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Chapter 6

Lactation Responses toward Milk Indigenous Enzymes

Aneela Hameed, Majid Hussain and Saeed Akhtar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66392

Abstract

Milk being a highly nutritious food in its natural form provides energy. There are various factors influencing the composition of milk: breed, stage of lactation, nutritional status, health, and milking intervals. A number of indigenous enzymes present in milk are being affected by stages of lactation period. Their concentration varies during early, mid and late lactation periods. This varied behavior ultimately affects the quality of dairy products. In this chapter, the level of milk enzymes: lipases and esterases, plasmin (PL), plasminogen (PLG) phosphatases (alkaline phosphatase ALP; acid phosphatase (ACP), lysozyme (LZ), lactoperoxidase (LP), xanthine oxidoreductase (XOR), and catalase (CAT) will be reviewed with respect to the stages of lactation periods.

Keywords: milk, indigenous enzymes, lactation stages, parity, season

1. Introduction

Milk is one of the perfect, complete, and primitive dairy food known by mankind. It is white and nutritious physiological secretion from the mammary glands of mammals, serves as nourishment for their neonates [1, 2]. It is a major product obtained from healthy and highly productive dairy animals. Physiological and nutritional provisions of each species are more or less distinctive. The breed, health, nutritional status, stage of lactation period, and milking intervals are some of the factors that affect the milk composition [3, 4]. The variation in constituents occurs entire lactation period. Lactation stage is the prime factors that affect the

milk properties and some of the enzyme activities [5, 6]. Solids-non-fat (SNF) content is frequently highest throughout first 2–3 weeks of lactation.

2. Milk indigenous enzymes

Numerous enzymes have been indigenously identified in milk from 1924 to 1970 [7]. A large number of enzymes with multiple functionalities are present in milk. Additional enzymes contribute in quality of milk products and also perform an antibacterial action (LP). In bovine milk more than 70 enzymes are detected [8, 9]. A 50–60 substantial number of milk enzymes with multiple functions are present in abundance in milk and are concerned with processing stability and general customer safety [10] and additionally processing suitability (ALP). Some enzymes (LP) having antibacterial characteristics are with significant importance in preservation of milk and milk products and some, e.g., plasmin (PL) and lipoprotein lipase (LPL) connected with the serum, plasma, fat globules, casein, or leukocytes are important in maintaining of quality of milk and milk products. More than 40 enzymes have been recognized in cow milk [9, 11].

Lactation period in animals involves colostrum, developed milk, peak, and production with compositional variations. Numerous indigenous enzymes present in milk are secreted by epithelial cells and their composition changes with the lactation stages.

In already recognized indigenous enzymes in milk [7], almost 20 enzymes have been well characterized and the rest of the 40 enzymes are of little significance but can be identified through their activity. These enzymes indicate the efficient process of milk pasteurization (ALP, γ-glutamyl transferase GGT) or of mastitis (phosphatases, CAT). Additional enzymes can be of significance in processing and ultimately providing safety to human beings. They play an antibacterial activity (LP) and contribute quality to milk products (e.g., LPL, PL) associated with the serum, plasma, fat globule, casein, or leukocytes.

2.1. Lipases and esterases

Lipolytic enzymes have capability to hydrolyze triacylglycerols are considered as carboxylesterases [12, 13]. Those enzymes that can hydrolyze acyl glycerol having <10 carbon atom fatty acids are known as esterases or carboxylases (Enzyme Commission, EC 3.1.1.1) while those can hydrolyze ≥10 carbon atom fatty acids are considered as lipases, or triacylglycerol acyl hydrolases (EC 3.1.1.3) [14, 15].

Esterases are different from lipases due to their functions for being relatively soluble compared to emulsified ester substrates. Several esterases are present in milk [15, 16], the most prominent are carboxylesterase (EC 3.1.1.1), acetylcholinesterase (EC 3.1.1.7), and cholinesterase (EC 3.1.1.8). In bovine colostrum, lipase is not connected with casein and not activated by blood serum, therefore exhibited low lipase activity and showed slight lipolysis in early lactation. However, after few days of calving, normal milk from early lactation exhibited higher lipase activity [17, 18].

http://dx.doi.org/10.5772/66392

Lipases are naturally a critical group of enzymes since they are connected with the fat digestion system. Lipases are more dynamic at pH 8–9 and catalyze the advancement of hydrolytic rancidity in milk. Investigation of lipases is more alluring in the light of the fact that it would add to our comprehension about the properties and modes of these enzymes [19, 20].

