Milk processing involves biochemical reactions and is influenced by genetic polymorphism in major proteins

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Abstract. In last decades, researches conducted on cow's milk recorded a special dynamic, from a basic identification of milk constituents up to molecular characterization of gene variants involved in milk proteins encoding. Furthermore, various associations between different allelic variants and some parameters of cow's milk quality and quantity were also established. This is an important progress, nowadays being possible to use different allelic variants of milk proteins as genetic markers in the process of animals' selection. This is an economically efficient instrument, both for farmers and milk factories, with results in milk yield and quality increasing. The aim of this paper is to present the chemical composition of cow's milk, the genetic polymorphism of its major proteins and various associations between different allelic variants and milk processability. This work is important, considering the aim of dairy cattle breeding for cost-effective milk obtaining, and the aim of dairy industry for a high efficiency of milk processing.

Key Words: proteins, allelic variants, dairy products, processability, chemical composition.

Introduction. The milk sector in Romania is considered a priority, cattle breeding taking place mainly in farms designed to provide milk. At the end of 2005, the total number of cattle ranks our country in top ten of European Union Members States (EU-25) and Bulgaria (NIS 2007). Significant decreases in the number of cattle were reported until 2010, but from 2010 until 2014 the cattle number was maintained at a constant level (NIS 2015).

Although the Romanian people traditionally consume milk, compared with other countries of EU, the consumption of milk and dairy derivatives is still considered lower. These data can be changed by education and finding cost-effective ways for cattle breeding and milk obtaining.

Since milk is a perishable food and its production was traditionally seasonal, over the time became necessary to transform its surplus in more stable products such as fermented milk, cheese or butter. In recent decades, these products were diversified by new ones, such as condensed milk, milk powder, ice cream, different formulas for children etc. These are obtained by implementing various technological processes, such as milk centrifugal separation (to produce cream and, subsequently, butter, and skim milk), concentration, ultrafiltration and drying (to produce concentrated milk, milk powder), enzyme or acid clotting (to produce cheese, whey) (Fox & McSweeney 1998).

Almost half of the Romanian milk market was reported in 2008 to be represented by cheese products and the remaining, by drinking milk and yogurts (Giurcă et al 2008). Although the milk market is a dynamic one and these data may be changed from year to year, the Romanian interest for cheese consumption is well-known.

Over the time were performed many investigations which took into account the chemical composition of cow's milk, the genetic polymorphism of its major proteins and various associations between different allelic variants and some parameters of milk quality and quantity (Grădinaru et al 2013; Marchini et al 2010; Çardak 2005; Caroli et al 2004; Jakob & Puhan 1994; Aleandri et al 1990). The reported results showed the possibility to use these genetic markers in selection programs for cattle breeding in order to increase the protein, fat, and milk yields, and the subsequently milk processing efficiency. The presentation of such results in this paper may be useful for cost-effective milk obtaining and for a high efficiency of its processing.

The aim of this paper is to present the chemical composition of cow's milk, the genetic polymorphism of its major proteins and various associations between different allelic variants and milk processability.

The chemical composition of cow's milk, the genetic polymorphism of its major proteins, and various associations between different allelic variants and milk traits

A. Water is the major component of cows' raw milk (87.8%). Due to its polar trait, water provides dissolution of various constituents which contain groups likely to be bound (water form hydrogen bounds with polar solutes). Water also exerts a hydrophobic effect on non-polar molecules, revealed by their arranging in the form of conglomerates (in aqueous solutions, nonpolar molecules tend to cluster together). Amphipathic substances are particularly arranged with hydrophilic groups (polar) to the water wherewith interacts. Their hydrophobic (nonpolar) regions tend to avoid the contact with water, forming a cluster in which all of them are sequestred from water. In such a manner, amphipatic compounds are arranged in stable structures named micelles (with hundreds or thousands of molecules) (Nelson & Cox 2004; Lupea 2000).

B. Fats of cow's milk consist of triglycerides (98.3%), diglycerides (0.3%), monoglycerides (0.03%), free fatty acids (0.1%), phospholipids (0.8%), sterols (0.3%), carotenoids (trace), fat-soluble vitamins (trace) and favour compounds (trace) (Walstra & Jenness 1984 cited by MacGibbon & Taylor 2006). In terms of chemically features, glycerides are considered esters of fatty acids with glycerol, their aggregation status being closely related to fatty acids composition (for example, those rich in inferior and unsaturated acids are liquids) (Lupea 2000).

