hamza Sajid[1], Misbah Murshid[1], Muhammad Javid Iqbal[1]

Abstract

Raw milk and commercially available milk samples were tested for the alkaline phosphatase activity to evaluate the efficacy of the pasteurization of milk. A colorimetric assay used for the kinetic determination of serum alkaline phosphatase to detect ALP in the targeted samples. The colorimetric assay uses p-nitrophenyl phosphate as the reaction substrate. When ALP acts upon this substrate, it breaks down into a phosphate group and p-nitrophenol which produces the characteristic yellow colour in the solution. The increase in absorbance of the solution measured at 405 nm is directly related to the ALP activity in the respective sample. Three raw milk samples were tested firstly as a whole and then after fat removal through centrifugation at 4000 rpm for 30 min. Seven UHT and three naturally pasteurized commercial milk samples available in the local market were used for the ALP detection in this study. Significant values for ALP activity in raw milk samples was observed whereas, for all the pasteurized samples, the test was observed to be negative that indicate the effective pasteurization process before the milk was packed for marketing. Casein was the major protein found in milk as 3.43g per 100 ml of milk sample, whereas the values for standard deviation and relative standard deviation were 0.08 and 2.54 respectively. Based on the negative results for ALP and actual yield of casein found to be closer to the theoretical yield for all the samples it can be suggested that all the targeted milk brands were safe to be consumed.

Keywords: *Alkaline phosphatase (ALP), Pasteurization, Bovine milk, UHT treatment, Milk casein, Thermal processing.*

Composition of Raw milk

Milk is considered an essential part of the human diet as it contains several constituents required for the proper growth and functioning of our immune system and is consumed by people of all ages around the world. It is a lacteal secretion

[1] Department of Chemistry, COMSATS University Islamabad, Lahore Campus, Pakistan.
[2] Department of Biosciences, COMSATS University Islamabad, Islamabad Campus, Pakistan.
[3] Department of Biochemistry, Minhaj University Lahore, Pakistan.

https://doi.org/10.58932/MULH0013

produced by mammals and it is the only food content taken in by their infants for a specific time duration in their initial growing years. The components present in milk include enzymes, growth factors, hormones, lactose, vitamins, proteins, and valuable minerals necessary for bone development. As milk is rich in nutrients having a pH between 6.5 to 6.7 which makes it a suitable and favourable medium for the microbial community to grow so it becomes necessary to treat milk thermally to eliminate the growth of such pathogens (Ritota, Di Costanzo, Mattera, & Manzi, 2017). Alkaline phosphatase (ALP), γ-glutamyl transferase, lacto-peroxidase (LP), and leucine arylamidase are the enzymes commonly found in milk. If pasteurized milk is accidentally mixed with raw milk, microbial communities can grow in the pasteurized milk rendering it contaminated (Kabariya & Ramani, 2018). The major components present in raw milk are water, milk proteins, milk fats, milk carbohydrates, vitamins, and inorganic components. The milk found for sale in the commercial markets should contain a specific least amount of fats and solids and according to US legal limits this least amount of non-fat solid content should be 8.25% and that of fat content should be 3.25% while, the specific legally allowed limits vary depending on the country and state. Several factors control the composition of nutrients in milk. The diet given to the animal (cow, sheep, or goat), genetic makeup, diseased or stressed conditions, environmental and physical factors, medications, stage of lactation, calving season, breed, and age of cow greatly influence the concentration of different nutrients in milk (Mungkarndee, Techakriengkrai, Tumcharern, & Sukwattanasinitt, 2016). Milk proteins include casein, lactalbumin, and lactoglobulin among which the first two types are present in relatively high amounts. The ratio of casein and lactalbumin in cow's milk is observed to be 3:1 while in human milk this ratio is 1:2. Being the major protein content of milk, casein is present in the concentration of 35 g/L. It is not considered as a single compound rather it is a heterogeneous mixture of phosphorous-containing proteins. In milk, casein is found as a salt named calcium caseinate. The four major types of casein include alpha, beta, gamma, and kappa. Lactalbumin and lactoglobulin are distinctive albumins and globulins that coagulate thermally. All three major milk proteins have all essential amino acids that holds their biological importance.