The phenomenon of lipolysis is correlated with the lactation days. Higher activity is associated with its presence in fat fraction of milk. Activity of lipase in milk fat increases with the advancement in lactation stages [21]. The lipolysis process is of major apprehension in the dairy industry, as rancid off flavors are produced in milk and milk products during this phenomenon [22].

Earlier research has well established that milk lipase is sensitive to heavy metals. Copper, cobalt, and nickel have been shown to be more powerful inhibitors of lipase than iron, chromium, manganese, and silver. Enzyme activity is stimulated by blood serum albumin, ammonium, calcium ions, and mercaptoethanol. The buffer solutions, citrate, acetate, and phosphate buffers damage the enzyme activity, whereas borate and barbiturate buffers do not [23, 24].

LPL in cow milk is altered due to the breed, lactation phase, feed and fodder, season, and milk yield [22, 25]. Lipase activity increased from 0.32 to 2.98 U/mL of milk. At the point when milk fat globule membrane (MFGM) is damaged, lipolysis takes place rapidly and leads to hydrolytic rancidity and ultimately may cause variations in functionality and flavor of dairy products throughout storage period [15]. LPL found in goat milk is of low concentration in the early and late lactation stage [26].

The membrane lipase is available in higher concentration in milk from dairy animals in late lactation [27]. They additionally reported that lipase action in milk showed inclined pattern with reference to lactation stages. Hameed et al. [28] reported the expanding pattern of lipase activity with lactation stages in bovine milk. Lipase action (1.55 U/mL) was recorded higher ($p < 0.01$) in milk, examined at the last of lactation, followed by other lactations (1.29 and 1.16 U/mL, respectively).

2.2. Plasmin

Plasmin (PL; EC 3.4.21.7) is an alkaline serine proteinase enzyme that proteolytically cleaves the blood clots [29]. This enzyme has affinity toward arginine (Arg) and lysine (Lys) residues, specifically breaks the Arg-X and Lys-X bonds [30, 31]. The activity of enzyme is increased with the multiple factors that include lactation stage, lactation number and severity of mastitis infection [32–34].

On the basis of origin, the PL and plasminogen (PLG) are considered to be migrated from blood to milk, and higher activity of PL in peak lactation designating more conversion of PLG into PL in bovine milk [35–37].

PL is basically released in the form of PLG in normal milk. The concentration of PLG (0.8–2.8 μg/mL) in fresh milk is varied and its concentration is 2–30 times higher than that of PL (0.1–0.7 μg/mL). It is activated by storage or when milk is stayed in the lumen of mammary glands

before milking. A considerable interest has been involved in the activation of PLG, upon which activity of PL depends [38–41]. It promptly hydrolyzes the bonding of β-casein, α_{s2}-casein, and α_{s1}-casein and affects the quality of dairy products [32, 42].

Advancing lactation stage is an essential factor that influences PL activity and percentage, suggesting that more PL activity in milk from goat and older cows is a result of increased PLG activation [43–45]. However, the relevant information about the varied concentration and activity of PL during lactation stages is controversial. Leitner et al. [46] declared significantly higher activities of PL in infected glands of sheep.

Caroprese et al. [47] and Albenzio et al. [33] found that there was decrease in PL activity in ewe's milk from the early to the late lactation stage whereas Koutsouli et al. [48] and Bianchi et al. [49] announced that PL activity significantly affected by udder health status and found an increased level of PL activity due to more somatic cell counts (SCCs) during the late lactation period.

The variation in PLG-derived activity and total PL plus PLG-derived activity is greatly influenced by lactation stage and seasonal changes. It is linked with reduction of milk yield and advancement in lactation stage [45, 50, 51]. Due to increased activity of plasminogen, more entry of PL occurred from blood to milk inside the mammary glands [52]. The PL and PLG activities were significantly increased in the advancement of lactation and a nonsignificant decrease in their ratio (PL:PLG) was observed as compared to camel milk [53, 54].

