Pak. j. sci. ind. res. Ser. B: biol. sci. 2023 **66B**(1) 91-100

Review

Biochemical Changes in Ultra-high Temperature Milk: A Review

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(received October 10, 2019; revised March 27, 2021; accepted May 8, 2021)

Abstract. In developing countries, cold chain facilities are not present in all areas. Ultra-high temperature (UHT) milk is usually manufactured in these countries for catering the needs of all areas. In UHT treatment, sterile milk is filled in sterile packages in a sterilized environment. The operation is usually performed at 140 °C to destroy vegetative forms of bacteria. Shelf life of UHT milk very high as compared to raw milk at room temperature without the need of cold storage. However, UHT treatment and subsequent storage lead to several undesirable biochemical reactions such as alteration in the structure of whey proteins, formation of Maillard reaction products and lipid oxidations etc. Proteolytic events in milk consequences in the production of bitter flavour and age gelation, which is led to limit the life of UHT treated milk. Alkaline proteinase, plasmin are the proteolytic enzymes of milk origin, while psychrotrophic bacteria also produce extracellular proteinases which causes breakdown of protein in ultra-high temperature milk. In UHT milk, sedimentation can happen right after thermal treatment or it takes place during the storage time. Sediment is composed of denatured protein, minerals, lactose and lipids with wide variation in composition. Degree of sedimentation depends upon the raw milk quality, processing and storage temperature.

Keywords: UHT milk, maillard reaction, sedimentation, furosine, age gelation

Introduction

Ultra-high temperature (UHT) milk. Long shelf life of milk can be attained through UHT treatment of milk for a few second 135-140 °C with (Fitria *et al*., 2015; Kelly *et al*., 2012). Pathogenic and spoilage bacteria growth in milk were limited or destroyed by using the UHT treatment (Rauh *et al*., 2014). UHT milk is quite popular in developing countries, where cold chain facilities are lacking (Chove *et al*., 2013; Chavan *et al*., 2011). The shelf life of UHT milk is about six months but it depends on the quality of raw milk, physico-chemical and biochemical operations and subsequent storage conditions (Pulkkinen *et al*., 2014; Lacroix *et al*., 2008). The shelf life is highly dependent on time-temperature combination. There are two types of heat treatment adopted in UHT processes, direct and indirect. When direct heat treatment is applied, steam injected into milk and as a result heat exchange occurred, where as indirect heating method heating media and product are not direct contact to each other (Pak, 2003). The effect of both treatment on milk is different, which is reported by Perkin *et al*. (2005) the content of ketone, cooked flavour, and furosine have been observed less in direct heat treatment as compared to indirect heating of milk during storage. Thermal treatment leads to the partial dissociation of k-casein that results in the reduction of size of casein micelles. Beta-lactoglobulin, alpha-lactalbumin, serum protein immuno-globulin is the major whey protein of milk that is about 20% of total milk protein. In addition to these major proteins, numerous minor proteins are also present in milk these include osteopontin, portease peptone fraction and lactoferrin about 60 enzymes are also associated with whey proteins (Le *et al*. (2006). In UHT milk, sedimentation can happen right after thermal treatment or it takes place during the storage time. Sediment is composed of denatured protein, minerals, lactose and lipids with wide variation in composition. Degree of sedimentation depends upon the quality of raw milk (Hulmi *et al*., 2010). Maillard reaction is influenced by time, pH, water activity, lower pH values lead to increase the reactivity with carbonyl groups of reducing sugars and promotes the formation of furfurals etc (Yong *et al*., 2021). Rate of Maillard reaction is mainly governed by the magnitude of carbohydrates and proteins in the processing and storage of milk. For the characterization of early and terminal stages of Maillard reaction (Troise *et al*., 2016).

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Effect of UHT treatment on milk proteins. UHT treated milk was characterized by structural changes such as protein denaturation. Exposure of casein to UHT treatment leads to dephosphorylation, which affected the micelle structure (Belitz *et al*., 2004). UHT treatment can lead to the formation of peptides, αs1, αs2 and β-casein are susceptible to hydrolysis. Heat treatment can also induce inter and intra-molecular cross-linkage of caseins due to the high reactivity of certain amino acids, such as lysine and tyrosine with in presence of milk sugar. Heat treatment caused de-stabilization of milk proteins which led to promote higher sedimentation during the storage of UHT milk (Lewis *et al*., 2011). UHT treatment result in the formation of casein and whey protein aggregates, consequences in increased gelation (Datta and Deeth, 2001). Mode of heat treatment had also a significant effect on the proteins, indirect UHT treatment had more deleterious effect on proteins as compared to direct mode of UHT (Datta and Deeth, 2003; Marchesseau *et al*., 2002). Lin *et al*. (2010) result showed that whey protein was decreased 23% and 85% in pasteurized and UHT heat treatment, respectively.