Milk carbohydrates include lactose, glucose, and galactose among which lactose is the major carbohydrate while the other two are found in minute quantities. As milk is the only food for the infants of mammals for specific time duration so lactose produces galactose for the preparation of glycolipids needed for brain development. Milk fats are found as triglycerides with different fatty acids as side chains and these are present in milk in emulsified form. The prime fatty acid chains

are of palmitic acid and oleic acid whereas free fatty acids and cholesterol are found in minute concentrations. The milk fats found in goat milk and sheep milk contain shorter chained and medium chained triacylglycerols (TAGs) which influence the taste of milk from these species. Buffalo milk fat includes higher concentrations of TAGs having medium chains in comparison with the cattle milk fat, which contains significant concentrations of longer-chained TAGs. Because shorter or medium-chained TAGs are thought to be more readily digested by lipases, these variances are responsible to decide the milk digestive patterns of fats in the milk of different species. Long chains of free saturated fatty acids are not readily absorbed by the body. The fat content is found to exist in the form of little round droplets known as globules with a diameter between 0.2 to 15µm. The size of these fat globules depends on the species producing the milk and due to these varying sizes, these fat molecules are digested differently (Roy, Ye, Moughan, & Singh, 2020).

Inorganic components and Vitamins

Milk contains all the required nutrients excluding iron, needed to our body for proper growth and functioning of the immune system. These essential nutrients include sodium, calcium, riboflavin, phosphorous, potassium, magnesium, sulphur, and chloride. Among all these minerals, magnesium and sulphur are found in minute concentrations whereas the presence of some other elements can also be observed. Milk is a good source of vitamin A, pantothenic acid, and riboflavin. Vitamin C and D are the only vitamins that are considered to be added to the diet for an infant because the rest of the required vitamins come from the mother during the foetal period and the gut microflora (Gupta, 1992).

Microbiota of Raw milk

Raw bovine milk has a pH of 6.5 to 6.7 which is almost neutral. This is one of the favourable conditions for the microbial community to grow and survive. There is a variety of microbes existing in the raw milk originating from different sources which may include the outer udder surface, animal feed, soil, grass, air, and the milking equipment. The microorganisms existing in the raw milk right after milking may include lactic acid bacteria e.g. *Streptococcus*, *Lactococcus*, and *Lactobacillus* species. Technologically these species show importance in the dairy industry but sometimes these can cause spoilage of the products. This bacterial population is termed psychrotrophic bacteria and it can spoil germ-free, antiseptic, unadulterated, and ultra-high temperature treated milk and dairy products having a longer shelf life. The most observed contaminants in the cold raw milk that can produce these spoilage

enzymes are Pseudomonas and Serratia species (Machado et al., 2017). Raw milk or unpasteurized milk has been considered as a major cause of food-borne illness across the globe because raw milk contains strains of pathogenic bacteria such as *Salmonella enteric*, *Escherichia coli*, *Listeria monocytogenes*, and *Clostridium botulinum.* Pasteurization is the process that eliminates these bacteria and protects the consumers from these disease-causing pathogens (Ziobro & McElroy, 2013).

Alkaline Phosphatase

Alkaline phosphatase (ALP) exists in nature in an abundant form and can be seen in human body tissues such as liver, bone, kidney, and blood cells. Its presence can be observed in milk and other bodily fluids with varying levels among organisms. It was identified and characterized for the first time by Suzuki *et al*. in 1907 (Rankin, Christiansen, Lee, Banavara, & Lopez-Hernandez, 2010). There are more than 60 enzymes that are present in milk naturally; alkaline phosphatase is one of them (Schlimme, Kiesner, Lorenzen, & Martin, 1997). ALP in its pure form shows a molecular mass of 187 kDa and its maximum activity is observed at 37^{o}C in the pH range 9.65-10.1 (Rankin, Christiansen, Lee, Banavara, & Lopez-Hernandez, 2010; Vega-Warner, Wang, Smith, & Ustunol, 1999). ALP exists naturally in raw milk and its concentration indicates the efficacy of the pasteurization process. Non-pasteurized milk contains a higher concentration of this enzyme. If this milk is consumed it can lead to gastrointestinal bacterial infections. Pasteurization denatures the ALP and makes the milk safe to be consumed (Kabariya & Ramani, 2018). The ALP level in the raw milk depends on the source from where the milk is obtained and keeps varying accordingly within the range of 6.0-28 U/L. While pasteurized milk contains a very low activity of 0.001 to 0.006 U/L of ALP. So, if the high activity of ALP is observed then it is a clear indication of a poor pasteurization process (Sharma, Sehgal, & Kumar, 2003). The U.S. and European public health limit for alkaline phosphatase in pasteurized drinks is set to be 350 mU/ liter (Park, Bae, Kim, & Lee, 2011).