2.3. Phosphatases

2.3.1. Alkaline phosphatase

In 1925, for the first time, phosphatase enzyme in milk is documented by Demuth and then considered as an alkaline phosphatase (ALP; EC 3.1.3.1) indigenous to milk by Graham and Kay [55]. It became recognizable when it was confirmed that the requirement for time-temperature relationships to inactivate the ALP required slightly higher as compared to kill *Mycobacterium tuberculosis* [56, 57]. Almost 40% activity of ALP in raw cow milk is declared to be linked with the milk fat globule membrane (MFGM) in the cream phase, though the rest is soluble or dispersed in whey membrane particles (WMP) in skim milk [58]. Between individuals and herds, higher ALP levels vary significantly and its concerned activity is correlated with lactation stages and mastitis [59, 60]. Magnesium and zinc ions are promoter of ALP while tin, copper, cobalt, and ethylenediaminetetraacetic acid (EDTA) have inhibitory action and iron has no effect on activities of ALP [61].

ALP activity is in inverse relationship with yield but the other factors, e.g., fat content, breed, and feed, have no effect. For ovine milk, the ALP content is contrarily linked with milk production and directly to the milk fat substance, while infected milk (mastitis) has higher ALP activity [62, 63]. It is reported that ALP activity is low at the mature milk production stage, increased to maximum activity during the peak production stage and again decreased at the end production stage [28]. ALP activity in cow increases as lactation stage proceeds. Immediately after parturition, there is a decrease in ALP activity with a further sharp decrease after

http://dx.doi.org/10.5772/66392

the first milking period. ALP activity then continues to decrease and noted minimum at the first week. Then increased slowly and found maximum by the 28th week of lactation [64]. In another study, ALP activity in milk was found lowest in the early lactation stages and progressed along with advancement of lactation stages and milk yields decreased. These ALP activities were also noticed greater in milk samples from evening milk as compared with morning milk [65].

2.3.2. Acid phosphatase

Acid phosphomonoesterase (ACP; EC 3.1.3.2) in milk was initially identified by Huggins and Talalay [66] and affirmed by Mullen [67], declared that ACP was ideally in the active form at 4.0 pH. It was thermally stable and for complete inactivation it required 88°C for 10 min. ACP in bovine milk hydrolyzes the phosphate group of casein particles [68]. There are some components that act as inhibitor and activator. Fluoride acts as an inhibitor for ACP activity but slightly activated by Mn^{2+}. In milk, the ACP level is just ~2% that of the ALP level. Approximately 75% of ACP was found generally in the skim milk phase and 20% of ACP in the MFGM [68, 69]. Reducing agents, ascorbic acid and 2-mercaptoethanol increases the ACP activity by 100% in skim milk, whereas the ACP activity in MFGM is unaffected by these agents. Casein acts as a substrate for the activity of ACP and major casein fractions αs (αs1 + αs2) > β > κ also serve as competitive inhibitors as the ACP enzyme binds with the phosphate group of casein. The ability to bind calcium with κ-casein to form micelles is reduced by dephosphorylation of casein [61].

ACP in milk might be of innovative significance due to three reasons. First, ACP exhibits thermal stability and because of this property it may be used as an indicator for severe heat treatment rather than normal. Second, numerous milk items may have a pH near to that of its optimum. Third, phosphoproteins such as caseins might be dephosphorylated readily. Technological milk properties and development of dairy products depend on the integrity of casein micelles. The enhanced activity of ACP may create problem in the inactivation of ACP without affecting nutritional qualities as it is linked with gelation of ultra-high temperature (UHT) and development of cheese flavor [70, 71].

Specific activity of ACP is greater in cream; however, about 80% ACP of milk is present in skimmed milk [60]. ACP levels in milk of Sahiwal dairy animals showed a declined pattern alongside lactation stages [28]. Shakeel-ur-Rehman and Farkye [72] observed the higher activity of ACP at 5–6 days postpartum, and afterward observed declined trend up to the end of lactation stage. Nevertheless, the range of ACP levels in their study was presented from 2.6 ± 10^{-4} to 2.6 ± 10^{-3} U/mL in normal cow milk. The ACP level is 4–10 times more in mastitis milk than normal cow milk [73, 74].

