Comparative Effects of Heating and Acidification on the Composition and Physicochemical Properties of Buffalo and Cow Milk and Milk Whey Protein – A Review

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Abstract

Background: Buffalo and cow milk differ significantly in their composition, with buffalo milk containing higher levels of fat, protein, and minerals. The physicochemical properties of these milks under thermal and acidic conditions are critical for various dairy processes, such as cheese production. Methods: Milk samples from both species were analyzed for compositional and physicochemical changes during heating and acidification. The molecular structure of milk proteins, whey protein fractions, and bacterial counts were evaluated. Additionally, the effects of thermal treatments on casein and whey protein stability were examined. Results: Buffalo milk exhibited higher buffering capacity, protein content, and mineral concentration compared to cow milk. Heating and acidification induced significant changes in the molecular structure of milk proteins, with buffalo milk showing greater stability. The heat treatment increased casein solubility while denaturing whey proteins, impacting coagulation and cheese yield. Conclusion: Buffalo milk demonstrated superior stability under heat and acidification, suggesting its enhanced suitability for dairy processes requiring higher thermal resistance and protein retention compared to cow milk.

Keywords: Heating effects, Buffalo milk, Cow milk, Acidification, Milk protein denaturation

Significance | This review discusses the differential responses of buffalo and cow milk to heat and acidification, impacting dairy production.

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Introduction

Milk is a complex biological liquid that provide bioactive components and nutrients. It helps to stimulating digestive maturation, cellular growth; symbiotic microflora and development of gut-associated lymphoid tissues are successful postnatal adaptation of the newborn infant. Milk proteins, peptides, probiotic lactic acid bacteria, calcium and other minerals can significantly reduce blood pressure and milk fat contains several components having functional properties.

The importance, strength and number of bioactive compounds in milk and fermented milk products are probably greater than previously thought. They include certain vitamins, specific proteins, bioactive peptides, oligosaccharides, organic (including fatty) acids. Some of them are normal milk components; others appear during digestive or fermentation processes. Fermented dairy products and probiotic bacteria decrease the absorption of cholesterol. This protein plays an important role in the sensitization capacity of cow's milk, and its modification might be a way to reduce the allergenicity of cow's milk. It can also play a role in reducing these social and economic costs and have several putative

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biological effects when ingested that including an anticancer action. Milk composition of mammalian species varies widely with reference to genetic, physiological and nutritional factors and environmental conditions (Malacarne et al., 2002). This is achieved by proteins such as lysozyme, peroxidase, lactoferrin and immunoglobulins. Proteinases, such as plasmin and lipases, and serum albumin or β-lactoglobulin (β-Lg), may aid digestion and nutrient absorption (Creamer, 1996).

Proteins are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. There are many proteins present in different source of food such as milk, meat, egg, soy, wheat, etc. In different forms of milk contains different proteins but I study the effect of heat and cold on whey protein from cow and buffalo milk and its effects on human health.

This review discusses on the structural properties of milk and milk proteins and how these properties are influenced by environmental factors for example, pH, temperature, and salts in manifesting characteristic functional properties, such as gelation, foaming, and emulsifying activity, which are important in food applications. Proteins are important structural components in many foods and they are also used as ingredients because of their nutritional value and physicochemical properties. The market for functional protein-rich ingredients is expanding and is currently supplied by various proteins. Proteins obtained from dairy products have traditionally been one of the main protein sources for humans. Currently, a selection of high-protein dairy powders for example, caseinates, coprecipitates and whey powders are used, yet these display a highly variable range of functional properties because of differences in composition and processing treatments. However, whey protein preparations vary immensely in functional behavior and are presently relegated to limited use as functional ingredients in the food industry. This need not be the case since conventional and new technologies permit rigorous control of production protocols for example, careful control of heat treatments can result in the production of whey protein preparations with consistent, reliable functional properties (Kinsella in Whitehead, 1989).

