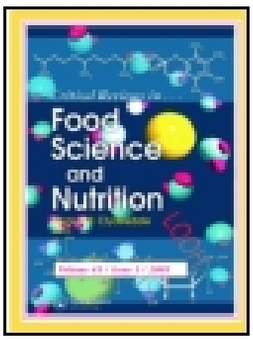

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Effect of processing on nutritive values of milk protein

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Abstract

Milk is an essential source of nutritionally excellent quality protein in human, particularly in vegan diet. Before consumption, milk is invariably processed depending upon final product requirement. This processing may alter the nutritive value of protein in a significant manner. The processing operations like thermal treatment, chemical treatment, biochemical processing, physical treatments, non-conventional treatments, etc. may exert positive or negative influence on nutritional quality of milk proteins. On one side, processing enhances the nutritive and therapeutic values of protein while on other side intermediate or end products generated during protein reactions may cause toxicity and/ or antigenicity upon consumption at elevated level. The review discusses the changes occurring in nutritive quality of milk proteins under the influence of various processing operations.

Keywords

Milk proteins; milk processing; protein nutritive value; milk protein allergy, bioactive milk peptides

1. Introduction

Milk is one of the best food endowed to human beings. It is contains most of the nutrients and also acts as energy source. In a country like India, where people avoid non-vegetarian foods, milk acts as the only source of complete protein. Milk proteins i.e. casein and whey proteins have been found important for various human metabolic, health and other nutrition functions. Milk proteins have also been known to show antioxidant activities, immune enhancing properties, and are able to prevent gastro-intestinal infections. Milk proteins are currently the main source of many biologically active peptides, even though other animal and plant proteins contain potential bioactive sequences (Wu and Ding, 2002). The milk proteins have been extensively studied for their bioactivities and mining of these to prepare bioactive peptides as such and/ or utilization of these peptides by formulation of functional foods, is the recent trend, which is growing at an impressive rate across the globe.

Protein quality is generally associated with the ability of protein to satisfy nitrogen and essential amino acids requirement for tissue growth and maintenance, energy homeostasis and other life essential processes. Thus, nutritive value may be called as the ability of a protein source to achieve defined metabolic actions relating to optimal health. The quality of protein becomes especially important during pregnancy and lactation, when protein requirements are elevated. FAO/WHO/UNU Expert Consultation (2007) group have recommended average protein requirement of 0.66 g/kg body weight/day to achieve zero Nitrogen (N) balance in a healthy adult. Properties relevant to dietary protein quality are- amino acid composition, protein digestibility, amino acid bioavailability and subsequent amino acid and protein metabolism. At present, protein and N digestibility data, growth measurements, N retention, N balance, etc.

aspects are taken into consideration for determination of protein quality (Tome, 2010). Protein quality is largely dependent upon the amino acid composition of the protein. Based on nutritional significance in humans, amino acids are classified as nutritionally essential/indispensable, non-essential/dispensable and conditionally indispensable (Table 1). Since milk is good source of many essential amino acids, the nutritional significance of milk proteins increases manifold.

Milk, being rich in array of nutrients serves as excellent medium for growth of bacteria. The milk spoiling bacteria are ubiquitous in environment. Therefore, processing of raw milk becomes essential to achieve food safety (microbiological quality to requisite standards) as well as food quality (aesthetics, nutrition, texture/ rheological aspects, shelf value, preservation value) of the milk. Also, various processing methodologies aid in preparation of specific dairy products by inducing desirable changes and increasing yield, formulation of new product and preserving/ contributing toward the functionality and nutrition of the product. Unlike fat and carbohydrate in milk, protein is the only major nutritional as well as functional component that is affected during various stages of processing and storage.

The protein consumption pattern among the developed countries and developing countries is depicted in the Table 2. The consumption of quality protein, which are basically animal proteins, in the developed countries are significantly high as compared to developing countries. The cereal proteins, being cheaper, has replaced most of milk proteins as well as meat proteins in the developing countries. Onis *et al*., (1993) conducted survey on the protein-energy malnutrition status of developing countries and covered 87% of total population under 5 years of age. The PEM was observed among more than third of population. It can be attributed to lack of awareness regarding protein nutrition among the developing countries.

Since the processing of milk is an absolute necessity, the processing must not adversely affect the protein functionality and nutritional values as milk is an almost inseparable components of many milk based health and functional foods/ drinks. The very intent of this critique is to provide comprehensive knowledge about the effects of various milk processing operations on the nutritive value of the milk proteins which could be beneficial in facilitating the research and development of foods having milk protein as an essential-nutritional component as well as processing operations for better management of milk proteins' attributes. The review addresses changes occurring in milk proteins when subjected to various processing treatments (thermal, non-thermal, chemical, enzymatic and non-conventional) in a stepwise manner.

2. Nutritive value of milk protein

Milk protein contains complex mix of different protein components in varying proportions in different mammalian species. In bovine milk, nitrogen is distributed in form of protein fraction called casein, whey proteins and non-protein nitrogen fractions (Table 3). The protein quality is essentially related to amino acid bioavailability i.e. degree of dietary protein digestibility and absorption in the gastrointestinal tract. Hence, the difference between nitrogen intake and nitrogen losses is expressed as the digestibility of particular protein (Tome and Bos, 2000). In context to the nutritive value, proteins are indexed based on several scales/ methodologies like Biological Value (BV), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), True Digestibility (TD), Protein Digestibility Corrected Amino Acid Score (PDCAAS) and Digestible Indispensable Amino Acid Score (DIAAS). All these assays consider the amino acid profile of the protein. The nutritive value of milk proteins has been compared to various other food proteins and presented in Table 4. Apart from the amino acid profile, various

casein fractions are well known for their mineral carrying bioactivity and helps in the passive absorption of several minerals namely calcium, zinc, copper, iron, phosphate etc. which are essential during all life stages. Likewise, β-lactoglobulin (β-Lg) component of milk whey protein acts as a carrier for the retinol (Vitamin A). Not just milk protein as a whole, bioactive peptides derived from various milk protein fractions have been known to possess numerous health benefits. Milk protein based bioactive peptides are non-functional when buried inside the native conformation of proteins, however, upon enzymatic hydrolysis, they are liberated to contribute amino acid sequence specific bioactivity (Gobbetti *et al.*, 2002). The health benefits of bioactive peptides have been well reviewed by Korhonen and Pihlanto (2006); Nagpal *et al*. (2011) and Udenigwe and Aluko (2012). Some of the major beneficial effects of milk derived bioactive peptides are depicted in Table 5. The surge in research and development of milk derived bioactive peptides have given rise to number of commercial products claiming various health attributes linked to specific bioactive peptides. However, being a food component, bioactive peptides must pass through safety and regulatory aspects (Phelan *et al*., 2009). Casein hydrolysates were studied extensively for their safety and regulatory status using in-vivo studies. No adverse health effect was noticed in relation to organ toxicity (Maeno *et al*., 2005; Mizuno *et al*., 2005; Nakamura *et al*., 2005), mutagenicity (Ponstein-Simarro Doorten *et al*., 2009) and reproductive performances (Kurosaki *et al*., 2005; Dent *et al*., 2007) of casein hydrolysates (specifically Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP)).