Milk fats contain approximately 400 different fatty acids. The feeding of animals with seasonal fodder can influence the level of essential fatty acids in milk. In summer, for example, the saturated fatty acids are in a lower amount (when cows are grazing) compared to their sharing in the milk obtained in the cold season (due to indoor feeding). The content of the unsaturated fatty acids shows the opposite pattern with the highest amount in the summer (Mansson 2008).

The type of milk fatty acids and their sharing give particular characteristics of the alterative process. In general, the unsaturated fatty acids decrease the resistance of milk fat to rancidity. Their feeding incorporation in the form of capsules increases the susceptibility to oxidation and to unsaturated lactones obtaining in dairy products (Lupea 2000).

The phospholipids sharing in cow's milk varies between 0.2 and 1%, the main representatives (lecithin/cephalin/sphingomyelin) being in percentage ratio of 30/45/25. Phospholipids are part in the structure of fat globule membranes, contributing to the stability of the fat emulsion and to the fat globules binding to different proteins (Bondoc 2007; Lupea 2000).

Since the fat of milk has a lower density than milk aqueous phase, when milk is left to stand, the fat globules gradually ascend to the surface, forming a layer of cream. This property is industrially used for cream obtaining.

C. The protein substances of milk are classified considering their degree of solubility in caseins and whey proteins (Formaggioni et al 1999).

<u>Caseins</u> are the major representatives of milk proteins, with ~80% share of the total amount of proteins (Ng-Kwai-Hang et al 1986). Their average content in cow's milk was reported between 2.4 and 2.9% (Bondoc 2007). Caseins are with an exceptional nutritional value, due to essential amino acids including in their structure (for example, lysine, valine, leucine, isoleucine, phenylalanine, tyrosine, proline). Caseins are phosphorylated proteins, the presence of phosphoric acid confering an acidic character and, subsequently, the property to form salts. The milk caseins are most often found in the form of calcium salts, and less in salts with magnesium and citrate. Increasing the concentration of calcium ions in solution will result in agglomeration of hundreds or even thousands of molecules of caseins in a micellar form (a complex mainly consisting by insoluble calcium phospho caseinate) (Chandan 2007; Rusu 2005; Lupea 2000).

The diameter of casein micelles was reported between 5 and 10 µm. Their size is variable, depending on the species from which milk was obtained. For example, big micelles are found in sheep milk, mid-sized micelles in cow milk and small micelles in human milk. Not all caseins are found in micellar form, about 3-10% of the total casein being soluble in whey (Bondoc 2007; Rusu 2005).

Research conducted by immunoelectrophoresis revealed the existence of several casein fractions and subfractions, such as casein alpha (α) with two subfractions αS_1 and αS_2, casein beta (β), casein gamma (γ), and casein kappa (κ). These fractions are different in their amino acids and phosphorus contents. Casein alpha (α) represents 50% of the total casein, both of its subfractions being particularly important in terms of milk technology, considering their rich content in phosphorus (0.85 to 1.4%) and increased sensitivity to the action of calcium ions. Casein beta (β) represents 34-36% of total casein, having a phosphorus content of 0.6%. It is not sensitive to the action of calcium ions at lower temperatures than 20^0C. Casein gamma (γ) represents 3-4% of total casein; it has very low phosphorus content and its properties are similar to immunoglobulins. Casein kappa (κ) represents 12-13% of the total casein; it has a phosphorus content of 0.2%, is insensitive to calcium ions and contributes to the stability of casein micelles (Bondoc 2007; Lupea 2000).

Caseins do not coagulate at boiling, exerting a protective role on albumins and globulins which are maintained uncoagulated. Caseins are of special importance in terms of milk technology being involved in acidic dairy products and cheese obtaining. In the case of acidic dairy products, casein coagulates under the action of lactic acid derived from lactose by the fermentative action of lactic acid bacteria. In the case of cheese obtaining, the curd formation is due to the action of gastric renin on the casein kappa (κ), which is cleaved at Phe105-Met106 bond. The resulted κ–casein (named para-κ-casein) is no longer able to stabilize the casein micelles which will aggregate and produce the coagulum. In the coagulation process, the presence of calcium ions is also needed, the newly formed product being a calcium para caseinate (Fox et al 2017; Brown 2013).