2.4. Lysozyme

Lysozyme (LZ; EC 3.2.1.17; muramidase) is a single polypeptide chain (14.3 KDa M.W.), cross-linked by four disulfide bonds [75, 76]. It is an important bacteriolytic protein in milk, component of the antibacterial system, that kills bacteria by cleaving the β-1,4-glycosidic bond

between N-acetyl muramic acid and N-acetyl glucosamine residues in peptidoglycan of the bacterial cell wall [77, 78].

It helps in improving the human health status, especially neonate, to protect them from infections of invading pathogens with the promotion of gut microbiota until their own immune system is developed [79–81].

Basically, there are two types of LZ: hen egg-white (C-LZ) and goose egg-white (G-LZ). However, both C-LZ and G-LZ forms may be present in cow milk as these forms are present in other body fluids and in stomach tissue of the cow [82].

LZ is available at higher concentration (0.420 g/L) in human milk as compared to buffalos (3.85 μg/mL), cow (0.0013 g/L), and goat (0.0025 g/L) milk [83–86].

The activity of LZ was in greater extent and more stable in buffalo milk as compared to cow milk. However, colostrum possessed 5 times higher activity as compared to mature milk. It was also observed that various factors: parity of animal and lactation stage not influences the activity of LZ but it was increased during the peak summer and winter seasons [86–88]. A substantial increase of milk LZ in mastitis among different bovine species suggested that the neutrophils are the most probable source of LZ due to inflammation of mammary gland [89–91]

2.5. Lactoperoxidases

Lactoperoxidase (LP; EC 1.11.1.7) is the second most abundant enzyme after xanthine oxidase in bovine milk. The most generally prescribed industrial utilization of LP systems is the preservation of raw fresh milk during transportation and storage in dairy plants [92, 93]. It received a considerable attention as an optimum indicator of super-pasteurized milk [94]. Its level in bovine milk is about 30 mg/L constituting approximately 1% whey protein [95]. The LP system (LP-thiocyanate-H_2O_2) is a natural preservation system and has antimicrobial characteristics. Oxidation of thiocyanate in the presence of H_2O_2 is catalyzed by activated LP and produce hypothiocyanate (OSCN) or higher oxides (antimicrobial compounds). These compounds exhibited their antimicrobial properties by oxidizing the sulfhydryl groups of proteins to disulfides [96].

LP enzyme activities are affected by various factors, i.e., sexual cycle, season, lactation, diet, and breed [95, 97]. LP activity in bovine milk (1.2–19.4 U/mL) is about 20 times higher in peroxidase action than human milk [98]. LP levels in dairy animals milk is ranged from 1.5 to 2.7 U/mL with a general mean of 2.3 U/mL [99]. The LP level is low in colostrum of dairy animals, after that adopted inclined trend rapidly after 3–5 days postpartum [95]. LP enzyme activity is a precursor to diagnose the mastitis disease in dairy animals. The activity of LP increases as the somatic cell count (SCC) increases [100, 101].

The LP activity of cows adopted declined trend along with lactation stages. The activity of LP decreases with the advancement in lactation stages (9.64–6.66 U/mL) [28]. The decreasing trend along with lactation stages was also observed by Althaus et al. [102] who reported significant reduction in LP activity from the early stage of lactation toward the end of lactation. Reiter [95] observed a significant increase in LP activity between 4 and 5 days after calving of the lactation

period, followed by a gradual decrease toward close of lactation. The reduction in action of LP activity in cow milk could be due to increase in the thiocyanate content as Fonteh et al. [99] described that the LP level promoted with an increase in 2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or ABTS contents but reduced with an increase in thiocyanate contents. They also reported that LP activity is enhanced with whey protein, lactose, magnesium, sodium, and calcium chlorides, and reduced in occurrence of casein.