Importance of bovine ALP

ALP holds its importance in the dairy industry due to its thermal inactivation properties as it tends to be a bit more heat resistant to the treatment provided to milk than the bacteria. The process of thermal inactivation of milk ALP is co-related to the microbial death caused by the thermal treatment. This correlation has led to the hypothesis that ALP assay could be used to indicate the efficiency of pasteurization (Rankin, Christiansen, Lee, Banavara, & Lopez-Hernandez, 2010). ALP activity is used as an index of proper pasteurization, and it has become a common practice to

determine the ALP activity to assess the quality of pasteurized milk and the pasteurized products for quality control and assurance. The sensitivity of the ALP test depends on the initial concentration of ALP present in the raw milk so, if there is a higher initial concentration then the test becomes more sensitive. The sensitivity also depends on the method through which the residual enzyme activity is determined therefore alkaline phosphatase is considered as the most important indicator for the assurance of the proper milk pasteurization ensuring the safety and hygiene of pasteurized milk (Stănciuc, Ardelean, Diaconu, Râpeanu, Stanciu, & Nicolau, 2011).

Milk Casein

Milk is highly nutritious being a mixture of a variety of constituents. These can be isolated from milk and used for different applications in the food and beverage industry. Micellar caseins and milk-derived whey proteins can now be generated from skim milk through microfiltration owing to the developments in filter technology and these have a distinctive flavor and special functional properties when used in different food-related applications (Carter, Cheng, Kapoor, Meletharayil, & Drake, 2021). Some of the important industrial factors that can destabilize micelles are hydrolysis of the κ -casein by selected proteinases, which is used in the production of different types of cheese varieties; acidification to about pH 4.6, which is used in the production of some cheeses, fermented milk, and functional caseinate products; ethanol (or other alcohol); anionic detergents (e.g., SDS); high pressure. Despite the extensive research, a definitive model for the casein micelle structure has yet to be developed however, different models based on the characteristics and behaviour of individual caseins and casein micelles have been developed.

Research methodology

Alkaline phosphatase activity was tested in the 3 raw milk samples, 7 UHT treated milk samples, and 3 naturally pasteurized samples available in the local market from Lahore. This research work has been done in Food and Biotechnology Research Centre, PCSIR Labs Complex Lahore.

Materials required for ALP detection.

ALP test reagent R1

Diethanolamine	**pH 9.8, 1.0 mol/L**
Magnesium chloride.$6H_2O$	0.5 mmol/L

ALP test reagent R2

p-Nitrophenyl phosphate	10 mmol/L

The high purity distilled water was used.

ALP test reagents of MTD Diagnostics Company were provided by PCSIR labs complex and the milk samples were purchased from the local market.

Milk samples that were tested are listed below:

Table 1. ***List of Samples for Analysis***

Sr no.	**Sample**	**Category**
1	Mixed milk	Raw
2	Cow milk	Raw
3	Buffalo milk	Raw
4	Olpers milk pack	UHT
5	Dairy omung milk pack	UHT
6	Nurpur milk pack	UHT
7	Dairy pure milk pack	UHT
8	Nestle milk pack	UHT
9	Haleeb milk pack	UHT
10	Day fresh milk pack	UHT
11	Anhaar milk	Pasteurized
12	Prema milk	Pasteurized
13	Adams milk	Pasteurized

The adopted method was Kinetic determination of serum alkaline phosphatase ("Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions," 1972; Vassaul, Grafmeyer, Naudin, Dumont, Bailly, Henny, Georges,1986).

Principle

p – Nitrophenyl phosphate + H_2O ⟶ phosphate + p – nitrophenol

The increase in absorbance of the solution, measured at 405 nm is directly proportional to the activity of the ALP in the sample.