Biochemical changes in UHT milk. Bacterial proteases and lipases are more heat resistant than indigenous enzymes of milk, these enzymes retain about 30 to 70% of their activity after UHT treatment (Datta and Deeth, 2001). During the storage of UHT milk, proteases and lipases induced several undesirable flavours in UHT milk such as bitter, unclean, fruity, yeasty and metallic (Datta and Deeth, 2003). Biochemical changes in UHT milk have negative impact on the nutritional characteristics, flavour, physical stability, pH and acidity (Hassan *et al*., 2009). The biochemical reactions reported by Dupont *et al*. (2007) caused undesirable changing in UHT milk such as oxidation flavour, age gelation, bitterness and appearance defect. Tamime (2008) studied that plasmin, protease and lipases activity enhanced the biochemical changes in UHT treated milk such as sedimentation and fat separation. Many studies reported that UHT heat treatment greatly effect the sensory and nutritional profile of milk. During UHT heat treatment cause thermal degradation the milk protein, lipid and milk sugar. At high temperature milk protein and sugar caused the Maillard reaction in UHT treated milk. Therefore, to keep the low processing temperature to avoid the degradation of milk component but in other hand spore forming bacteria cannot inactive at low temperature (Elliott *et al*., 2003; Datta *et al*., 2002). Vazquez-Landaverde *et al*. (2006) studied that UHT treatment promoted the sulfide, aldehyde, ketone and off-flavour by rising the processing temperature.

Proteolytic activity. Raw milk naturally contains plasmin and plasminogen at the concentration of about 0.3 g/L and 2.7 g/L, respectively. Protein hydrolysis was due to action of enzymatic activity in milk and dairy products (Bhatt, 2014; Clare *et al*., 2005). Plasmin reduced stability of UHT milk because they produced heat- stable enzymes and survived at UHT treatment (Datta and Deeth, 2001). UHT treated milk had long shelf life when less microbial load in raw milk (Tamime, 2008). When raw milk stored at lower temperature then less amount of psychotrophic bacteria were there and less biochemical reactions occurred. Chove *et al*. (2013) reported that milk serum, casein micelles fat globule membrane were associated with indigenous microbes with in milk. Vijayakumar (2012) reported that lipases and proteases deteriorated the quality of UHT milk. Pulkkinen (2014) studied that psychotropic bacteria in dairy industry have important role with respect to quality and stability of UHT milk after processing. Samaržija *et al*. (2012) studied that the temperature between 20-30 °C is known optimum temperature for growth of bacteria. Heat resistant enzymes inactivated during UHT treatment and after processing they activated, ultimately cause spoilage the milk at ambient temperature (Richards *et al*., 2014). Heat resistant enzymes were survived in UHT treated milk and reduced the stability of milk (Topçu *et al*., 2006). The refrigeration of raw milk before processing is necessary in bulk tanks but even that temperature accelerated the growth of psychtrophic bacteria. The refrigeration temperature did not prevent the growth of psychrotrophic bacteria even at 4 °C their growth rate was less than as compared to high temperature (O'Brien and Guinee, 2011). Plasmin and plasminogen are effective milk components, which promoted the milk protein particularly caseins. Peptides, proteose-peptones were produced by the action of plasmin. Age gelation in UHT milk was due to activation of plasminogen and that released by plasmin during storage of milk at low temperature, as a result, proteolysis occurred in UHT milk (Cilliers, 2007). Plasmin attaches all types of protein but favourite target is caseins. It cleaves β-casein, αs1-casein and αs2-casein at risk for proteolysis by plasmin in UHT milk. Plasmin cannot completely stope because plasmin are survived at UHT treatment of milk (Gazi *et al*., 2014). Proteolysis in UHT milk enhanced by indigenous plasmin of raw milk.