Apart from positive nutritional significance discussed above, milk proteins are implicated in causing allergy. Milk protein allergy is an immunological mechanism in which clinically abnormal adverse reactions occur upon consumption of milk proteins (Bu *et al*., 2013). The

allergenic milk protein components are β-Lg, α-Lactalbumin (α-La) and casein fractions. Bovine serum albumin and lactoferrin are also known to exert antigenicity (Sharma *et al*. 2001; Fritsche, 2003). However, their lower concentration in milk and milk products make them least allergic component. Fiocchi *et al*. (2010) reported cow's milk protein allergy as the most prevalent allergy for 2% infants or 7.5% young children among various countries. There are chances that any structural or conformational change in protein could possibly alter the allergenicity. Any processing treatment leading to generation of minimal allergic components, can be employed successfully for the commercial production of many niche products, especially infant milk powder, infant formula and other baby foods. Therefore, discussion regarding the effect of processing techniques (thermal/ non-thermal) becomes pertinent at present.

3. Processing operations relevant to milk

Milk proteins are excellent source of quality protein, rather complete protein. However, differences exists among the qualities of milk proteins obtained from different milk products, due to the processing treatments given to the milk and milk products. The processing treatments in dairy industry can be classified as below:

- Thermal treatment: Heating, Extrusion cooking, Ohmic heating
- Chemical treatments: Acidification, Oxidation
- Biochemical treatments: Fermentation, Enzymatic hydrolysis
- Physical treatment: Homogenization, High pressure homogenization, High Pressure Processing (HPP)
- Non-conventional treatments: Microwave processing, Irradiation, Membrane filtration, Ultrasound processing, Supercritical fluid extraction

Aforementioned treatments may alter the nutritive value of milk protein either in positive manner (improved bioavailability of amino acids, reduced allergenicity, generate bioactive peptides) or negative manner (loss of amino acids, formation of anti-nutrients, toxic compounds or allergic compounds). This transformation may influence the digestion, absorption, excretion, metabolism and/or retention of proteins. Therefore, processing of milk certainly governs the nutritional aspects of milk proteins. Thus, literature pertaining to the effect of milk processing operations on nutritional aspects of milk proteins is of paramount importance. These effects are discussed in upcoming sections.

3.1 Effect of thermal processing on the nutritive value of milk protein

Thermal processing is an integral step of almost all dairy products manufacturing process. Till date, heat processing is the unit operations with no feasible alternative at the industrial level. The preferred aim for the thermal treatment is to improve the microbial quality of milk by killing the microflora and thus, safeguarding against food borne diseases. The process inactivates enzymes, brings some product-desired physico-chemical changes and often aids in processing. Many times, it also causes undesirable changes in nutritive and physico-chemical properties of milk and milk products. Thermal processing affects the nutritive value of milk proteins to the greatest extent among all dairy processing treatments. However, much of the detrimental effect of heat thermal treatment on nutrients depends on the severity of the treatment and on environmental conditions. Protein undergo various chemical as well as physical alterations upon heating namely denaturation, glycation, β-elimination reactions, racemisation, iso-peptide bonds formation, etc., which are significant with respect to nutrition.

Heat denaturation temperatures of milk protein fractions vary from 62 to 78°C (Singh and Havea, 2003) and the order of sensitivity to thermal denaturation is- immuneoglobulins > serum albumin > β-lactoglobulin > α-lactalbumin > casein. Therefore the properties of milk proteins are altered upon various thermal treatments. Though, changes occur in milk proteins during course of heating, at commercial pasteurization time-temperature (72°C for 15s) combination, the protein retain their bioactivities (Fox and Flynn, 1992; Finot, 1997). Quality of the protein is degraded as a whole if thermal load is very high.

The heating of milk affects the nutritive properties of milk by involving amino acids in various reactions. The most reactive amino acid residues in milk protein includes lysine, tryptophan, threonine, glutamine, asparagine, phosphoserine and sulphur containing amino acids (cysteine and methionine). During heating, tryptophan and glutamic acid may form mutagenic derivatives. Tryptophan, particularly thus, is destroyed during heat treatment. Arginine may be converted into citrulline and ornithine upon severe heat and/or alkali treatment. Glutamine and asparagine gets de-amidated whereas, sulphur containing amino acids like cysteine and cystine are de-sulphured to form hydro-alanine. These reactions modifies amino acids in often reactive forms, that further react to form intra- or inter- chain cross links namely, lanthionine, lysino-alanine, iso-peptides and ornitho-alanine. Involvement of the lysine and dehydro-alanine in these reactions deteriorates the protein digestibility and bioavailability (Fig. 1). The end products of such reactions are toxic in nature (Tome, 1995). Milk with added alkali (for neutralization of developed acidity) upon heating may have decreased arginine content on account of its conversion to citrulline or ornithine. Whey proteins show improvement in digestibility following thermal treatment due to denaturation which exposes the sites for the enzymatic hydrolysis of the

protein. But when denaturation becomes too prominent, digestibility is reduced as enzymes are unable to attack the substrate site buried inside protein aggregates via covalent interactions among proteins (Alkanhal *et al*., 2001).

Apart from causing reduction in digestibility of milk proteins, heating may render lysine- one of the essential amino acid, unavailable. The major cause for the reduction of lysine is Maillard reactions. These browning reactions involve interaction between lysine of a proteins and reducing sugars to produce brown coloured pigments called melanoidins (Fig. 2). It causes loss of nutritive value of protein, since the amino acid upon reacting with sugar is unavailable for metabolism. Table 6 represents status of lysine content in various dairy products as a result of processing interventions. The thermal treatment like pasteurization or UHT to milk exerts negligible effect on lysine availability, however, at milder thermal treatment loss of available lysine would be greater when the product is containing higher solids. Thus, protein content of product and presence of lactose (reducing sugar) play important role in the lysine availability and overall nutritional value of the product.

The Maillard reaction is not only responsible for reducing lysine bioavailability, but also causes production of toxic compounds. The intermediate and end-products of Maillard reactions i.e. furosine, hudroxymethyfurfural, acrylamide (Fig. 3), carboxymethyllysine exert toxicological consequences. Some of the produced compounds are known to be potent neuro-toxicants to human. Lubec *et al*. (1989) stated that heating milk at 174-176°C for 10 min caused formation of cis-3-hydroxyproline, cis-4-hydroxyproline and D-proline. The latter is found to be neurotoxic, hepatotoxic and nephrotoxic. Even though such drastic thermal treatment is not commercialized in dairy industry, ultra-high temperature processes (UHT, sterilization, drying) need to be

monitored for any such toxicants. Microwave processing, is invariably a process where temperature control is difficult to achieve; therefore is cause of concern. Metta and Johnson (1956) studied the effect of heat and radiation sterilization on the nutritive value of evaporated milk. The percent apparent digestibility (AD) -86.4, true digestibility -97.9 and biological value-89.5 of evaporated milk reduced to 85.2, 96.7 and 84.3 respectively, upon heat sterilization of evaporated milk at 242°F for 14 min. Chatterton *et al*. (2004) confirmed the heat treatment led resistance of casein to digestion. Almaas *et al*. (2006) observed faster digestion of raw milk by human proteolytic enzymes compared to high heat (100°C, 1 min) treated milk. Dupont *et al*. (2010) highlighted the impact of milk processing on the casein digestion pattern under simulated infant digestion system. The high heat treatment given to sterilized milk (120°C/10 min) and milk for yogurt making (92°C/10 min) led greater number of peptides generation from the casein as compared to raw milk products.