Caseins are strongly hydrophilic, especially by the nature and proportion of contained amino acids. The carboxyl group confers hydrophilic function, while the chain of carbon atoms confers hydrophobic function. In amino acids with short radical prevail the function of carboxylic group, but in those with a high number of carbon atoms, the hydrophobic function of the radical is significant. The hydrophilic function is more pronounced in the presence of two carboxyl groups added to a short radical (for example, glutamic acid and aspartic acid). The hydrophilic strength of casein is manifested weaker at acidic pH. In this way, by replacing the acid whey in which cottage cheese (feta) is preserved, with brine prepared with tap water (pH 7), it is created a conductive environment for water attracting and not for its removing from cheese into whey. This practice is fraudulent because it favors an illicit increasing of cheese weight, decreases the nutritional value by water content increasing, and reduce the time of preservation by creating favorable conditions for the development of damaging microbial flora, with the possibility of food poisonings (Mihaiu & Mihaiu 1998).

The casein synthesis, as of any other protein in the organism, is based on processes of transcription and translation performed in cell nucleus and in cytoplasm, in which the genetic information encoded in segments of DNA named „genes" is transcripted in a molecule of messenger RNA which will be subsequently translated in a sequence of amino acids. In the case of milk proteins is well-known the phenomenon of polymorphism which represents a change in the DNA sequence which is present in at least 1-2% of a population (Buckingham 2007). The most important caseins (α_{S1}, β, α_{S2}, and κ CN) are encoded by four genes located on autosome chromosomes. For example, in cows these genes are located on the 6^{th} chromosome, their segregation occuring together, in a group of linkage (Caroli et al 2009; Formaggioni et al 1999). In buffalo, the involved genes in caseins synthesis are located on the 7th chromosome (Barłowska et al 2012), in sheep and goat on the 6th chromosome, in mice on the 5th chromosome, in rabbits on the 12th chromosome, and in humans on the 4th chromosome (Vilotte et al 2013). At different loci of milk protein genes are found various allelic variants, some of them being associated with yield traits, compositional and technological milk properties. During the last decades, many investigations on these types of associations were performed, especially in cattle. The use of certain genetic variants of milk proteins as markers in the process of animal selection for milk yield and quality became an important factor for dairy industry profitability and for an economically positive impact on the dairy cattle breeding (Grădinaru et al 2015, 2013). For example, the B allele of α_{S1}-CN was reported to be associated with higher milk, protein, fat and cheese yields (Miceikienė et al 2006; Aleandri et al 1990) and the C allele with a shorter rennet clotting time and a higher firmness of the gel (Çardak 2005; Buchberger & Dovč 2000). The B allele of β casein was associated with higher levels of milk proteins and with an improved property of coagulation (Marchini et al 2010; Buchberger & Dovč 2000; Ng-Kwai-Hang et al 1986). Considering A^1 and A^2 allelic variants of β casein, the A^1 allele was associated with higher amounts of caseins and total proteins in milk (Jakob & Puhan 1994; Ng-Kwai-Hang et al 1986). The κ-CN A allele is associated with increased milk yields and κ-CN B allele with higher yield of proteins and fats, and others parameters related to milk processability in cheese and hard cheese (Marchini et al 2010; Caroli et al 2004; Jakob & Puhan 1994; Aleandri et al 1990).

Whey proteins are soluble proteins which remain in whey after the precipitation of caseins at pH 4.6. In cow's milk, this fraction represents 18 to 21% of the total proteins and 17% of nitrogen chemicals, being represented by albumins, globulins, proteozopeptones and a special group of minor milk proteins.