Procedure for ALP detection

Thirty milliliters of fresh raw milk were taken and stored in the refrigerator. Approximately 25 ml of this milk sample was taken in the centrifuge tube and centrifuged at 4000 rpm for 30 minutes. The fat layer was carefully removed with a clean and dry spatula. Then the milk sample was filtered to remove the remaining fat particles. This serum was observed to be less turbid than the original milk. 400 µL of R1 and 100 µL of R2 were withdrawn using the micropipette with a range of 10 to 100 microliters. This was the working reagent. 1.5 mL of double distilled water was needed to be added to the working solution to make an overall volume of 2 mL to be observed under the spectrophotometer. Milk sample, distilled water, and the working reagent were heated in a water bath to attain the temperature of 37^{o}C. 500 µL of the working reagent, 1.5 mL of distilled water, and 10 µL of the milk sample were mixed properly and then the absorbance was measured at 405 nm using SPECORD 200 spectrophotometer.

Method to isolate casein

Casein was separated using the method explained by David T Plummer in "An introduction to practical biochemistry" (Yates, 1978). 100 ml of milk were taken in a 500 ml beaker and warmed to 40 ^{o}C. 100 ml of acetate buffer were also warmed and slowly added to the warm milk with stirring. Then this suspension was cooled to room temperature and leftover for five minutes before filtration. Precipitates were formed which were washed by using a small volume of water. These washed precipitates were then suspended in 30 ml of ethanol. Filtration was done to separate the precipitates out and then washed using a mixture of equal volumes of ethanol and ether. Then 50 ml of ether was used to wash the precipitate. Finally, the precipitate was removed and spread over a petri plate and dried. Casein was then weighed and the percentage yield was calculated. This process was repeated for all the targeted milk samples.

Results and discussion

ALP activity analysis

Raw milk (whole)

Table 2. ***Absorbance Values Observed for Raw Milk (Whole) Sample***

First reading right after mixing	1.8110	ΔAbs
Reading after 1 min	1.8515	0.0405
Reading after 2 min	1.8971	0.0456
Reading after 3 min	1.9441	0.047
Reading after 4 min	1.9760	0.0319

Raw milk (Centrifuged)

Table 3. ***Absorbance Values Observed for Raw Milk (Centrifuged) Sample***

First reading right after mixing	0.4237	ΔAbs
Reading after 1 min	0.4588	0.0351
Reading after 2 min	0.4907	0.0319
Reading after 3 min	0.5227	0.032
Reading after 4 min	0.5583	0.0356

Cow milk (whole)

Table 4. ***Absorbance Values Observed for Cow Milk (Whole) Sample***

First reading right after mixing	0.6419	ΔAbs
Reading after 1 min	0.7605	0.1186
Reading after 2 min	0.8626	0.1021
Reading after 3 min	0.9659	0.1033
Reading after 4 min	1.0632	0.0973

Cow milk (centrifuged)

Table 5. ***Absorbance Values Observed for Cow Milk (Centrifuged) Sample***

First reading right after mixing	0.4702	ΔAbs
Reading after 1 min	0.5645	0.0943
Reading after 2 min	0.6643	0.0998
Reading after 3 min	0.7604	0.0961
Reading after 4 min	0.8553	0.0949

Buffalo milk (whole)

Table 6. ***Absorbance Values Observed for Buffalo Milk (Whole) Sample***

First reading right after mixing	0.6368	ΔAbs
Reading after 1 min	0.6957	0.0589
Reading after 2 min	0.7614	0.0657
Reading after 3 min	0.8456	0.0842
Reading after 4 min	0.9442	0.0986

Buffalo milk (centrifuged)

Table 7. ***Absorbance Values Observed for Buffalo Milk (Centrifuged) Sample***

First reading right after mixing	0.4886	ΔAbs
Reading after 1 min	0.5345	0.0459
Reading after 2 min	0.5854	0.0509
Reading after 3 min	0.6377	0.0523
Reading after 4 min	0.6913	0.0536

Olpers milk pack

Table 8. ***Absorbance Values Observed for Olpers Milk Pack Sample***

First reading right after mixing	1.1052	ΔAbs
Reading after 1 min	1.0947	-0.0105
Reading after 2 min	1.0895	-0.0052
Reading after 3 min	1.0876	-0.0019
Reading after 4 min	1.0842	-0.0034