Antigenicity of bovine casein is not affected even if milk is thermally processed at much higher temperatures (120°C for 15 min) owing to thermal stability of casein structure. However, antigenicity of bovine serum albumin (BSA) and immunoglobulins is thermally labile and is lost completely at when milk is heated i.e. 70-100°C (Hanson and Mansson, 1961). Fiocchi *et al*. (1998) reported 30% reduction in antigenicity of BSA upon heating at 100°C for 5 minutes in 10 children having atopic dermatitis. Heppell *et al*. (1984) studied possibilities of heat treatment to produce infant formula from cow milk. During in-vivo study on guinea-pigs, they noticed unheated β-Lg and bovine IgG components of whey to be antigenic, whereas heat denatured whey proteins were unable to induce antibody production in animals; however, some animals produced serum antibodies against β-Lg and α-casein, although at very low levels. Rytkönen *et*

al. (2002) observed that immunological responses of heat denatured (90°C/30 min) β-Lg were more intensive than untreated β-Lg. The change in antigenicity of α-La and β-Lg in whey protein isolate (WPI) during heating has also been reported by Kleber and Hinrichs (2007) and Bu *et al*. (2009). These workers reported increased antigenicity of α-La and β-Lg upon heating in temperature range of 50-90°C. Heat induced unfolding of protein molecules arguably caused increase in milk protein allergenicity upon heating from 50-90°C by exposing the allergenic epitopes buried inside the native conformation. However, heating above 90°C, decreased the antigenicity. The antigenicity of α-La was further decreed to 25% of control sample when heated at 120°C for 20 min. Nowak-Wegrzyn *et al*. (2008) estimated the tolerance level of heated milk among patients with milk allergy and reported that out of 100, extensively heated milk was effectively tolerated by 68 children.

Extrusion cooking is a different kind of thermal processing treatment in which food material is exposed to high-temperature, under very high shear, for short-time followed by pressurized extrusion through small diameter orifice/die in order to cook it. The food components, in particular, starch undergo swelling, gelatinization and subsequent plasticization, whereas protein is denatured under the influence of heat. The moisture is removed when the product exits the die due to pressure and temperature difference (Havck and Huber, 1989; Castells *et al*., 2005). The shear offered to the food material disrupts the covalent bonding in biopolymer leading to structural modifications at molecular level. (Asp and Bjorck, 1989; Carvalho and Mitchelle, 2000). This causes inactivation of enzymes and destruction of anti-nutrients like haemagglutinin, phytate, tannin, trypsin inhibitors, etc. (Fellows, 2000; Bhandari *et al*. 2001). Milk proteins, being nutritionally superior, have been blended with many cereals to

optimize the extruded snacks with milk protein content up to 25% (Singh *et al*., 1991; Matthey and Hanna, 1997; Onwulata *et al*., 1998, Onwulata *et al*., 2001 and Patel *et al.,* 2016). While texturizing whey protein powders (Whey Protein Concentrate-WPC, WPI and whey albumin) using twin screw extrusion process at temperature of 35, 50, 75 and 100°C, Onwulata *et al*. (2003) noticed reduction in percent digestibility of texturized protein from 89.6 (35°C) to 84.5 (100°C). Severe extrusion processing conditions (temperature >180°C, shear/screw speed >100 rpm and moisture <15%) favour the Maillard reactions (Cheftel, 1986). Bjorck *et al.* (1983) studied the effect of extrusion cooking on the nutritional value of protein in a biscuit. A mixture containing 22% protein (26% wheat protein, 25% casein and 49% soy protein) when processed using twin screw extruder (170-210°C for 42-44 s), the available lysine content was reduced upto 37% during the most severe process. The loss of available lysine was in line with processing temperature. The sulphur containing amino acids were also reduced. i.e. reduction in methionine was 26-28% whereas cystine was reduced by 17%. Arginine content was decreased upto 20% whereas tryptophan loss was 10% at 210°C. However, the moisture content had protective effect on these amino acid losses. BV and NPU of the sample were also reduced under the drastic extrusion conditions.

Owing to severe multiple processing factors like high temperature, high shear and low moisture, the allergic milk protein components may undergo structural and functional alterations. Cheftel (1986) reported the application of extrusion for the reduction of anti-nutrients, toxic components and enzymes in oilseed meals and soy flours. The extrusion processing, thus could possibly be employed for the reduction of antigenicity of milk protein. However, no such confirmatory research work has been conducted till date. There exists a great scope for the

technical interventions using extrusion to reduce the antigenicity of various milk protein fractions.

Ohmic heating (OH) is the thermal processing technology in which heating is achieved by allowing alternating current (AC) to flow through the food. Ohmic processing achieves heating of materials at rapid rates (few seconds to few minutes) (Sastry and Barach, 2000). When electrical current flows through a conductive food, the motion of charges within the polar matrix results in agitation of molecules (or atoms), which results in temperature rise. Electrical conductivity of liquid milk is >0.05 S/m, which makes it suitable for OH (Goullieux and Pain, 2005). One of the major technical innovation for this technology was the continuous-flow Ohmic heater (Skudder, 1988). The potential applications of OH includes pasteurization/ sterilization of milk or other particulate milk foods. Sun *et al.* (2008) studied the effect of OH on the microbial counts as well as denaturation of proteins in milk and reported insignificant protein denaturation during OH as compared to conventional heating. Due to the thermal nature of treatment, OH is expected to exert the same effect on the nutritive value of milk proteins. However, there is dearth of literature showing effect of OH on nutritive value of milk proteins.

3.2 Effect of chemical treatments on the nutritive value of milk protein

Acidification is the most common chemical phenomenon observed in the milk and milk products. In general, protein hydrolysates are prepared by either chemicals or enzymes. Hydrolysis gives prospect of removing/ altering allergenic milk components. Chemical hydrolysis is carried out either by acid, or alkali. Protein hydrolysis by acidification or fermentation is known to improve protein digestibility. Whey proteins and whey protein hydrolysates are used for specialised nutrition applications, particularly in infant formulae and in

specialist enteral nutrition formulations. β-Lactoglobulin has been identified as a major potential allergen in bovine milk, and does not occur in human milk. Hydrolysates have particularly been favoured for enteral nutrition and hypoallergenic infant formulae because the hydrolysis process breaks down possible allergenic structures. Dieptides particularly, Ile-Val, Leu-Val, Val-Leu, Ile-Ile, Leu-Ile, and Ile-Leu arising from whey protein digestion are important. These peptide fractions, particularly Ile-Leu, have been found to stimulate glucose uptake rate in myotubes of rats (Morifuji *et al*. 2009), which stimulated glucose uptake in isolated skeletal muscles from exercise-trained rats. Another important aspect of amino acid nutrition relates to tryptophan uptake in the brain. Tryptophan is a precursor of serotonin and uptake of tryptophan by the brain for serotonin synthesis is considered to be important for sleep. To be effective, tryptophan must cross the blood-- brain barrier. The transport system that takes tryptophan across this barrier is specific only for large neutral amino acids (Phe, Trp, Tyr, Leu, Ile, Val), so the ratio of tryptophan to total large-neutral-amino-acids (Trp: LNAA) will determine its effectiveness. The branched-chain amino acids will compete with tryptophan and limit the rate of tryptophan uptake. This helps in fighting fatigue (Blomstrand, 2006). In contrast, α-La has a high Trp:LNAA ratio and has been promoted as sleep enhancer (Silber and Schmitt, 2010). Therefore, balance between the major whey proteins is important in any nutritional formulation aimed at altering levels of fatigue or promoting sleep.