2. **Studies the effects of heating and freezing**

Effect on Milk

2.1 ***Heating effects***

2.1.1 ***Effect of pH and acidification on composition of buffalo and cow milk***

Composition and physico-chemical properties of buffalo and cow milks were compared at their initial pH and during acidification. As compared to cow milk, buffalo milk was richer in fat, lactose, protein and minerals such as calcium, magnesium and inorganic phosphate. Along with these differences of major components, the capacity of milk to be acidified (named buffering capacity) was higher for buffalo milk than for cow milk. The precipitation/aggregation of caseins at their isoelectric pH, solubilization of calcium and inorganic phosphate and decrease in hydration of casein as a function of decrease in pH were significant for both milks. For both species, these molecular changes were qualitatively similar but quantitatively different. These quantitative differences during acidification were related to the differences of composition between the milks (Ahmad in drugi, 2008).

2.1.2 ***Effect of coagulation and variation in composition of milk***

The objective of this study was to evaluate the effect of variations in milk protein composition on milk clotting properties and cheese yield. Milk was collected from 134 dairy cows of Swedish Red and White, Swedish Holstein and Danish Holstein-Friesian breed at 3 sampling occasions. Concentrations of αS1-, β-and κ-casein (CN), α-lactalbumin, and β-lactoglobulin (LG) A and B were determined by reversed has liquid chromatography. Cows of Swedish breeds were genotyped for genetic variants of β- and κ-CN. Model cheeses were produced from individual skimmed milk samples and the milk clotting properties were evaluated. More than 30% of the samples were poorly coagulating or noncoagulating, resulting in weak or no coagulum, respectively. Poorly and noncoagulating samples were associated with allow concentration of κ-CN and a low proportion of κ-CN in relation to total CN analyzed. Furthermore, the κ-CN concentration was higher in milk from cows with the AB genotype than the AA genotype of κ-CN. The concentrations of αS1-, β-, and κ-CN and of β-LG B were found to be significant for the cheese yield, expressed as grams of cheese per one hundred grams of milk. The ratio of CN to total protein analyzed and the β-LGB concentration positively affected Cheese yield, expressed as grams of dry cheese solids per one hundred grams of milk protein, whereas β-LG A had a negative effect. Cheesemaking properties could be improved by selecting milk with high concentrations of αS1-, β-, and κ-CN, with high κ-CN in relation to total CN and milk that contains β-LG B (Wedholm in drugi, 2006).

2.1.3 ***Effect of whey protein fraction on their composition from buffalo milk***

In the study conducted by (Buffoni in drugi, 2011) that the whey protein fractions from 120 Mediterranean water buffalo individual milks were analyzed by microchip electrophoresis (MCE), reverse-phase high-performance liquid chromatography (RP-HPLC) and mass spectrometry (ESI-MS). Validation procedures were carried out for both MCE and HPLC. The chromatographic analysis allowed the complete separation of the whey protein fractions, resulting in a well-defined peak structure; the adopted RP-HPLC and ESI-MS protocols provided identification of β-lactoglobulin (18,266 Da), α-lactoalbumin (14,236 Da) and serum albumin (66,397 Da). The calculated mean concentrations were 4.04 g/l, 2.45 g/l and 0.35 g/l, respectively.

2.1.4 ***Counting of bacteria and physicochemical properties of milk***

A study was conducted by Markiewicz et al (2013) with the objective to determine the whey protein profile somatic cell count, total bacteria count, chemical composition and some physicochemical properties of late lactation milk produced by Polish Cold-Blooded Horses mares between 141- and 210-days post-partum. The whey protein profile, expressed as a percentage of the total sum of the six whey proteins considered, was characterised by high proportions of lysozyme (11.6%), lactoferrin (14.6%) and immunoglobulin (15.8%). Whey proteins represented in greatest amounts included β-lactoglobulin (29.2%) and α-lactalbumin (25.4% of the total whey protein fraction). Mares' milk was characterised by very low numbers of somatic cells and total bacterial counts. Results presented confirm that late lactation mares' milk constitutes a valuable source of bioactive components (Markiewicz-Kęszycka in drugi, 2013).