Dairy Omung milk pack

Table 9. Absorbance Values Observed for Dairy Omung Milk Pack Sample

First reading right after mixing	0.7417	ΔAbs
Reading after 1 min	0.7228	-0.0189
Reading after 2 min	0.7143	-0.0085
Reading after 3 min	0.7089	-0.0054
Reading after 4 min	0.7046	-0.0043

Nestle milk pack

Table 10. ***Absorbance Values Observed for Nestle Milk Pack Sample***

First reading right after mixing	0.9138	ΔAbs
Reading after 1 min	0.9035	-0.0103
Reading after 2 min	0.8980	-0.0055
Reading after 3 min	0.8946	-0.0034
Reading after 4 min	0.8912	-0.0034

Haleeb milk pack

Table 11. ***Absorbance Values Observed for Haleeb Milk Pack Sample***

First reading right after mixing	1.1490	ΔAbs
Reading after 1 min	1.1333	-0.0157
Reading after 2 min	1.1256	-0.0077
Reading after 3 min	1.1221	-0.0035
Reading after 4 min	1.1222	0.0001

Nurpur milk pack

Table 12. ***Absorbance Values Observed for Nurpur Milk Pack Sample***

First reading right after mixing	1.1616	ΔAbs
Reading after 1 min	1.1520	-0.0096
Reading after 2 min	1.1420	-0.01
Reading after 3 min	1.1364	-0.0056
Reading after 4 min	1.1357	-0.0007

Day Fresh milk pack

Table 13. ***Absorbance Values Observed for Day Fresh Milk Pack Sample***

First reading right after mixing	1.0306	ΔAbs
Reading after 1 min	1.0233	-0.0073
Reading after 2 min	1.0198	-0.0035
Reading after 3 min	1.0180	-0.0018
Reading after 4 min	1.0165	-0.0015

Dairy pure milk pack

Table 14. ***Absorbance Values Observed for Dairy Pure Milk Pack Sample***

First reading right after mixing	1.1525	ΔAbs
Reading after 1 min	1.1421	-0.0104
Reading after 2 min	1.1337	-0.0084
Reading after 3 min	1.1308	-0.0029
Reading after 4 min	1.1318	0.001

Adams milk

Table 15. ***Absorbance Values Observed for Adams Milk Sample***

First reading right after mixing	1.1964	ΔAbs
Reading after 1 min	1.1789	-0.0175
Reading after 2 min	1.1671	-0.0118
Reading after 3 min	1.1632	-0.0039
Reading after 4 min	1.1619	-0.0013

Prema milk

Table 16. ***Absorbance Values Observed for Prema Milk Sample***

First reading right after mixing	1.3473	ΔAbs
Reading after 1 min	1.3330	-0.0143
Reading after 2 min	1.3256	-0.0074
Reading after 3 min	1.3230	-0.0026
Reading after 4 min	1.3182	-0.0048

Anhaar milk

Table 17. ***Absorbance Values Observed for Anhaar Milk Sample***

First reading right after mixing	1.2783	ΔAbs
Reading after 1 min	1.2630	-0.0153
Reading after 2 min	1.2573	-0.0057
Reading after 3 min	1.2518	-0.0055
Reading after 4 min	1.2497	-0.0021

Table 18. ***ALP Values Measured for Raw Milk Samples And Their Mean, Standard Deviation, and Relative Standard Deviation***

	Mixed milk	**Cow milk**	**Buffalo milk**
Whole	111.24 U/L	288.037 U/L	214.081 U/L
Centrifuged	90.084 U/L	262.879 U/L	137.16 U/L
Mean	100.662 U/L	275.458 U/L	175.6205 U/L
Standard deviation	.95955106	17.7893924	54.39136072
Relative standard deviation	1414.86117012	6.458114268	30.97096336