It was responsible for releasing the βK-complex which was known preliminary step for age gelation and sedimentation (Bavarian *et al*., 2010). The mastitis milk undergoes for UHT process reported by Chavan *et al*. (2011), it showed short shelf life, fast age gelation. Plasminogen level in mastitis milk is more than normal milk so, activity of protease was faster than normal UHT milk. Valero *et al*. (2001) reported that heat stable enzymes were survived during heat treatment and caused several undesirable biochemical changes in UHT milk at room temperature. Datta and Deeth (2003) reported that the origin of heat stable bacteria from psychotropic bacteria with in raw milk. Proteolysis of the UHT milk was the consequence of released of tyrosine in milk which led to the development of objectionable flavor. Bagliniere *et al*. (2013) reported that gelation and bitterness in UHT milk due to proteolysis of protein in milk due to plasmin. Proteases degradation of casein micelles, beta-casein to k-casein and proteose-peptones. It was reported that when milk stored at refrigerator temperature more then 72 h and might be formation of thermo-resistant becteria (proteases) and later heat-stable and indigenous psychotrophic bacteria caused proteolysis in heat treated milk (Richards *et al*., 2014). Whey proteins undergo different changes during processing temperature as well as by the action of bacteria.

Gaucher *et al.* (2011) studied that caseins micelle degraded by proteolytic activity in UHT milk. Bavarian *et al.* (2010) studies that β-casein faster degradation than αs1-casein by action of proteases. Chove *et al*. (2013) reported that proteolysis at high temperature was increased in UHT treated milk during storage. Proteolytic enzymes were more problematic than indigenous enzymes (Forsbäck, 2010). Protease enzymes produced by psychotrophic bacteria mostly attack on casein protein instead of whey protein. Caseins protein more sensitive of protease than the whey protein such as β-casein and κ-casein. β-casein, κ-casein were affected by action of protease enzymes (Samaržija *et al*., 2012). The proteolysis destabilized milk protein by *Pseudomonas flurescens* reported by Bagliniere *et al*. (2013) which was produced at chilling temperature before processing of milk. In UHT milk fat separation is a limiting the life of milk. The proteases remain active after the treatment of UHT in this process protein attached with surface of fat. Many studies described that plasmin cause the separation of fat (Zhang *et al*., 2020).

Lipase activity. Free fatty acids concentration was increased in UHT milk by the lipolytic activity of lipases enzymes. Lipases origin from psychotrophic bacteria and have ability disturb the native membrane structure of fat globule and ultimately result degradation of fat within milk (Samarzija *et al*., 2012). Lipases attack on first and third position in di and tri- monoglycerides of milk and liberate fatty acids as well as free fatty acids and development of hydrolytic rancidity in UHT milk (O'Brien and Guinee, 2011). The lipase enzymes partially inactivate by heat treatment and activated during storage temperature and start their hydrolysis of fat as result off-flavors, rancidity, oxidation, bitter tastes and formation of soapy flavours in UHT milk and it is reported by Richard *et al*. (1014). Lipase caused serious defects in UHT milk such as changes the viscosity, thickness, off-flavours, breakdown of lipid and short shelf life of milk. During processing of UHT treatment some lipase enzymes survived (Hassan *et al*., 2009). Samaržija *et al*. (2012) reported that heat stable enzymes break down the major components of UHT milk. Psychrotrophic bacteria are not major component of bovine udder however, become the part of milk due to contamination of equipment during or after milking (Derakhshani *et al*., 2020).