2.1.5 ***Effect of heat treatment on molecular structure of milk proteins***

Heat treatment affects the molecular structure of milk proteins at the interfaces of oil-in-water emulsions and in aqueous media. Experimental evidence of the impact of thermal processing on milk protein structure is presented and the contribution of whey proteins and caseins at film formation during emulsification is discussed. Recent advances in understanding the effect of heat treatment in milk protein functionality at emulsion interfaces are reviewed with emphasis on the emulsifying ability of whey proteins with or without the presence of the casein fraction. The major findings regarding the destabilizing mechanisms of oil-in-water emulsions brought about by heat-induced denaturation of milk proteins are presented. This paper aims to combine recent knowledge on how thermal processing of milk proteins affects their molecular configurations in bulk and particularly at interfaces, which in turn appear to be important with respect to the physico-chemical properties of milk protein-stabilized emulsions (Raikos, 2010).

2.1.6 ***Effect of heat treatment milk protein of bovine milk***

(Zamberlin in drugi, 2010) aim of study was to investigate the effect of heat treatment of ovine milk at (60°C/5min and 90°C/5) min (control group) on the compositional and sensory properties of set yoghurt. The concentration of apparent casein and total whey protein were significantly higher while sensory properties (except consistency) were not significantly different from the yoghurts in control group ($P<0.05$). The results showed that ovine set yoghurt produced by heat treatment at low temperature possessed higher amount of preserved inherent functional and nutritional properties of milk than yoghurt produced by heat treatment at high temperature.

2.1.7 ***Effect of Heat treatment for stability of milk protein***

A subjective test for the determination of the stability of milk protein to heat was described by (Davies in White, 1966). In the test, the time required for particles of coagulated protein to become visible throughout a 2·5-ml sample of separated milk maintained at 135°C in a glass tube rocking at 8 c/min is taken as a measure of stability. The precision of the test was such that single determinations were generally adequate. Coagulation time decreased by about 12% as rocking speed was increased over the range 4–12 c/min and increased by a factor of about 3 for a decrease in heating temperature of 10°C over the range 140–105 °C; with some milks the Q10 °C value increased to 5–8 a temperature decreased. As sample volume was increased over the range 1–3 ml coagulation time increased, especially with milks whose coagulation was poor (initial clots small). This volume effect appeared to be a consequence of the accompanying decrease in the proportion of headspace oxygen to volume of milk. Different composition of why protein shown in Table 1, Table 2.

2.1.8 ***Effect cooling, and storing treatment of milk***

A study by (Dzurec Jr in Zall, 1985), determined that Cottage cheese yields increase as a result of heating (74°C, 10 s), cooling (3°C), and storing (7 days) milk prior to cheese making. Protein analysis of milk indicated that casein was higher and whey proteins lower in experimental heated, cooled, stored milk as compared with un-heated, fresh (<2 days old) control milk. Heating caused some proteins normally in whey to associate with casein by some undetermined mechanism. Samples of control and experimental milk subjected to centrifugation (21,000 x g/120 min) and gel electrophoresis showed that solu- ble l~-casein decreased with heat treat- ment and cold storage of milk. Pretreating raw milk with nitrogen- ethylmaleimide or ethylenetetraacetic acid prior to heat treatment and storage and subjecting these samples to electro-phoresis indicated little or no decrease of whey proteins compared with milk heated without added nitrogen-ethylmalei- mide or ethylenetetraacetic acid. Heat- treating, cooling, and storing milk seemed to cause part of the/3-casein to be trapped physically in the casein micelle. These treatments also caused portions of whey proteins to associate with casein micelles via disulfide bridging and calcium link- ages. protein changes in skim milk due to heat treatment & cold storage is shown in table 3.

2.1.9 ***Effect of heat treatment of whey protein during manufacturing of baby milk***

(Kilshaw in drugi, 1982) conducted experiments on guinea-pigs, and suggested that heat treatment applied during the manufacture of baby milk formulae reduces the immunological sensitising capacity of the cows' milk proteins. This immunological benefit must be weighed against possible damage that heat treatment may cause to the nutritional quality of the products. Severe heat treatment of skimmed milk (121°C for 20 min) destroyed all the vitamin B12, about 60% of the thiamin and vitamin B6, 70% of the ascorbic acid, and about 30% of the folate. Available lysine was reduced by 21 % and lactulose was formed (166 mg/100 ml). Despite extensive denaturation of the whey proteins the milk

retained its capacity to sensitise guinea-pigs for systemic anaphylaxis when administered orally. Animals drinking heated milk also produced circulating antibodies to 3-lactoglobulin and casein, although titres were lower than for unheated milk. Unlike skimmed milk, heat-treated diafiltered whey failed to sensitise guinea-pigs orally. It caused the production of trace levels of antibodies in some of the animals, but these were specific for residual casein. We suggest that it may be possible to produce a non-sensitising baby milk without casein based on heat-denatured whey. The nutritional quality could be preserved by removing low molecular weight nutrients before heat treatment and adding back appropriate quantities later. Effect on Mil Whey Protein