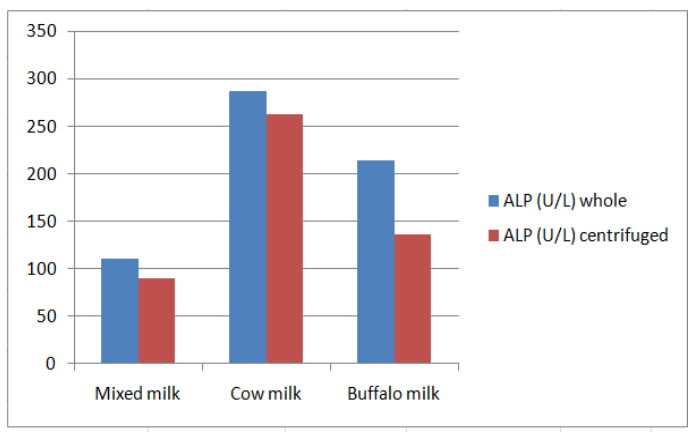

Fig 1. ALP activity in raw milk samples

The above graph shows ALP values in raw milk samples. The blue bars show the ALP concentration in whole milk samples, whereas the red bars represent the ALP concentrations in centrifuged milk samples. It is found that ALP is present in a slightly high concentration in whole milk samples as compared to centrifuged samples.

Casein concentrations in respective samples

Table 19. ***Obtained Casein Concentrations in Respective Samples and Their Mean, Standard Deviation and Relative Standard Deviation***

Sample	Casein in grams isolated / 100 ml	Percentage yield
Raw milk	3.61	100%
Olpers milk pack	3.31	94.54%
Dairy omung milk pack	3.39	96.85%
Nestle milk pack	3.40	97.14%
Haleeb milk pack	3.48	99.42%
Nurpur milk pack	3.38	96.57%
Day fresh milk pack	3.33	95.14%
Dairy pure milk pack	3.50	100%
Adams milk	3.51	100%
Prema milk	3.43	98%
Anhaar milk	3.47	99.14%
Mean	**3.437272727**	-
Standard deviation	**0.087531812**	-
Relative SD	**2.546548364**	-

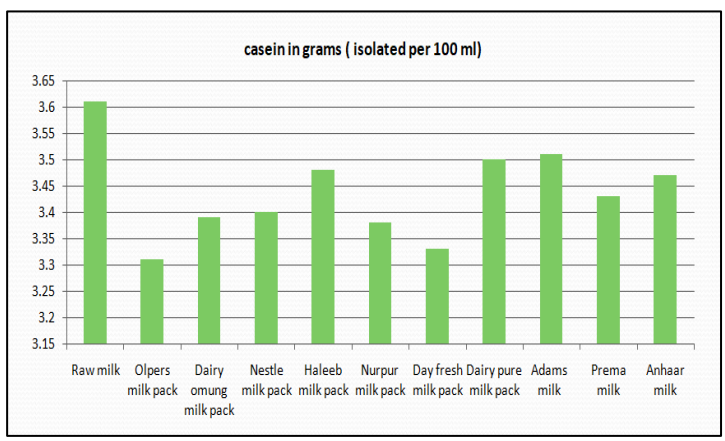

Fig 2. Casein isolation data

Table 20. ***Overview of the ALP Values and Casein Concentrations in the Respective Samples***

No.	Sample	ALP activity in U/L	Concentration of casein in grams (isolated per 100 ml)
1	Raw milk (whole)	111.24	3.61
2	Raw milk (centrifuged)	90.08	-
3	Cow milk (whole)	288.03	-
4	Cow milk (centrifuged)	262.87	-
5	Buffalo milk (whole)	214.08	-
6	Buffalo milk (centrifuged)	137.16	-
7	Olpers milk pack	-20.33	3.31
8	Dairy omung milk pack	-31.42	3.39
9	Nestle milk pack	-18.05	3.40
10	Haleeb milk pack	-20.95	3.48
11	Nurpur milk pack	-20.33	3.38
12	Day fresh milk pack	-12.19	3.33
13	Dairy pure milk pack	-20.12	3.50
14	Adams milk	-24.26	3.51
15	Prema milk	-20.53	3.43
16	Anhaar milk	-20.19	3.47

Milk and dairy products are crucial elements of a balanced diet, but unpasteurized milk and dairy products might provide a health risk due to the possibility of harmful bacteria contamination. This microflora might come from clinically healthy animals that produce milk, from the external conditions in which the animal exists, or from the milking equipment used during milking and storage afterward. This contamination might be reduced by improving the bovine and milking hygienic conditions, but it cannot completely eradicate the chances of milk-borne diseases. The most efficient method to improve the microbial quality of milk is said to be pasteurization. Pasteurization has no effect on the nutritious value of milk, despite popular belief. If the consumer avoids intake of raw milk, chances of milk-borne diseases can be greatly reduced, and several organizations have issued guidelines and declarations on milk pasteurization to preserve human health. Only pasteurized milk must be provided in the market for consumer utilization, according to the American Medical Association (policy H-150.980). Similarly, the American Veterinary Medical Association recommends selling only pasteurized milk and milk products for human consumption (LeJeune, Rajala-Schultz, Xe, & Ivi, 2009).