2.6. Catalases

Catalase (CAT; H_2O_2:H_2O_2 oxidoreductase; EC 1.11.1.6) dismutates hydrogen peroxide (H_2O_2) into water (H_2O) and free oxygen (O_2) [8, 103]. CAT was among the first enzymes present in milk. Babcock and Russell [104] portrayed that an extract of separator slime can break down H_2O_2. The CAT activity in milk fluctuates with feedstuff and lactation phase, level expanded particularly during mastitis [103, 105]. CAT has the ability to degrade the surplus hydrogen peroxide and reduce oxidative infection caused by reactive oxygen species (ROS) [106].

CAT and SCC contributed in the mastitis risk markers. Risk level of mastitis and losses in milk production increase with the advancement in parity, phase of lactation, and also in spring and winter seasons [107]. Measurement of CAT activity plays a distinct role in monitoring the health status of udders in cow. The antioxidant activity of enzyme CAT increases when SCC increases [100, 108, 109]. CAT antioxidant activity is higher in colostrum, then reductions occur as the lactation stage proceeds and again high in the late lactation [110, 111]. Its absence in milk is an indication of an efficient pasteurization process [7].

2.7. Xanthine oxidoreductase

Xanthine oxidoreductase (XOR; EC 1.13.22; 1.1.1.204) is a milk indigenous enzyme having capability of oxidizing hypoxanthine to xanthine and xanthine to uric acid with the reduction of O_2 to H_2O_2 [7, 112]. This protein is initially presented in milk; in 1902, Schardinger reported that this compound is competent for oxidizing aldehydes to acids by the lessening the methylene blue and after that generally called this chemical as "Schardinger enzyme." XOR has been established to require FAD^+ and Mo^{++} for its optimum catalytic action [103, 113, 114].

XOR is concentrated in MFGM, which is the second most abundant protein constituting, 20% of the MFGM protein. Milk is a good source of XOR, some of its portion is shifted to mammary glands by means of the blood circulation system. The XOR level in milk differs recognizably during lactation. However, bovine milk contains significant levels of XOR (1.4–1.8 U/mg) as compared to goat (0.27 U/mg) and sheep (0.69 U/mg) milk and camel (nd) milk because enzyme molecules lack molybdenum (Mo^{++}) [115–118]. This level can be amplified by complementing the diet with Mo^{++} [7].

In buffalo milk, XOR (0.75 U/mg) exists in the catalytically inactive form because of higher concentration of demolybdo and desulfo forms. Structural factors and lower contents of Fe/S might be the possible reason of lowering enzymatic activity of XOR in buffalo [119].

Surprisingly, camel milk exhibited no detectable XOR activity and its Mo^{++} contents were comparable to human and goat milk [120].

Being significant part of lactating cells, the levels of XOR mRNA began to increase during mid-pregnancy, turned upward at the onset of lactation and diminished quickly in constrained involution [121]. XOR expression remained constant, while specific activity enhanced at the initial lactation phase that facilitates in milk synthesis [122]. Physiologically, XOR contains hydrogen peroxide, nitric oxide, and superoxide ion, mainly functions as in the activation of various metabolic pathways [123]. XOR contributes to an antimicrobial defense mechanism in the gastrointestinal tract (GIT) tract and plays a significant role in the immune system of mammary glands [111, 124, 125]. XOR activity increases during infectious diseases and its cytotoxic action is useful for the defenses against bacteria [123].

3. Conclusions

Conclusively, intensive review of enzyme activities has revealed the significance of indigenous milk enzymes with varied concentration behavior during lactation stages. Lactation stage has a prominent effect on enzymes activities and ultimately it may affect the technological behavior of milk composition.

Generally, colostrum formation contains higher enzyme activities than during the established lactation period. Mastitis or several other progressions that increase leukocytes in milk increase enzyme activities such as CAT. LP, ACP, and LP decrease while lipase activity increases with progress of lactation. ALP activity first increases then decreases at the end of lactation. PL activity increases in the late lactation because of that it makes milk less suitable for cheese making.

This varied behavior of enzyme activities at early, mid, and late lactation stages can be a troubling problem for manufacturing of milk and milk products in various regions of the world. As enormous animals in late lactation periods and considerable seasonal variations affect the ultimate quality of milk and have a better increased choice to process the specified valued dairy product. Furthermore, milk from mid lactation would be a balanced source of energy to maintain the health status of the individuals.

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