2.1.10 ***Effect of heat and pressure treatment on whey proteins***

(Baier in drugi, 2015) studied on the structural changes in micellar caseins and whey proteins due to high pressure--low temperature treatments (HPLT) were investigated and compared to changes caused by high pressure treatments at room temperature. Whey protein isolate (WPI) solutions as well as micellar casein (MC) dispersions and mixtures were treated at 500 MPa (pH 7.0 and 5.8) at room temperature, -15 °C and -35 °C. Surface hydrophobicity and accessible thiol groups remained nearly unchanged after HPLT treatments whereas HP treatments at room temperature caused an unfolding of the WPI, resulting in an increase in surface hydrophobicity and exposure of the thiol groups. For HPLT treatments, distinct changes in the secondary structure (increase in the number of β-sheets) were observed while the tertiary structure remained unchanged. Large flocs, stabilized by hydrophobic interactions and hydrogen bonds, were formed in casein containing samples due to HPLT treatments. Depending on the pH and the applied HPLT treatment parameters, these interactions differed significantly from the interactions determined in native micelles

2.1.11 ***Effect of heat treatment on whey protein***

There is a great interest in the production of heats table and clear beverages containing high levels of whey proteins. A challenge of incorporating whey proteins in sports beverages is hot-fill treatment (88 °C, 2 min). The objective of this research was to analyze the effects of thermal treatment on the profile of whey proteins in a whey protein beverage (WPB). WPB were prepared mixing 5 % whey protein with 0.04 % potassium sorbate and 0.5 M phosphoric acid was used to adjust pH to 3.0 and 7.0. The protein particle size and zeta-potential were tested using a spectrophotometer. Finally, the protein profile of beverages containing whey was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Hot-fill treatment had a negative impact on the physiochemical properties of whey proteins. The formation of protein complexes produced an increase in particle size and absolute zeta potential in WPB formulations at both pH 3.0 and 7.0 (Quant in drugi, 2019).

2.1.12 ***Effect of heat treatment for stabilization of whey protein***

The effect of heating has been studied for whey protein-stabilised oil-in-water emulsions (25.0% (w/w) soybean oil, 3.0% (w/w) whey protein isolate, pH 7.0). These emulsions were heated between 55 and 95 °C as a function of time and the effect on particle size distribution, adsorbed protein amount, protein conformation and rheological properties was determined. Heating the emulsions as a function of temperature for 25 min resulted in an increase of the mean diameter (d32) and shear viscosity with a maximum at 75 °C. Heating of the emulsions at different temperatures as a function of time in all cases resulted in a curve with a maximum for d32. A maximum increase of d32 was observed after about 45 min at 75 °C and after 6–8 min at 90 °C. Similar trends were observed with viscosity measurements. Confocal scanning laser micrographs showed that after 8 min of heating at 90 °C large, loose aggregates of oil droplets were formed, while after 20 min of heating compact aggregates of two or three emulsion droplets remained. An increase of the adsorbed amount of protein was found with increasing heating temperature. Plateau values were reached after 10 min of heating at 75 °C and after 5 min of heating at 90 °C. Based on these results we concluded that in the whole process of aggregation of whey protein-stabilised emulsions an essential role is played by the non-adsorbed protein fraction, that the kinetics of the aggregation of whey protein-stabilised emulsions follow similar trends as those for heated whey protein solutions and that upon prolonged heating rearrangements take place leading to disaggregation of initially formed large, loose aggregates of emulsion droplets into smaller, more compact ones (Sliwinski in drugi, 2003).