Pasteurized or UHT milk carry high consumer demand and the reason for this demand is their higher shelf life. The pasteurized milk offer a shelf life of about 14 days but it is to be stored at refrigeration temperature which ranges between 4 to 7 ^{o}C. UHT shows higher shelf life of about 6 to 12 months at ambient temperature. During the storage duration, the physical damage to the packages or the post-pasteurization contamination with raw milk can greatly influence the quality of the packaged milk (Machado et al., 2017). ALP is found to be a bit more thermally resistant than the pathogenic bacteria which are targeted to be killed in the pasteurization process (specifically, *Coxiella burnetii* and *Mycobacterium tuberculosis*), if there is a great reduction in the ALP activities then it can be referred that pathogenic population also got removed, confirming that the legitimate thermal specifications were achieved. If ALP activity values are found high in the assay, then it can be referred that the pasteurization was not effective, or the milk might be got in contact with the raw milk after pasteurization and it might also indicate the existence of bacterial ALP or the biochemical reactivation of ALP. There are different methods to detect ALP activity in the milk like Colorimetric, Fluorometric, Chemiluminescent, and Immunochemical methods (Hilal).

Vial-based immediate detection is also possible for the ALP activity determination. It is quicker, convenient, and economic method and includes a reaction between p-nitrophenyl phosphate and ALP in the presence of water which results in a colour change from light blue to green. This colour change confirms the presence of ALP in the particular sample and it can be seen by the unaided eye.

Biostrips also provide fast and convenient way for the sample analysis and these have been widely used for illness detection and testing of various chemical and biological parameters. These dry reagent strips work by liberating p-nitrophenol and inorganic phosphate from the ALP reaction with p-nitrophenyl phosphate. As a result of reaction of p-nitrophenol with certain chromogen, the blue colour of strip turns green. This colour change is visible with the naked eye. The sensitivity of the strip is greater than 0.5 U/L and is stable for over a year. The strips are highly useful for the distant places especially in the developing countries where complex and costly tools and machines are unavailable (Sharma, Sehgal, & Kumar, 2003).

Casein micelles are the principal part of milk that helps in the structure formation and their interaction qualities are critical in the conversion of milk into dairy products for example ice cream, yogurt, and cheese. Whey proteins, lactose, fat globules, and mineral components play their role in structure formation along with casein. Casein micelles assemble themselves and form clumps of proteins constituting

the major part of milk protein content. The rest of the protein part of milk is contributed by water-soluble globular whey proteins. Temperature, ionic strength, shear, pH, and water content all affect the microstructure of milk. This is significant from an industrial standpoint since milk microstructure is linked to the processability of dairy products as well as their physicochemical and sensory qualities, such as mouthfeel, spread ability, and firmness. Structure formation of milk proteins can be beneficial in some situations such as in yogurt or cheese, but it can also be detrimental in others like gelation during aging of UHT milk. Whey proteins quickly denature when heated, resulting in binding to casein micelles, but casein micelles are stable towards heat.

Caseins present in milk account for the milk structural qualities during processing and storage. Caseins assemble them into nanometer-sized colloids. These have been studied using SAS and these studies focus on the micelle's internal structure. Later studies of USAXS analysis focus on the micrometer scale so the inter-particle interactions that exist among the casein micelles can also be determined. This study considers the micelles as the sticky spheres. This stickiness influences USA and USAXS measurements and it also restricts the use of simple approximations, which presume that particles do not interact with each other (Smith, Brok, Christiansen, & Ahrné, 2020).

Conclusion

In this study, a colorimetric assay used for the kinetic determination of serum alkaline phosphatase was employed to detect ALP in the targeted samples. Raw milk samples showed the presence of a significant amount of ALP whereas all the pasteurized samples were tested negative for the ALP activity confirming that the pasteurization done was effective and efficient. Casein protein from the milk samples was also isolated and measured using 100 ml of each sample and its mean value turned out to be 3.43 g and the relative standard deviation was 2.54.