Age gelation. Age gelation in UHT treated milk is a undesirable. Gelation in UHT milk was due to whey protein denatured by activity of protease enzymes which decrease electrostatic repulsion with in protein and formation of gel (Tijssen *et al*., 2007). The protein formed self-aggregate and complex network with β-LG and α-lactalbumin (α-La) as a result age gelation was occurred and it is reported by Chove *et al*. (2013). Age gelation and bitterness were defect of UHT milk which directly related with quality of milk before heat treatment. By the activity of enzymes casein micelle disperses and form ακ-complexes and as a result viscosity of UHT milk is gradually increased with activity of proteolysis of proteins (Richards *et al*., 2014). In age gelation fluidity of milk is decrease and viscosity of milk increase and as a thick white gel can be observed within UHT milk container. Age gelation is big problem in dairy industry because is short shelf life of UHT milk and also negative impact on customer acceptance. The pH of UHT milk is decreased by action of bacteria which lead towards the gelation. Age gelation rate in UHT milk was greater at high temperature as compared to low temperature (Holland *et al*., 2011). The viscosity of UHT treated milk is stable up to 4 weeks and continue increases at storage temperature (Fernandez *et al*., 2008). Proteolysis affected the quality of dairy products such as shorten the shelf life of UHT milk. Proteolysis in UHT milk was changed the flavor and texture as a result affect acceptance (Prado *et al*., 2006). Pulkkinen (2014) studied showed that age gelation mechanism involved major two steps in first phase some structural changes in proteins and second stage some physio-chemical reaction takes place to decrease the stability of proteins within UHT milk. Milk contains more than 70 indigenous enzymes so it is very biological active product (Gazi *et al*., 2014). Samaržija *et al*. (2012) reported that UHT treatment was promoted the age gelation due to break down of fatty acids as well as tri-acylglycerol of UHT milk at storage.

Sedimentation. Datta *et al*. (2002) studies that sedimentation composition contains aggregates of denatured protein, fat, inorganic salt and lactose. The composition of sedimentation varies types of heat treatment. Sedimentation affected the acceptability of UHT treated milk. The amount of sedimentation depends quality of raw milk, activity of bacteria before and after processing of UHT treatment and storage temperature. Chavan *et al*. (2011) reported that sedimentation mostly depend on the biological quality of raw milk. Schalk *et al*. (2013) reported that sedimentation is major problem as a result of indirect heat treatment as compared to direct heat treatment process. Lewis *et al*. (2011) studied that when milk was heated at 140 °C for 2 s then sedimentation was enhanced due to de-naturization of protein. In UHT treatment κ-casein highly hydrated which lead to an increase in casein micelle density and formation of sedimentation. Sedimentation and flocculation occurred in UHT milk and ultimately decreased the shelf life of milk (Abdel-Aziz *et al*., 2012). UHT heat treatment cause aggregation in proteins which promote the formation of emulsion, instability of protein and sedimentation (Liang *et al*., 2013). Hassan *et al*. (2009) studied that rejection of UHT milk due to development of sedimentation, bitter taste and increased in thickness. Stability and quality of UHT milk is affected by the presence of enzymes because they are promoted biochemical degradation within milk and sensory defects, rheological defects, sedimentation. Samaržija *et al*. (2012) studied that protease attack on casein because casein locates on micelle surface as well as casein have more open structure than whey so more exposed to enzymatic cleavage. The activity of milk enzymes decreased the pH value of UHT milk and at low pH caseins micelles isolate from casein micelle and caused sedimentation and result showed by Lewis *et al*. (2011) that ionic calcium level more than 2 mM caused significant sedimentation in UHT milk.

Lipid oxidation. Free fatty acids are the precursor of objectionable flavours such as, oxidized, cardboard, bitter, rancid, soapy, unclean and metallic. The role of free fatty acids in the acceleration of auto-oxidation was scientifically established (Datta and Deeth, 2003). Milk contains about 21-23% oleic acid (C18:1), which is susceptible to auto-oxidation, rate of auto-oxidation of C18:1 is fifteen times greater than stearic acid (C18:0). Oxidative stability of unsaturated fatty acids mainly depends upon the processing and storage temperature. Average ambient temperature of Pakistan is high, storage of UHT milk at ambient temperature may lead to oxidative deterioration, which can limit consumer's acceptability. UHT milk samples were analyzed for protein, fat, lactose and fatty acid profile. Concentration of fat protein, fat and lactose decreased in UHT treated milk, concentration of SCFA, MCFA and USFA were lower in UHT treated milk (Miguel *et al*., 2015). Lipid oxidation in fresh and recombined UHT treated milks were studied, samples were stored at 6, 20 and 35 °C using same manufacturing facility. Fat separation was higher in fresh milk as compared to recombined milk, during the storage period, recombined milk became more viscous than fresh UHT milk, extent of lipolysis was similar in both types milk (Hassan *et al*., 2009). Lipid oxidation in recombined UHT milk estimated using FAP, PV, AV and TBARS. Value of all these parameters intensified in the storage, major deviations in fatty acid profile were also recorded (Meshref and Rowaily, 2008). Unsaturated fatty acids in milk are more oxidation than saturated by heat treatment (Costa *et al*. 2011). Oxidation of lipid is promoted in the presence of transition metal, light and enzymes (Shahidi and Zhong, 2010). Hydroperoxide are produced by primary oxidation of lipids. Free radical is produced by decomposition of peroxide and lipid molecules initiate propagation process which is known autoxidation (Elias *et al*., 2008). Primary oxidation of hydroperoxide secondary oxidation products is produced such as ketone and aldehyde. Psychrotrophic bacteria at cold temperature formed heat stable lipase and proteases and contribution of lipids and proteins degradation. Zamora and Hidalgo (2005) studied that Maillard reaction is also promoted the lipid oxidation in UHT milk during storage of milk. In UHT milk ketone, aldehyde, phospholipid, dicarbonyls (glyoxal, methylglyoxal) and free radicals are formed by lipid oxidation and highly reactive with protein during Maillard reaction.