Whey protein hydrolysates are also utilized in paediatric nutrition. A high level of whey protein is desirable in infant formula, to better reflect the amino acid composition of human milk; however, some whey proteins, notably β-lactoglobulin (absent in human milk) are considered to be potential allergens. Hydrolysates have particularly been favoured for enteral nutrition (tube

feeding) and hypoallergenic infant formulae because the hydrolysis process breaks down possible allergenic structures. Hydrolysis of whey proteins for paediatric use should result in hydrolysis of the β-lactoglobulin so that no remaining peptides large enough to contain epitopes. To achieve this a high degree of hydrolysis is desirable, but too high DH may cause high osmotic load there by upsetting gastrointestinal tract and also bitter taste. Hydrolysed whey protein is also used in sports nutrition. Pre-digested protein produced from controlled hydrolysis are more easily ingested due to rapid uptake of amino acids (Farnfield *et al*. 2009).

Unlike acid treatment, alkali treatments are rarely employed in the dairy industry. Alkali treated milk protein undergoes different set of chemical changes. Alkali racemization of amino acid occurring during alkaline treatment influences protein quality because the resultant D-amino acids formed are poorly available for the absorption in humans. The alkaline treatment causes loss of protein digestibility due to the formation of cross-linkage between amino-acids such as lysino-alanine, lanthionine and loss of enzyme attack sites (Hayashi and Kameda, 1980). Henderson and Snell (1948) reported complete destruction of arginine and threonine and partial destruction of valine, histidine and isoleucine upon hydrolysis of casein with barium hydroxide. Alkaline racemization decreased the utilization of threonine (Pollock and Frommhagen, 1968), due to its conversion to D-form. In addition, D-serine has been reported to be nephrotoxic (Ganote *et al*., 1974). Though racemization does not possess major problem in the dairy processing, the products containing alkaline ingredients like sodium caseinate, alkali hydrolysed proteins may cause adverse health implications. Alkali treatment causes formation of dehydro-alanine (DHA) residues from cysteine and serine residues (Fig. 1) (Patchornik and Sokolovsky, 1964). Further, DHA formed may react either with ε-amino group of lysine, to form lysino-

alanine (LAL), or with intact cystine residue to form lanthionine. Thereby, reducing the nutritive value of milk proteins. Lysino-alanine formation depends not only on pH (Touloupais and Vassiliadis, 1977) but also on hydroxide ion concentration (Sternberg and Kim, 1977). The loss in protein quality correlates well with the severity of alkali treatment. Casein nitrogen digestibility was reduced by 19% when treated with 0.2 M NaOH (40°C, 4 h). The digestibility was reduced to 43% when alkali concentration was increased to 0.5 M (40°C, 4 h). Dakin and Dudley (1913) reported that alkali treated casein was not hydrolysed by trypsin or pepsin when fed to dogs, and these were eliminated as such in the faeces. Furthermore, alkali treated casein and fish meal proved toxic when fed to rats (Ramasarma *et al*., 1949) and chickens (Carpenter and Duckworth, 1950). Thus, lysino-alanine, available lysine and cysteine may serve as good indicators of alkali damage to proteins, with former being most sensitive (deGroot and Slump, 1969).

3.3 Effect of biochemical treatments on the nutritive value of milk proteins

Traditional knowledge describes fermentation as process that improves the nutritive and therapeutic value of milk. Fermentation is used to produce variety of milk based products like *dahi*/ yogurt, cheese, *kefir*, *kumis*, acidophilus milk, etc. Yogurt and cheeses are the world's most recognized milk products and quality of both depends upon quality and amount of milk protein. Improved nutritive and therapeutic values of milks upon fermentation is well established science since long. The proteins of fermented milks like acidophilus milk, bifidus milk, yogurt and buttermilk are more digestible than that of unfermented milk (Hargrove and Alford, 1978).

The proteolysis induced by microbial enzymes produces free amino acids which are readily absorbed and metabolized in human system. The proteins in cheese are more digestible

than milk protein owing to proteolysis during ripening. The most ripened cheese varieties possess almost 100% TD; which is higher than that of whole milk. During rat feeding trial, utilization of cheese protein (89.1%) in terms of essential amino acids, was faster as compared to native casein (85.7%). PER values of cheddar cheese (3.7) was higher than casein (2.5) (Kotula *et al.,* 1987). Ripening process also helps in releasing several biologically active peptides buried within casein chains i.e. angiotensin converting enzyme (ACE) inhibitor, casomorphins and phosphopeptides (FitzGerald and Meisel, 2003).

Ripening process yields free amino acids. In case of surface ripened cheese varieties, extensive decarboxylation of free amino acids leads to formation of biologically active amines i.e. cadaverine, histamine, phenyl-ethylamine, putrescine, tryptamine, tyramine, etc. Ripened cheeses may contain 10-30 mg/ 100g histamine and 15-40 mg/ 100 g tyramine. The concentration is dependent upon period of ripening. Although these biogenic amines are rapidly oxidized to aldehyde and acids by oxidases via oxidative-deamination reaction but they are toxic in nature and their toxicity threshold values vary widely (tyramine: 10-80 mg, histamine: 70-100 mg). Tyramine and phenyl-ethylamine are hypertensive in nature whereas histamine is bestowed with hypotensive effect. Patients lacking monoamine oxidase, consumption of these kinds of cheeses can cause health implications like cheese syndrome. Biogenic amines are also associated with intolerance reactions called pseudo-allergies (sudden onset of anaphylaxis-like non-immunologic reactions associated with food ingestion). These kinds of health conditions cannot be addressed by immunity development (Renner, 1993). However, allergenicity caused by cheese protein is still not reported in any literature.

During lactic fermentation, the hydrolysis improves the digestibility of milk proteins, however, the reduction in protein antigenicity largely depends on the species of starter culture and fermentation conditions. Proteolysis during course of fermentation can break some epitopes thereby causing decrease in milk allergenicity (Cross *et al*. 2001; Bertrand-Harb *et al*. 2003). Jedrychowski and Wroblewska (1999) studied 399 starter culture strains for their properties to reduce the allergenicity of milk protein and screened *Lactococcuc lactis* ssp. *lactis, Leuconostoc mesenteroides* ssp. *creamoris, Lactococcuc lactis* ssp. *creamoris, Lactococcuc lactis* ssp. *diacetilactis, Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus helveticus, Streptococcus salivarius* ssp. *thermophiles, Lactobacillus casei, Lactobacillus delbrueckii* ssp. *lactis, Lactobacillus acidophilus,* etc. strains, having ability to reduce the antigenicity of whey proteins in fermented coagulum by 99% as compared to raw milk. Kleber *et al*. (2006) reported reduction in β-Lg antigenicity during lactic fermentation of skim milk and sweet whey by 70% and 90%, respectively. Bu *et al*. (2010) studied the synergistic effect of various lactic acid bacteria and reported greatest reduction in antigenicity of α-La and β-Lg in skim milk upon fermentation with *Lactobacillus helveticus* and *Streptococcus thermophilus*.

The fermentation of milk involves glycolysis, proteolysis and lipolysis depending upon fermentation time. The glycolysis, the initial biochemical reaction is usually followed by proteolysis and to a lesser extent lipolysis. The protein hydrolysis by enzymes liberated by micro-organisms produces several bioactive peptides from the caseins, and to lesser extent whey proteins. The degree of hydrolysis and types of peptides liberated depend on the types of starter culture and fermentation parameters (Marshall and Tamime, 1997).