References

Carter, B. G., Cheng, N., Kapoor, R., Meletharayil, G. H., & Drake, M. A. (2021). Invited review: Microfiltration-derived casein and whey proteins from milk. *J Dairy Sci*, *104*(3), 2465-2479.

Hilal, A. Validation of milk product pasteurization by Alkaline Phosphatase Activity. Con Dai & Vet Sci 1 (3)-2018. *CDVS. MS. ID*, *113*.

LeJeune, J. T., Rajala-Schultz, P., xe, & ivi, J. (2009). Unpasteurized Milk a Continued Public Health Threat. *Clinical Infectious Diseases, 48*(1), 93-100.

Machado, S. G., Baglinière, F., Marchand, S., Van Coillie, E., Vanetti, M. C. D., De Block, J., & Heyndrickx, M. (2017). The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. *Frontiers in Microbiology, 8*(302).

Mungkarndee, R., Techakriengkrai, I., Tumcharern, G., & Sukwattanasinitt, M. (2016). Fluorescence sensor array for identification of commercial milk samples according to their thermal treatments. *Food Chemistry, 197*, 198-204.

Park, L., Bae, H., Kim, Y.-T., & Lee, J. H. (2011). Rapid monitoring of alkaline phosphatase in raw milk using 1,1′-oxalyldiimidazole chemiluminescence detection. *Analytical Methods, 3*(1), 156-160. doi:10.1039/C0AY00383B

Rankin, S. A., Christiansen, A., Lee, W., Banavara, D. S., & Lopez-Hernandez, A. (2010). Invited review: The application of alkaline phosphatase assays for the validation of milk product pasteurization. *J Dairy Sci, 93*(12), 5538-5551.

Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. (1972). *Z Klin Chem Klin Biochem, 10*(6), 281-291.

Ritota, M., Di Costanzo, M. G., Mattera, M., & Manzi, P. (2017). New Trends for the Evaluation of Heat Treatments of Milk. *Journal of Analytical Methods in Chemistry, 2017*, 1864832.

Roy, D., Ye, A., Moughan, P. J., & Singh, H. (2020). Composition, Structure, and Digestive Dynamics of Milk from Different Species—A Review. *Frontiers in Nutrition, 7*(195).

Schlimme, E., Kiesner, C., Lorenzen, P. C., & Martin, D. (1997). Chemical process parameters for thermal inactivation of alkaline phosphatase in milk. *Kieler Milchwirtschaftliche Forschungsberichte, 49*(3), 207-219.

Sharma, S. K., Sehgal, N., & Kumar, A. (2003). Dry-reagent strips for testing milk pasteurization. *LWT - Food Science and Technology, 36*(6), 567-571.

Sharma, S. K., Sehgal, N., & Kumar, A. (2003). Dry-reagent strips for testing milk pasteurization. *LWT-Food Science and Technology, 36*(6), 567-571.

Smith, G. N., Brok, E., Christiansen, M. V., & Ahrné, L. (2020). Casein micelles in milk as sticky spheres. *Soft Matter, 16*(43), 9955-9963.

Stănciuc, N., Ardelean, A., Diaconu, V., Râpeanu, G., Stanciu, S., & Nicolau, A. (2011). Kinetic and thermodynamic parameters of alkaline phosphatase and γ—glutamyl transferase inactivation in bovine milk. *Dairy Science & Technology, 91*(6), 701.

Vassault, A., Grafmeyer, D., Naudin, C., Dumont, G., Bailly, M., Henny, J. . . . Georges, P. (1986). Protocole de validation de techniques. *Ann Biol Clin, 44*(686), 45.

Vega-Warner, A. V., Wang, C. H., Smith, D. M., & Ustunol, Z. (1999). Milk alkaline phosphatase purification and production of polyclonal antibodies. *Journal of Food Science, 64*(4), 601-605.

Yates, D. W. (1978). An Introduction to practical biochemistry (2nd edition): By David T. Plummer. Pp 362 McGraw-Hill Book Company (U.K.) Ltd., London 1978. Softback, £6.00. *Biochemical Education, 6*(3), 71-71.

Ziobro, G. C., & McElroy, K. M. (2013). Fluorometric detection of active alkaline phosphatase and gamma-glutamyl transferase in fluid dairy products from multiple species. *J Food Prot, 76*(5), 892-898.