Edvaldo *et al*. (2011) result showed that fatty acids profile of raw milk was different than UHT milk. In UHT milk, unsaturated fatty acids were decreased after heat treatment and also during storage of milk at ambient temperature. The ratio of omega-6 and omega-3 fatty acids in raw, pasteurization and commercially sterilized (UHT) milk were 2.10, 2.07 and 1.97, respectively.

Maillard reaction in UHT milk. In UHT treated such as dairy products are under go different chemical and sensory changes. The heat treatment in milk causes the lipid oxidation and protein degradation. In presence of primary and secondary oxidation milk fat and proteins Maillard reaction is promoted after heat treatment. In Maillard reaction, amino acid (lysine) reacts with reducing sugar and as a result decreased the nutritional value of food products. Maillard reaction de-stabilized the milk protein heat treatment and caused defect in milk products *in vivo* and *in vitro* studies (Gilani *et al*., 2012). Volatile thiols (2-propenal and methanethiol) were source of off-flavored in UHT treated milk (Al-Attabi *et al*., 2014). Maillard reaction was started by condensation of amino group on protein with carbonyl groups on reducing sugars as a result in Schiff base formation and rearrangement to Amadori products (Hellwig and Henle, 2014). When melanoidins polymerization was formed, this leads to the formation of browning, structural, compositional and functional changes in food components including sugars, protein and amino acids. Maillard reaction significantly affected the color, taste and digestibility of food (Pischetsrieder and Henle, 2012). Maillard reaction promotes bitter and cooked flavoured in heat treated milk and food products. Browning reaction was accelerated with heat treatment and storage temperature. When α-dicarbonyls and α-amino group of amino acids reacts to each other as a result Strecker aldehydes and off-flavoured produced in food, beverage and UHT milk. Proteolysis was occurred in many foods either due to heat-processing or naturally and as a result increased the free amino acid in milk and food items (Jansson *et al*., 2014). Heat treatment hydrolyzed the lactose into glucose and galactose which were more reactive than lactose. Some lactase hydrolysis into lactose and protease, and react with amino acids as a result Strecker aldehydes were produced (Troise *et al*., 2016). When depletion of amino acids residues formed melanoidins, as a result browning, sensory defects in UHT during storage temperature (Meltretter *et al*., 2014). Maillard reaction changed the functionality properties of protein including stability, solubility, emulsifying, foaming and structural changes (Lee *et al*., 2017). Maillard reaction was promoted by many factors including, types of reactants, time temperature combination, pH and water activity (van Boekel, 2001). Low pH increased the reactivity with carbonyl groups of reducing carbohydrates and favoured the formation of acid-catalyzed sugar degradation. Martins *et al*. (2005) studied that Maillard reaction was influenced by temperature and metallic cations. Formation of coloured compound was due to chemical reaction of reducing sugars (glucose and fructose), protein and some water. Metal ions also play important role to accelerate the Maillard reaction after processing (Lund *et al*., 2005). The de-colourization on powder milk was due to Maillard reaction and caused deterioration in the functional properties such as foaming, solubility and emulsifying reported by Le *et al*. (2011).