2.1.13 ***Effect of temperature and pH on solubility of whey protein***

An integrated study was conducted on the effects of temperature and pH on the solubility of whey proteins. The solubility was determined experimentally in the range of 40–60°C for temperature and 3.5–7.8 for pH. The results showed that, both temperature and pH influenced in the protein solubility, and these properties had great interaction. Besides, for whey proteins, the solubility values were minimum at the pH value of 4.5, which is the isoelectric point of whey proteins, for all temperature values. It was also observed that at pH 4.5, the solubility decreased as the temperature increased, which indicated that the protein denaturation occurred. This behavior was also noticed in the neutrality (pH=6.8) (Pelegrine in Gasparetto, 2005).

2.1.14 ***Effect of pH, heat and ethanol on denaturation of whey protein***

(Nikolaidis in drugi, 2017) conducted a newly developed method of analysis of difference-UV spectra was successfully implemented in the study of the effect of heat, pH, ultrasonication and ethanol on the denaturation of whey protein isolate. It was found that whey proteins exhibit their highest stability against heat denaturation at pH 3.75. At very low pH values, i.e. 2.5, they exhibited considerable cold denaturation, while after heating at this pH value, the

supplementary heat denaturation rate was lower compared to that at neutral pH. The highest heat denaturation rates were observed at pH values higher than neutral. High power sonication on whey proteins, previously heated at 90°C for 30min, resulted in a rather small reduction of the fraction of the heat denatured protein aggregates. Finally, when ethanol was used as a cosolvent in the concentration range 20-50%, a sharp increase in the degree of denaturation, compared to the native protein solution, was observed.

2.1.15 ***Effect of denatured whey protein interaction with other milk***

This chapter reviews the literature on the denaturation of the whey proteins in milk and their interactions with other milk proteins and provides examples of relationships between denaturation/interaction reactions of the whey proteins and the functional behavior of the milk. Early studies were aimed at developing methods to assess denaturation levels and for determining the relationships between the denaturation of the whey proteins and the functional behavior of milk products in bakery and other applications. It has become increasingly apparent that a measure of the denaturation level is not a good predictor of functional performance. Therefore, further studies have investigated the interactions of denatured whey proteins with other milk proteins, with the casein micelles. Recent studies have indicated that manipulation of the interactions of the denatured whey proteins with other milk proteins may provide a tool for modifying/controlling the functional performance of milk protein products in some applications (Anema, 2020).

2.1.16 ***Effect of heat treatment on whey protein and their types***

(Akkerman in drugi, 2016) explored that previous standards in the area of effect of heat treatment processes on milk protein denaturation were based primarily on laboratory-scale analysis and determination of denaturation degrees by, for example, electrophoresis. In this study, whey protein denaturation was revisited by pilot-scale heating strategies and liquid chromatography quadrupole time-of-flight mass spectrometer (LC/MC Q-TOF) analysis. Skim milk was heat treated using 3 heating strategies, namely plate heat exchanger (PHE), tubular heat exchanger (THE), and direct steam injection (DSI), under various heating temperatures (VHT) and holding times. The effect of heating strategy on the degree of denaturation of β-lactoglobulin and α-lactalbumin was determined using LC/MC Q-TOF of pH 4.5-soluble whey proteins. Furthermore, effect of heating strategy on the rennet-induced coagulation properties was studied by oscillatory rheometric. In addition, rennet-induced coagulation of heat-treated micellar casein concentrate subjected to PHE was studied. For skim milk, the whey protein denaturation increased significantly as T and holding time increased, regardless of heating method. High denaturation degrees were obtained for T >100°C using PHE and THE, whereas DSI resulted in significantly lower denaturation degrees, compared with PHE and THE. Rennet coagulation properties were impaired by increased T and holding time regardless of heating method, although DSI resulted in less impairment compared with PHE and THE. No significant difference was found between THE and PHE for effect on rennet coagulation time, whereas the curd firming rate was significantly larger for THE compared with PHE. Micellar casein concentrate possessed improved rennet coagulation properties compared with skim milk receiving equal heat treatment. Denaturation degree of whey protein for heating skim milk is shown in table 4.