Proteolytic enzymes break down/ hydrolyze the protein based on specificity of enzyme, temperature, pH and co-factors. The desirability of enzymatic hydrolysis of protein depends on type of product and processing. For instance, controlled enzymatic hydrolysis causes most important and desirable bio-chemical changes in ripened cheeses, whereas uncontrolled enzymatic hydrolysis results in undesirable bitterness in cheese as well as other dairy products. Enzymatic hydrolysis can be used to address anti-nutrient and allergic components in protein rich foods (Heyman, 1999) via selective hydrolysis. Enzymatic degradation of antigenic fraction of protein is not a new to the researchers. In fact, it is the most effective process known to food processors that reduces the allergenicity of protein by disrupting their allergic epitomes. Pahud *et al*. (1985) observed reduction in the antigenicity of whey protein upon tryptic hydrolysis. Contrary to this, some researchers have even reported generation of new allergic components during enzymatic hydrolysis. Schmidt *et al*. (1995) investigated the antigenicity of milk proteins viz. α-La, β-La, BSA, bovine IgG and hydrolysates in the pH range 2-4. The antigenic properties of all proteins and hydrolysates but β-Lg, increased after hydrolysis at pH 4. The increase in antigenicity could be due to generation of low molecular weight (<3 kDa) peptides during hydrolysis (Puerta *et al*. 2006).

Tripeptides viz., VPP and IPP found in the sour milk (Nakamura *et al*. 1995) and ripened cheeses exhibit ACE-inhibitory activity. The bioactivity of tripeptides increased with cheese maturation, however, it tends to decrease upon extensive proteolysis (Meisel *et al.*, 1997). During cheese ripening, caseino-phosphopeptides (CPP) are also liberated from various casein fractions upon hydrolysis by plasmin and microbial protease (Roudot-Algaron *et al*., 1994; Singh *et al*., 1997). Laffieneur *et al*. (1996) reported immunomodulatory peptide derived upon

hydrolysis of β-casein lactic acid bacteria. During fermentation of milk using probiotic micro-organism, many bioactive peptides exhibiting ACE-inhibitory, opioid, immunomodulatory and other bioactivity are formed upon hydrolysis of casein (Sanders, 1994; Lee and Salminen, 1995; Brassart and Schiffrin, 1997; Buttriss, 1997 and Rokka *et al*., 1997). Peptides having opioid, ACE inhibitory, antithrombotic activity, immunomodulatory, antimicrobial, antioxidative, mineral binding, etc. activities have been detected upon hydrolysis of milk proteins during fermentation and enzymatic hydrolysis. Plenty of other bioactive peptides have been reported for their health attributes and possible nutraceutical applications. These bioactive peptides have been studied extensively and numerous products claiming such health benefits are available in global market.

3.4 Effect of physical processing treatments on the nutritive value of milk protein

Homogenization, developed by August Gaulin during early 20th century, is a process in which milk (at ~60°C) is passed under pressure (14-18 MPa) through very narrow gap between a valve and seat in order to achieve reduction in fat globule size. The whole process employs mechanism of shear stress, cavitation, inertial forces and sudden drop in pressure. Presently, 2-stage homogenization process is employed where second stage, operating at lower pressure (3-4 MPa), disrupts the newly formed aggregates following 1st stage. The altered protein structure in the homogenized milk is responsible for its soft curd forming ability in the stomach. The mechanism of improved digestibility of homogenized milk is well known since long. The homogenization results in finer coagulum of milk in the stomach than un-homogenized milk that helps in the transfer of milk protein to the small intestine much more easily. On the other hand, un-homogenized milk form a very firm coagulum in the stomach and consequently results in

slower gastric emptying rate (Meisel and Hagemeister, 1984; Sieber et al., 1997). The soft curd formation upon homogenization could be exploited in designing infant milks, which can be easily digested.

Ultra high pressure homogenization (UHPH) process works based on same principles and operational set ups as homogenization but at elevated pressure conditions. The pressure as high as 400 MPa results into very high shearing effect and greatest instantaneous pressure drop in the chamber, which inactivate the micro-organisms and modify the chemical structure of food constituents. The feasibility of UHPH has been studied as an alternative process to thermal processing in various dairy products like milk (Pereda *et al*., 2007), cheese (Zamora *et al*., 2007) and yogurt (Serra *et al*., 2007). Guerzoni *et al*., (1999) studied effect of high pressure homogenization (1000 MPa) on microbial and chemico-physical characteristics of goat cheeses and confirmed the enhanced proteolytic activity of rennet using FTIR spectroscopic analysis. The reduction in fat globules size upon UHPH is obvious and well established, however, there are ample chances of structural modification of proteins upon UHPH treatment. β-Lg was denatured more severely as compared to α-La, whereas activity of indigenous milk enzyme reduced by 85 and 95% at 150 and 250 MPa (45°C), respectively (Hayes and Kelly, 2003, Hayes *et al*., 2005); pressure being related to the reduction achieved. Pereda *et al*., (2008) also observed plasmin enzyme inactivation during UHPH (200-300 MPa at 30-40°C) in synchronization with increase in pressure. Further, degree of proteolysis in UHPH (300 MPa, 30°C) treated milk was greater than heat treated (90°C/ 15s) milk having same plasmin activity. UHPH treatment improved the susceptibility of casein to proteolytic enzymes. On the other hand, denaturation of β-Lg in heat treated milk was greater as compared to UHPH treated milk.

High pressure processing, also known as High Hydrostatic Pressure (HHP) or ultra-high pressure processing, is a relatively new non-thermal technology having good potential. In the HHP processing, food is exposed to elevated pressures (100-1000 MPa) to achieve microbial inactivation and/ or to alter the food attributes in order to achieve consumer-desired qualities. HHP offers unique advantages like retained food quality, intact natural freshness and extended microbiological shelf life. Microbial load of foods can be reduced to greater extent using HHP at 900 MPa even at room temperature, without degrading vitamins, flavour and colour (Polydera *et al*., 2005). Foods can also be treated with or without the addition of heat during HHP; the pressure requirement would be lower depending upon temperature of treatment.

Zeece *et al.* (2008) investigated the effect of HHP on β-Lg using *in-vitro* pepsin digestion under simulated gastric conditions. β-Lg, which is resistant to proteolytic digestion by pepsin and chymotrypsin in its native form, was hydrolysed slightly upon HHP treatment of 400 MPa for 10 min by pepsin. The hydrolysis/ digestion was rapid when treated with HHP at 600 and 800 MPa, i.e. less than 1 min of pepsin incubation. The liberated peptides were having molecular weight less than 1500 Da; the specificity can be targeted to reduce allergenicity in many foods. HHP (100 MPa) treated β-Lg showed complete hydrolysis by pronase and chymotrypsin as obtained by Izquierdo *et al.* (2005). Increased enzymatic hydrolysis of dairy protein by HHP treatment has also been reported by Stapelfeldt *et al.*, 1996, Chobert *et al.*, 1997 and Maynard *et al.*, 1998. Garcia-Risco *et al.* (2003) studied the effect of HHP treatment (200 & 400 MPa/ 15 min) on α_{s1}- and β-caseins digestion by plasmin. Faster rate of protein digestion for protein fraction were noticed when compared to control. Casein micelles are known to disintegrate and solubilize under the high pressure. This disintegration and solubilisation makes them susceptible to

proteolysis. Huppertz *et al.* (2004) also reported slight increase in proteolysis in HHP subjected milk (400 MPa at 51°C for 30 min) than untreated milk.

Upon treatment of whey proteins under high-pressure of 100-300 MPa followed by hydrolysis using trypsin, chymotrypsin and pepsin, Penas *et al*. (2006) observed enhancement in protein hydrolysis and reduction in the residual antigenicity of the whey protein hydrolysates. Kleber *et al*. (2007) reported elevated antigenicity of β-Lg in the HHP (200 to 600 MPa) treated WPI-solution, sweet whey and skim milk. The mechanism behind the increase in antigenicity is believed to be the same, as in case of low temperature thermal treatment. The pressure induced unfolding and aggregation, exposes epitopes buried within native protein structure and makes them accessible to antibodies.