Milk albumin represents about 9.2% of total nitrogen, its share in the total whey proteins being 78% (Bondoc 2007). The amount of albumin varies between 0.3 and 0.5% in normal cow's milk, but can reach up to 4% in colostrum. Compared to casein, albumin is less hydrophilic, so that the consistency of the extracted whey cheese (urda) is most severe. Biochemical analyzes revealed three factions of albumin: α-lactalbumin, β-lactalbumin (known as β-lactoglobulin), and serum albumin. α-lactalbumin is considered the regulatory subunit of lacto synthetase enzyme which contributes to the lactose formation from the monosaccharides. β-lactalbumin (β-lactoglobulin) contains the highest amount of cysteine residues in milk, being the main carrier of free sulfhydryl groups which are involved in the boiled taste of heat-treated milk. Alpha and beta fractions of milk albumin are synthesized in the mammary gland, as opposed to serum albumin which has origin in blood and does not undergo changes at the glandular epithelium level. Moreover, the latter is identical in its structure with blood albumin (Rotaru & Mihaiu 2001; Lupea 2000).

Amoung the polymorphism of whey proteins in cattle, that reported on β-lactoglobulin is the most important. The B allele of β-lactoglobulin was associated with favourable traits for the milk industry such as the fat percentage, fat and cheese yields, shorter coagulation time and a higher thermal stability of proteins (Jõudu et al 2009; Miciński & Klupczyński 2006; Kübarsepp et al 2005; Ikonen & Ojala 1995; Aleandri et al 1990). The locus of the gene involved in α-lactalbumin synthesis, like that of α_{S2} casein, was usually found as monomorphic in many investigated cattle populations, being not included in selection programs for milk traits improving (Grădinaru et al 2015, 2013; Bâlteanu et al 2010; Merlin & Di Stasio 1982).

Serum-albumin is synthesized in liver. Due to its high molecular concentration, serum-albumin represents a component which is responsible for 75-80% of the blood plasma osmotic pressure. It is also the carrier of many drugs and other xenobiotics (Dinu et al 1996).

Globulin is structurally similar to blood globulins. Due to its immunological properties is also named immunoglobulin. This fraction represents 3.3% of the total nitrogen in cow's milk; its share in colostrum is 6-12% compared to 0.08 to 0.1%, as it is in normal milk. By dialysis, milk globulin can be fractionated into euglobulin and pseudoglobulin. These globulin-antibodies do not cross the placenta but ingested by newborns, cross the intestinal wall entirely, reason why the colostral milk plays a vital role in the body defending (Rotaru & Mihaiu 2001; Mihaiu & Mihaiu 1998).

Proteozo peptones are found in the form of glycoproteins, forming a film on the surface of fat globules. In cow's milk they represent about 4% of protein nitrogen, do not precipitate at pH 4.6 and high temperature, and do not result from the degradation of other proteins, being a normal constituent of milk (Rusu 2005).

The minor proteins of milk are represented mainly by red protein (lactotransferrin or lactoferrin) and different enzymes (lysozyme, lactoperoxidase). The content of cow's milk lactoferrin is, on average, 0.2 g/L, with increases up to 1 g/L in the colostral milk. It fulfills antibacterial and anti-inflammatory functions. It is a metalloprotein which bind iron, ensuring its transport through the body and then through milk to the newborn in a non-toxic form and easy to be assimilated (Król et al 2012; Al-Jabri 2005).

D. The non-protein nitrogen substances of the cow's milk are found in small quantities, representing about 5% of the total nitrogen. They are represented by free amino acids, urea, uric acid, creatinine, ammonia. Their origin is blood, being in a significant share in mastitis milk due to permeability increasing of membranes for soluble nitrogen (Bondoc 2007; Mihaiu & Mihaiu 1998).

E. Milk sugar. Lactose is the characteristic carbohydrate of the milk, being found in a dissolved form in water. It is composed by a galactose molecule joined to one molecule of glucose (Fox et al 2015). In the structure of the lactose molecule participate a rest of β-D(+) galactopyranose and another of α or β-glucopyranose. These are 1,4 glycosidically linked, describing two isomeric forms of the lactose (α and β) which are different depending on the position of a hydroxyl group (HO-) on the C1 carbon of glucose (the chemical name of lactose is β-D-galactopyranosyl-D-glucopyranoside) (Izydorczyk 2005).

In the milk solution, lactose is in equilibrium of both isomers, their sharing being easily affected by temperature, and not by the pH of the medium. Thus, at a temperature of 20^0C, the equilibrium rate is 37.2% α-lactose and 62.7% β-lactose, the sharing of α-isomer increasing with milk temperature increasing (Stoicescu 2008; Lupea 2000).