2.1.17 ***Effect of heat treatment on solubility of whey protein and their types***

Structure and solubility of whey proteins are interrelated and affected by commonly used heat treatments. The relation between these characteristics, however, varies with the nature of the protein and the composition of the protein solution. After a brief analysis of structure and properties of the major whey proteins in the native state, attention is given to effects of thermal treatments (up to 150°C) on structure and solubility of the different whey proteins. It is pointed out that mild heat treatments up to 60°C may affect reversibly the solubility and foaming properties of whey proteins. Conformational changes, as reflected by differential scanning calorimetry and observed above 60°C for α-lactalbumin and near 80 and 140°C for α-lactoglobulin, however, exert more serious effects on the functional properties of whey proteins. Modifications of cysteine residues in the polypeptide chain are detected by amino acid anal~csis upon heat treatments above 100-C under identical heating conditions as used for differential scanning calorimetry. Special attention is given to the effect of changes of environmental conditions such as pH and the presence of lactose and calcium salts, to get more information on the complicated relation between structure and properties of proteins in whey (Dewit in Klarenbeek, 1984).

To investigate the production of antioxidant activity during fermentation with commonly used dairy starter cultures. To study the development of antioxidant activity during fermentation. Antioxidant activity was measured by analysing the radical scavenging activity using a spectrophotometric decolorization assay andlipid peroxidation inhibition was assayed using liposomal model system with afluorescence method. Milk was fermented with 25 lactic acid bacterial (LAB)strains, and from these six strains, exhibiting the highest radical scavenging activity was selected for further

Leuconostocmesenteroidesssp,cremorisstrains,Lactobacillusjensenii(ATCC 25258)andLactobacillus acidophi-lus(ATCC 4356) showed the highest activity with both the methods used. However, the radical scavenging activity was stronger than lipid peroxidationinhibition activity.

Table 1. Composition of types of whey protein

Types of whey proteins	composition	Lactose	FATS	Minerals
Whey protein concentration	70-80%	more	more	more
Whey protein isolate	90%	less	less	less
Whey protein hydrolysate	80%	absorb	absorb	absorb

Table 2. Composition of Whey Protein

S.No	**PROTEIN**	**APPRX CONTENT G/L WHEY**	**TOTAL WHEY PROTEIN %**
1	α-Lactoalbumin	0.6-1.7	20
2	β-lactoglobulin	2.0-4.0	55
3	Serum Albumin	0.2-0.4	5
4	Immunoglobulins	0.5-0.4	8
5	Proteose-peptone	0.2-0.4	12
6	Other (casein,glycoprotein	0.1	1

Table 3. protein changes in skim milk due to heat treatment & cold storage

	TOTAL PROTEIN		**WHEY PROTEIN**	
	X%	SD[1]	X%	SD
CONTROL	3.42	0.13	0.63	0.06
Heat-treated,freshed[2]	3.42	0.13	0.59	0.04
Heat-treated,stored[3]	3.42	0.13	0.55	0.05
[1]n=46				
[2]Milk heat-treated 74°:C/10 s, analysis completed within 24 h				
[3] Milk heat-treated 74°C, then stored 7 days at 3°C prior to analysis				

Table 4. Denaturation degree of whey protein for heating skim milk

Temperature Holding Time	**Relative RCT (%)**	**Denaturation of β-lactoglobulin**	**Denaturation of α-Lactoalbumin**
105/4	127.46 ± 13.19	0.79 ± 4.29	4.80 ± 1.21
115/4	138.92 ± 10.56	15.75 ± 11.36	9.23 ± 1.92
130/4	192.26 ± 40.79	25.29 ± 4.86	10.53 ± 5.65
145/4	420.81	39.35 ± 2.83	15.35 ± 2.97

Table 1. Denaturation of composition of whey protein

S.No	Protein	Apprx Content G/L Whey	Total Whey Protein %	Denaturation Temperature
1	**α-Lactoalbumin**	0.6-1.7	20	61
2	β-lactoglobulin	2.0-4.0	55	82
3	Serum Albumin	0.2-0.4	5	66
4	Immunoglobulins	0.5-0.4	8	72
5	Proteose-peptone	0.2-0.4	12	
6	Other (casein,glycoprotein	0.1	1	