3.5 Effect of non-conventional processing treatments on the nutritive value of milk protein

Microwave (MW) heating is based upon transmission of electromagnetic energy into food system and further conversion to heat. Microwaves generate heat within the food and rapidly raise the temperatures. The use of microwaves offers a number of advantages over conventional methods of heating. It has become popular in food industry for thawing, drying and baking foods, as well as for the inactivation of microorganisms in foods. The microwave radiation as such has no detrimental effect on the protein quality. The changes in the proteins upon microwave heating are function of temperature and similar to conventional heating.

Uncontrolled microwave heating may degrade protein but the temperature and pressure of processing are of great importance. Izquierdo *et al.* (2005) studied the effect of microwave irradiation at 15 W and 30 W on the enzymatic hydrolysis of β-Lactoglobulin. The microwave-assisted digestion by pronase was more effective as compared to digestion performed under

conventional heating. Enhanced chemical/enzymatic hydrolysis of proteins by MW irradiation has also been reported by Chen *et al*., 1987; Chiou and Wang, 1989 and Marconi *et al.*, 1995. El Mecherfi *et al*. (2011) reported a reduced immunoreactivity and improved enzymatic hydrolysis condition in terms of time (3 min) of microwave (200 W) treated β-lactoglobulin and bovine whey proteins.

Food irradiation is the process of treating food with a specific dosage of ionizing radiation. This treatment halts the spoilage by retarding enzymatic action or destroying microorganisms, both pathogenic and non-pathogenic. Radiant energy sources such as gamma rays, X-rays or electron beams are utilized for irradiation within appropriately shielded facility. Treating food with ionizing radiation kills micro-organisms that may otherwise cause food-borne illnesses. The food irradiation has shown great potential for its application in the dairy and food industry. In general, it is said that irradiation of proteins with gamma rays or high speed electrons cause denaturation, degradation, polymerization or molecular rearrangement. Changes in the secondary and tertiary structure of the protein largely depend upon the dose applied. Irradiating at dosage level of 35-40 kGy leads to modest destruction of amino acids via free radical formation; the formation of free radicals being greater in sulphur containing amino acids. Casein splits into small peptides at low doses but gets aggregated at high doses.

Svedburg and Brohult (1939) reported that proteins upon treatment with X-rays or UV rays can either split into smaller molecules or may form high molecular weight aggregates. Bellamy *et al*. (1993) noted changes in sedimentation of bovine serum albumin following irradiation. They further suggested splitting, cross-linking and/or polymerization of protein molecules upon irradiation. Such changes were also noticed previously by McArdle and

Desrosier (1955) following 7.4 Mrad irradiation of 2% aqueous solution of casein. These changes are due to alterations occurring in molecular structure because of increase in free sulphydryl group. The % AD (86.4), % TD (97.9) and BV (89.5) of evaporated milk was reduced to 85.3, 96.9 and 81.8 respectively upon sterilization of evaporated milk at 3 million Roentgen-equivalent-physical (rep) γ-radiation dosage (Metta and Johnson, 1956). Scheidegger *et al*. (2010) reported oxidative effects of UV and Fluorescent light on whole milk and skim milk protein. Carbonyl moieties produced upon oxidation of protein were detected after 1 h of UV treatment and after 4 h of fluorescent lighting. However, aggregation and cleavage of proteins occurring due to light exposure did not change the enzymatic digestion by pepsin and chymosin.

Membrane technology is novel processing technology in which separation of a molecules are achieved by means of semi-permeable membranes to concentrate or fractionate a composite liquid into permeate and retentate (Winston and Sirkar, 1992). By the virtue of selective passage, membrane processing has found its ground to isolate valuable components from milk and whey. In fact, the membrane process specifically ultrafiltration (UF) is the only processing technology that can harvest the milk proteins from milk in their native state. However, concentration polarization and membrane fouling phenomenon during UF restrict the concentration and fractionation process to 22-25% TS in retentate side. The microfiltration (MF, 0.2 μm pore size) can be efficiently employed to fractionate and concentrate native casein micelles from skim milk (Fauquant *et al*., 1985). Purity of the fractionated and concentrated casein micelles can further be improved up to 90% by employing diafltration. The application of casein concentrates with reduced mineral and lactose (upon DF) can be explored for pharmaceutical and edible purposes (Maubois and Ollivier, 1992). The undenatured whey proteins from the native whey can be

concentrated using UF to use as high functional property food additive (Maubois, 2002) in various products. WPC and WPI prepared from such native whey protein by UF concentration of native whey (Maubois *et al*., 2001) exhibit excellent functional properties namely solubility, gelation and foaming even after drying (Ostergaard, 2003). Native whey protein products, thus, find important application as an indispensable element of weight balancing products (Burton-Freeman, 2008). Since the native whey does not contain glycomacropeptides (threonine rich peptide liberated from k-casein upon rennet coagulation), their products can be used to formulate baby foods with low risk of hyperthreoninemia (Rigo *et al*., 2001). However, the reports regarding the effect of membrane processing on protein nutrition are not available primarily because of well-established gentle nature of treatment. Finot (1997) reported the membrane processing for their effects on protein nutrition. The protein fractionation may change amino acid composition of retained protein that can either improve or reduce its nutritive value. However, the fractionation and concentration cause enrichment of bioactive peptides that can enhance the nutritive value of milk protein concentrate. The application of membrane filtration to prepare liquid pre-cheese enables the inclusion of whey proteins in the cheese. Casein, major component of milk proteins lacks in sulphur containing amino acids. Therefore, nutritional value of casein (91-97) is lower than that of total milk protein (Renner, 1993). The infusion of whey proteins in cheese, as in heat-acid coagulated varieties and cheeses made from UF retentate, increases the nutritional value of its proteins because whey proteins are nutritionally superior to casein.

Ultrasound is another non-conventional processing technique that is extensively studied for various application, however it is yet to find its commercial ground in food processing. Although the use of ultrasound as the primary means of stimulating chemical reactions and

processes has been known for many years, this safe form of radiation has become increasingly popular during the last two decades along with the emergence of other stimulating techniques. Ultrasound has frequencies beyond human hearing, i.e., above 18 kHz. It is customary to divide ultrasound into two regions: conventional power ultrasound (20-100 kHz) and diagnostic ultrasound (1--10 MHz). Pressure variations in a flowing stream or the propagation of pressure waves (ultrasound) may generate the same phenomenon, cavitation, which in both cases is brought about by tension in liquids. The chemical and physical effects of ultrasound cannot result from the direct interaction of sound waves with matter.

The ultrasound was able to denature both whey proteins, and this effect was higher in whole milk than in skim milk. The denaturation of α-La and β-Lg was higher when the ultrasonic treatment was performed with heat as compared to the same treatment carried out without heating. This effect is synergistic and seems to be more important in the case of whole milk than in skim milk (Villamiel and Jong, 2000). The application of ultra-sonication is very limited, therefore, the information of effect of ultrasound on nutritive values of food, milk proteins in particularly is scanty.