Flavored milk. Consumption of flavoured milk is increasing in Pakistan, usually manufactured by blending milk with sugar, stabilizer, colour and flavour followed by UHT treatment and aseptic packaging. Now a day's different UHT dairy products are manufacturing such as tea whitener, cream as well flavoured milk. To improve the nutritional profile of milk, flavoured UHT milk is manufacture with fortified with fat and water soluble vitamins. Flavoured milk is excellent source of all milk nutrients and increasing the consumption among all age especially children. Flavoured UHT milk can be help to reduce the malnutrition, which caused by lack of essential nutrients. Flavoured milk was formulated with milk fat, lactose, whey powder, mineral, flavour and emulsifiers (Yanes *et al*., 2002). Yeung *et al*. (2017) studied that flavoured milk contains both sweetener (sucrose, glucose-fructose syrup) and non-calories sweetener which depend upon the type of flavoured milk. In flavoured milk addition of milk fat improved the creaminess in milk. Phillips *et al*. (2007) studied that addition of whey powder in pasteurized flavoured milk to full fill solid not fat (SNF) for great nutritional, biological and functional properties. The protein industry has started to supply whey powder for milk base beverages (Bariatrix, 2008). Opawumi and White (2004) studied that ingredients of flavoured milk (protein, flavour, colours, sweetener and carbohydrates) were played vital role for better nutritional value and better sensory attributes. Murphy *et al*. (2008) studied that pasteurized flavored milk, dairy drink which was formed by adding colouring agent's (artificial or natural), flavour agents and sweetener in milk. Kumari *et al*. (2016) reported that fruit base flavoured milk preparation was same as chocolate milk by adding flavour, colour, sugar, fruit (orange, pineapple, strawberry) and consumer warmly accepted. Bhargav (2013) sterilized oat flavoured milk with different concentration (1, 2 and 3%) with 7% sugar and he concluded that 2% oat with 7 sugar flavoured milk was more acceptable on the basis of colour, mouth feel, aroma and overall acceptability. Chatterjee and Patel (2016) prepared the sterilized flavoured milk by adding sugar, cocoa powder, carrageenan and oat in milk, founded that addition of oat improves the viscosity and mouth feel was more acceptable by sensory panel. Ultra-high temperature (UHT) heat treatment of milk caused chemical and biological changes in proteins, carbohydrates and vitamins (Gliguem and Birlouez-Aragon, 2005). In UHT treated milk products, chemical and biological changes are accelerated by addition of reducing sugar. During heat processing lactose is degraded into lactulose, acids and lateral chains of amino acids through β-elimination with the formation of de-hydroalanine that can be readily react with lysine and as result producing lysinoalanine. Heat processing was promoted to denature whey proteins, when β-lactoglobulin react with κ-casein (Fox *et al*., 2011). Maillard reaction was also occurred in UHT milk when free amino group of protein react non-enzymatically with reducing sugars. The Schifs is temporary stable product which was formed during Amodori rearrangements other products include fructose lysine and lactose lysine (Holland *et al*., 2011). Furosine is an indicator for thermal treatment in the milk. Furosine content in milk is directly proportion to severity of heat. The greater temperature produced high content of furosine in milk. Elliott *et al*. (2003) studied that furosine content was increased with elevation of storage temperature of UHT treated milk. Furosine was significantly increased both direct and indirect UHT treated milk but higher in indirect treated UHT milk. The amount of furosine had less value in de-hydrolyzed UHT milk as compared to normal milk. It was noted that when higher level of furosine as result lower level of bioavailability of lysine in milk (ISO, 2004). During the UHT heated milk and milk products, Maillard reaction, oxidation and proteolysis processes change the chemical composition of milk. It was noticed that degrading the lactose into galactose and glucose reactivity with protein was more than lactose (Jansson *et al*., 2014). Lactulose was formed by epimerization of lactose due to heat treatment. It was not present in raw milk therefore production of lactulose in milk was indication of heat severity (ISO, 2004).

Conclusions

Ultra-high temperature (UHT) treatment process is often used to process of milk for safety, shelf life and improving the quality. All over the world people mostly consumed process milk rather than raw milk. Heat treatment kill the bacteria in milk and improve the shelf life of milk. Milk proteins are two types casein and whey protein. Whey protein are heat sensitive and denature by UHT treatment. Sedimentation and age gelation occurred due to de-naturization of whey protein fractions. Maillard reaction is predominant during heat treatment in the presence of milk sugar. When milk is stored at room temperature heat resistant protease and lipase enzymes promotes the proteolysis and lipolysis of milk protein and fat. Long shelf life of UHT milk the quality of raw milk must be good and low microbial load as possible.

Conflict of Interest. The authors declare they have no conflict of interest.

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