Table 6. Effect of cold storage and mixing various lactating milk on the chemical composition of buffalo's and cow's milk

S.No	Treatment	Acidity, %		Total solids, %		Fat, %		Protein, %	
		Buffalo	cow	Buffalo	Cow	Buffalo	cow	Buffalo	cow
A	Fresh morning milk.	0.16	0.15	16.7	11.6	3.3	7.4	4.84	3.14
B	Cold milk at 4 -5°C for 10 hours	0.17	0.16	16.7	11.7	3.3	7.4	4.81	3.17
C	Fresh evening milk.	0.15	0.14	16.8	11.9	4	7.4	4.94	3.27
D	Mixing milk of treatments B and C.	0,.16	0.15	16.7	11.7	3.7	7.4	4.85	3,18
E	Milk of treatment D after storage at 4 -5°C for 24 hours	0.17	0.16	16.7	11.7	3.7	7.4	4.86	3.16
F	Milk of treatment A & E after cold storage 4 -5°C for evening next day.	0.18	0.16	16.6	11.6	3.7	7.2	4.77	3.17
H	Milk of treatment C & G after cold storage 4 -5°C for 48 hours	0.19	0.17	16.8	11.8	3.6	7,7	4.85	3.14

Table 2. protein changes in skim milk due to heat treatment & cold storage

	TOTAL PROTEIN		WHEY PROTEIN	
	X%	SD[1]	X%	SD
CONTROL	3.42	0.13	0.63	0.06
Heat-treated,freshed[2]	3.42	0.13	0.59	0.04
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[3] Milk heat-treated 74°C, then stored 7 days at 3°C prior to analysis				

The development of radical scavenging activity was connec-ted to proteolysis with four strains. Molecular distribution profiles showed thatfermentates with high scavenging activity also possessed a higher proportion of peptides in the molecular mass range of 4–20 kDa, while others had mostlylarge polypeptides and compounds below 4 kDa. In addition, the amount ofhydrophobic amino acids was higher in these fermenters. The development of antioxidant activity was strain-specific char-acteristic. The development of radical scavengers was more connected to thesimultaneous development of proteolysis whereas lipid peroxidation inhibitoryactivity was related to bacterial growth. However, high radical scavenging activity was not directly connected to the high degree of proteolysis. To the best of our knowledge, this seems to be the first report, which screens possible antioxidant activity among most common dairy LAB strains. Use of such strains improves nutritional value of fermented dairy products (Virtanen in drugi, 2007). Denaturation of composition of whey protein is shown in table 5.

2.2 ***Freezing effects***

2.2.1 ***Effect of cold storage on quality of the buffalo's and cow's milk***

Ammar,et.al (2010) studied about the effect of cold storage (4-5°C) for 48 hours and blinding different milkings to cold stored milk on some chemical composition, rheological properties and microbial quality of the buffalo's and cow's milk were studied. The results obtained indicate that no clear effects of cold storage of buffalo's and cow's milk on them TS, fat and total protein contents whereas the acidity values slightly increased. Cold storage of both kinds of milk increased the rennet coagulation time (RCT), curd tension and curd syneresis values. Also, preservation of buffalo's and cow's milk at 4-5°C increased total viable bacterial (TVBC), lactic acid, psychrophilic bacteria, proteolytic, lipolytic, colifom bacteria, sporeformers, and moulds and yeast counts. Adding evening and morning milk to cold stored buffalo's and cow's milk decreased the acidity and curd syneresis values and increased the pH, TS, fat, total protein, RCT and the curd tension values. Mixing evening and morning milk with cold stored milk lowered the above-mentioned microbial groups.