Supercritical fluid extraction is novel process that has found its ground for the recovery and production of high valued ingredients/ components. This technology can be used for extractions, micronization, cleaning, drying, etc. processing of vide range of food components like fat, protein, vitamins, etc. As compared to other conventional extraction processes, SCF technology offers greatest advantages like higher yield, better quality, less hydrocarbon pollution, greater safety, lower production cost and no degradation of heat-sensitive compounds. Thus, this technology has added new dimension to food processing sector. While extracting the

lipid component from whey protein concentrate powder, Catchpole *et al*., (2012) reported denaturation of whey protein in the process line. The little work has been carried out regarding the alteration in protein structure and nutrition upon SCFE treatment.

4. Conclusion

The changes occurring in nutritive quality of milk proteins upon processing were discussed and complied as per Table 7. Thermal processing, an invariably utilized process for making milk safe also degrades the nutritional aspects of milk protein. Thus these changes are of high significance. Also the loss of amino acids upon chemical hydrolysis specifically during preparation of hydrolysates cannot be ignored. The fermentation of milk improves the milk protein digestibility, liberates promising bioactive peptides; however, excessive fermentation has negative health consequences due to formation of biogenic amines. High pressure processing improves the protein digestibility and surface sterilization by irradiation may impair the protein quality due to formation of aggregates. Identification of suitable process markers to control nutritional losses in milk protein during processing can be of high importance. The change in antigenicity during milk processing is of paramount importance, as it could solely dictate the consumption behaviour of milk in future. Thus, there exists a great scope for production of minimally processed microbiologically safe milk products to utilize the full nutritive potential of native milk proteins.

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Table 1. Classification of amino acids based on their nutritional significance

Essential/Indispensable	**Conditionally indispensable**	**Non-essential/Dispensable**
Histidine (His)	Arginine (Arg)	Alanine (Ala)
Leucine (Leu)	Cysteine (Cys)	Aspartic acid (Asp)
Isoleucine (Ile)	Glutamine (Gln)	Asparagine (Asn)
Lysine (Lys)	Glycine (Gly)	Glutamic acid (Glu)
Methionine (Met)	Proline (Pro)	Serine (Ser)
Phenylalanine (Phe)	Tyrosine (Tyr)	
Threonine (Thr)		
Tryptophan (Trp)		
Valine (Val)		

Pellegrino *et al*., (2013)

Table 2. Protein consumption pattern (%) of developed and developing countries

Protein source	Developed	Developing
Milk (Dairy)	16.7	5.6
Eggs	4.3	1.6
Meat	26.4	8.6
Fish	7.3	4.1
Cereals	29.1	58.8
Pulses	1.7	7.4
Oilseeds	1.9	3.8
Vegetables	3.5	3.5
Starchy roots	3.2	3.1
Offal	2.2	1.2

Adapted from Friedman, 1996

Table 3. Protein components of milk

Protein	% of milk	% of protein
Casein	**2.6**	**78.3**
α_{s1}-Casein	1.07	32.0
α_{s2}-Casein	0.28	8.4
β-Casein	0.86	26.0
κ-Casein	0.31	9.3
γ-Casein	0.08	2.4
Serum proteins	**0.63**	**19.0**
β-Lactoglobulin	0.32	9.8
α-Lactalbumin	0.12	3.7
Serum albumin	0.04	1.2
Proteose peptone	0.08	2.4
Immunoglobulins	0.08	2.4

Adapted from Walstra *et al.*, 2006

Table 4. Indispensable amino acid content of various dietary proteins (mg of N/g of amino acid)

Sources	Trp	Phe	Met	Lys	Thr	Leu	Ile	Val	No. of limiting AA	TD	PDCAAS
FAO/WHO	90	180	140	270	180	300	270	270	-	-	
Milk	90	310	150	490	290	630	400	440	0	94	1.00
Meat	80	260	150	510	280	490	320	330	1	94	0.92
Egg	110	330	190	420	330	560	360	450	0	97	1.00
Wheat	80	290	100	170	180	400	240	270	3	86	0.54
Rice	90	320	140	220	240	510	270	370	1	88	0.55
Soya	80	300	80	390	240	480	330	320	2	91	0.91

(WHO, 2002)

Table 5. Milk protein derived bioactive peptides

Protein	Bioactive peptide	Bioactivity
Caseins		
α- and β- Casein	Casomorphin	Opioid agonist
	Casokinin	Antihypertensive
	Immunopeptide	Immunostimulants
	Phosphopeptide	Mineral carrier
	β- Casein f(60-70)	Immunomodulatory+Opioid+ACE inhibitory
αs1-, β- and k-Casein	Many di-, tri- and other peptides	ACE inhibitory
	Isracidin	Immunomodulatory
k-Casein	Casoxin	Opioid antagonist
	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met	Antioxidative
	Caseinophosphopeptide	Mineral carrier

	Glycomacropeptide	Detoxification
k-Casein, Transferrin	Casoplatelin	Antithrombotic
Whey proteins		
α-Lactalbumin	α-Lactophin	Opioid agonist
β-Lactoglobulin	β- Lactophin	Opioid agonist
Lactoferrin	Lactoferroxin	Opioid antagonist
	Lactoferricin	Antimicrobial

Korhonen *et al.*, 1998; Korhonen and Pihlanto, 2006; Phelan *et al.*, 2009

Table 6. Effect of processing treatment on loss of available Lysine content in various dairy products

Milk product	Treatment	Nature of Treatment	Blocked Lysine (% of total Lysine)
Market milk	Pasteurization	T	0.1--0.2
	UHT	T	3.0--6.5
	Retort	T	11.0--13.5
Skim milk powder	Drying	T	7.2--9.8
Milk based infant formulae	Drying	T	18.5--31.2
Yogurt	Fermentation	T + A + E	3.5--4.9
Cheese	Raw	T + E + A	0.2--0.3
	Processed	T + E + A + T	6.5--8.7

Adapted from Pellegrino *et al*., 2013

T = Thermal treatment, A = Acidification, E = Enzymatic

Table 7: Nutritional effects of various processing treatment on milk proteins

Product or Protein	Treatment	Nutritive value of milk protein		Reference
		Before treatment	After treatment	
Evaporated milk	Thermal; 242°F for 14 min	% AD - 86.4, % TC - 97.9, BV - 89.5	% AD -- 85.2, % TC -- 96.7, BV -- 84.3	Metta and Johnson (1956)
	Irradiation;3 M rep	% AD - 86.4, % TC - 97.9, BV - 89.5	% AD -- 85.3, % TC -- 96.9, BV -- 81.8	
β-Lg, BSA and Igs	Thermal; 70 to 100°C	Exhibited antigenicity	Lost antigenicity	Hanson and Mansson, 1961; Heppell *et al*., 1984
Texturized WP powders	Extrusion; 100°C	% Digestibility -- 89.6	% Digestibility -- 84.5	Onwulata *et al*., 2003
Protein rich biscuit	Extrusion; 170-210°C for 42-44 s	-	Loss of available lysine- 37%, Methionine -- 26-28%, Cysteine -- 17%, Arginine -- 20%	Bjorck *et al.*, 1983

Casein	Alkali; 0.2 M NaOH (40°C, 4 h)	-	Loss of digestibility by19%;	Groot and Slump, 1969
	Alkali; 0.5 M NaOH (40°C, 4 h)	-	Loss of digestibility by 43%	
Cheese	Fermentation	85.7% native casein utilization	89.1% cheese protein utilization	Kotula *et al.*, 1987
		PER of casein- 2.5	PER of cheddar cheese- 3.7	
Skim milk	Fermentation	-	Reduced antigenicity of β-Lg - 70%	Kleber et al., 2006
Sweet whey	Fermentation	-	Reduced antigenicity of β-Lg - 90%	
Milk	Fermentation	-	Generation of no. of bioactive peptides	-
β-Lg	HPP: 400 MPa	Resistant to digestion by pepsin	Slightly digested	Zeece et al., 2008
	HPP: 600 -800 MPa	Resistant to digestion by pepsin	Rapidly digested	