Effect of cold storage and mixing various lactating milk on the chemical composition of buffalo's and cow's milk is shown in table 6. Effect cooling, and storing treatment of milk

A study by (Dzurec Jr in Zall, 1985), determined that Cottage cheese yields increase as a result of heating (74°C, 10 s), cooling (3°C), and storing (7 days) milk prior to cheese making. Protein analysis of milk indicated that casein was higher and whey proteins lower in experimental heated, cooled, stored milk as compared with un-heated, fresh (<2 days old) control milk. Heating caused some proteins normally in whey to associate with casein by some undetermined mechanism. Samples of control and experimental milk subjected to centrifugation (21,000 x g/120 min) and gel electrophoresis showed that solu- ble l~-casein decreased with heat treat- ment and cold storage of milk. Pretreating raw milk with nitrogen- ethylmaleimide or ethylenetetraacetic acid prior to heat treatment and storage and subjecting these samples to electro-phoresis indicated little or no decrease of whey proteins compared with milk heated without added nitrogen-ethylmalei- mide or ethylenetetraacetic acid. Heat- treating, cooling, and storing milk seemed to cause part of the/3-casein to be trapped physically in the casein micelle. These treatments also caused portions of whey proteins to associate with casein micelles via disulfide bridging and calcium link- ages.

2.2.2 ***Effect of cold storage and heating of camel, cow and buffalo's milk***

The effect of cold storage (4°C/48 h.) and heating (85°C/5 min.) on some properties of camel's, cow's and buffalo's milks were studied. These included acidities, pH, nitrogen distribution, rennet coagulation time (RCT), surface tension (ST), foam expansion (FE), foam volume stability (FVS), buffer intensity, the electrophoretic properties and microstructure. Cold storage of all milk samples increased acidity, and decreased pH, ST, RCT (except camel's milk) and FE (except cow's milk). No significant effect on CN, NCN and WPN was found. Heating all milk samples increased acidity, casein nitrogen (CN) and RCT (except camel's milk), decreased non-casein nitrogen (NCN), whey protein nitrogen (WPN) and nonsignificant effect on ST. Also, heating decreased only FE of buffalo's milk and increased the others, while FVS of camel's milk was not recorded (after 15 min.). In all cases, buffer intensity curves showed same peaks and were the highest at pH (7-8) and (6.5-7.5) in buffalo's and cow's milk respectively. However, camel's milk had less buffering capacity compared with either buffalo's or cow's milk. Concerning, the electrophoretic patterns, some whey proteins especially β-lactoglobulin (β-Lg) disappeared on heating, whereas cold storage slightly decreased β-casein. In general, camel's milk showed different protein patterns. There was little microstructure difference between raw and cooled (4°C/48 h.) camel's, cow's and buffalo's milk; while heat treatment increased the size of casein micelles in all samples (Hassan in drugi, 2009). Protein changes in skim milk due to heat treatment & cold storage is shown in table 7

Influence of cold storage and mixing on properties of buffalo's and cow's milk:

The effect of cold storage (4-5°C) for 48 hours and blinding different milkings to cold stored milk on some chemical composition, rheological properties and microbial quality of the buffalo's and cow's milk were studied. The results obtained indicate that no clear effects of cold storage of buffalo's and cow's milk on them TS, fat and total protein contents whereas the acidity values slightly increased. Cold storage of both kinds of milk increased the rennet coagulation time (RCT), curd tension and curd syneresis values. Also, preservation of buffalo's and cow's milk at 4-5°C

increased total viable bacterial (TVBC), lactic acid, psychrophilic bacteria, proteolytic, lipolytic, colifom bacteria, sporeformers, and moulds and yeast counts. Adding evening and morning milk to cold stored buffalo's and cow's milk decreased the acidity and curd syneresis values and increased the pH, TS, fat, total protein, RCT and the curd tension values. Mixing evening and morning milk with cold stored milk lowered the above-mentioned microbial groups (Ammar in drugi, 2010).

Conclusion

Cold storage of all milk samples increased acidity, and decreased pH, ST, RCT and FE. Heat treatment decreased only FE of buffalo's milk and increased the others. Some whey proteins especially β-lactoglobulin (β-Lg) disappeared on heating, whereas cold storage slightly decreased β-casein. There was little microstructure difference between raw and cooled (4°C/48 h.) cow's and buffalo's milk; while heat treatment increased the size of casein micelles in all samples.

Author contributions

N.S. conceptualized the project and developed the methodology. U.N. and I. conducted a formal analysis and drafted the original writing and contributed to the methodology. S.Z. conducted investigations, provided resources, visualized the data and contributed to reviewing and editing the writing.

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Competing financial interests

The authors have no conflict of interest.

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