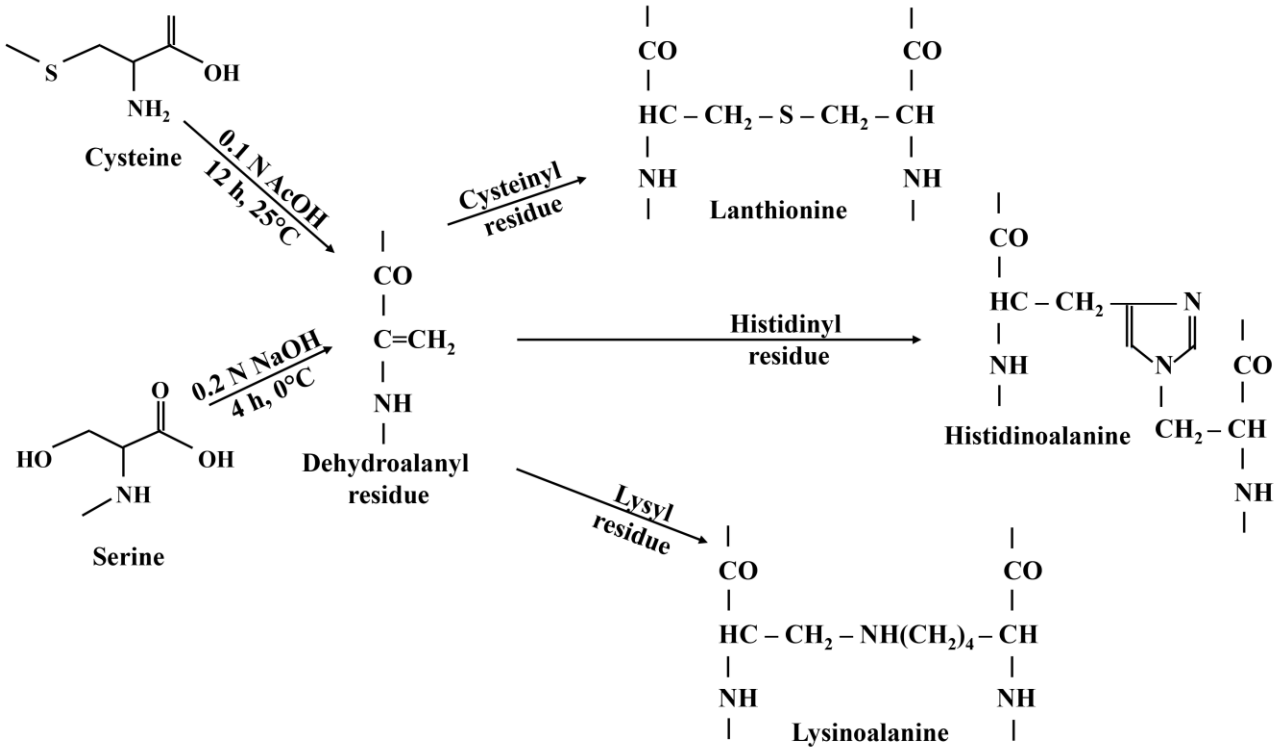

Figure 1

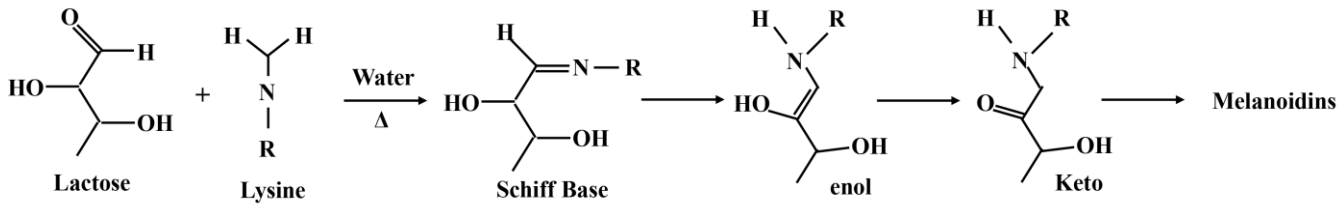

Figure 2

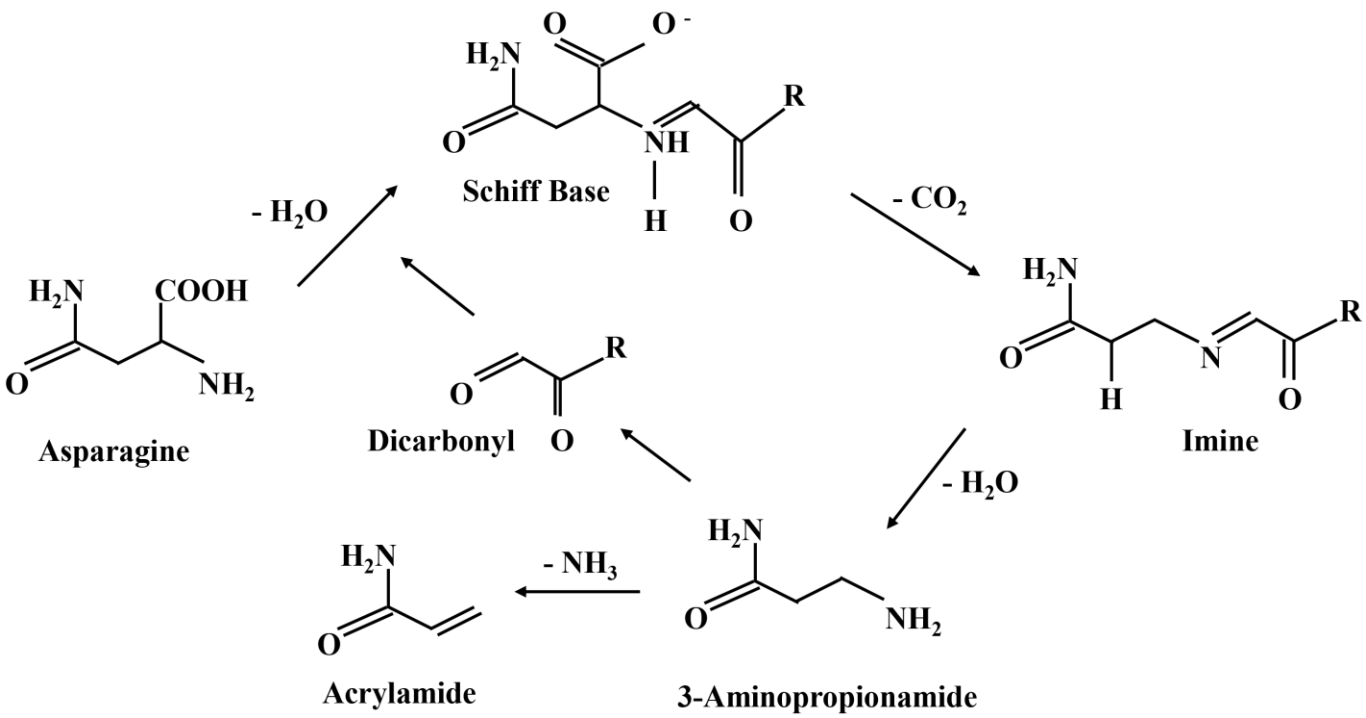

Figure 3