The lactose content in cow's milk varies from 4.4% to 5.2%, averaging to 4.8% (Corzo et al 2010). It is a substrate for the development of many species of bacteria and yeasts, by their intervention being possible four types of fermentation which are different by the resulted end-products (reviewed by Rotaru & Mihaiu 2001; Ţibulcă & Jimborean 2004):

a. <u>The lactic fermentation</u>. The hydrolysis of lactose under the action of lactic microflora lead, in a first stage, to glucose and galactose obtaining (reaction 1). Their each subsequently decomposition will generate two molecules of lactic acid (reaction 2). The lactic fermentation is involved in acidic dairy products and cheese obtaining.

$$\underset{\text{lactose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{lactase}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{galactose}}{C_6H_{12}O_6} \qquad \text{(reaction 1)}$$

$$2C_6H_{12}O_6 \xrightarrow{\text{lactic bacteria}} 4\ CH_3\text{-}CHOH\text{-}COOH \qquad \text{(reaction 2)}$$

b. <u>The alcoholic fermentation</u> takes place in the same time with lactic fermentation, contributing to kefir obtaining. In this type of fermentation, the resulted glucose and galactose (reaction 1) is decomposed under de action of different micro-organisms in ethanol and CO_2 (reaction 3).

$$2\ C_6H_{12}O_6 \xrightarrow{\text{yeast}} \underset{\text{ethyl alcohol}}{4\ C_2H_5OH} + 4\ CO_2 \qquad \text{(reaction 3)}$$

c. The propionic fermentation often occurs following the lactic fermentation, by the intervention of propionic bacteria in some types of cheeses with long maturation period (Swiss type) (reaction 4). The resulted propionic acid contributes to the taste improving. In the same time, the resulted CO_2 favors the appearance of fermentation empties which give a characteristic aspect. Simultaneously with lactic fermentation, some bacteria produce volatile substances such as diacetyl and various esters, which confer a characteristic flavor, especially for cream and butter.

$$3\ CH_3\text{-}CHOH\text{-}COOH \xrightarrow[\text{bacteria}]{\text{propionic}} \underset{\text{propionic acid}}{2\ CH_3\text{-}CH_2\text{-}COOH} + \underset{\text{acetic acid}}{CH_3\text{-}COOH} + CO_2 + H_2O \quad \text{(reaction 4)}$$

d. The butyric fermentation depreciates dairy products and causes distension of cheese by turning lactose into butyric acid (reaction 5).

$$2\ C_6H_{12}O_6 \xrightarrow[\text{bacteria}]{\text{lactic}} 2\ CH_3\text{-}(CH_2)_2\text{-}COOH + 4\ CO_2 + 4\ H_2 \quad \text{(reaction 5)}$$

A prolonged heating of milk promote the Maillard reaction due to the formation of a complex among lactose and amino residues of proteins, chiefly the ε–amino group of lysine. The new resulted compounds are responsible for milk browning. As the heating temperature is higher, the intensity of the color is more significant (Deeth & Hartanto 2009).

F. Milk enzymes are involved in flavor and stability of milk and milk products (lipase, lactase, lactoperoxidase, lactose dehydrogenase, xanthine oxidase, phospholipases C and D, hexose oxidases, milk protease, trypsin), in cheese maturation made from unpasteurized milk (protease, esterases), or in the diagnosis of udder diseases (catalase) (Bondoc 2007; Shipe et al 1975).

G. Milk is a food that contains soluble **vitamins** in water or in fat. Depending on how is milk processed, soluble vitamins in fat are concentrated in cream, butter and fatty cheeses. Water-soluble vitamins are the B complex, C vitamin and bioflavonoids (P vitamin). These include a polar structure, soluble in water, they are not accumulated as reserves in the body, and at the processing of milk they pass into skimmed milk and whey.

H. Milk mineral elements have a share in cow's milk of up to 0.9%, being found in the form of soluble or insoluble salts (chlorides, phosphates, citrates) or in a bound form with milk proteins, in particular, caseins. Their presence in milk has a double meaning, both by contributing to the development of living (micro)organisms, and through their involvement in technological processes of cheese and other milk derivative products (Rusu 2005; Țibulcă & Jimborean 2004).

Conclusions. The milk sector in Romania is considered a priority, in the last decades being observed an increased interest for cattle breeding, especially in farms designed to provide milk. A part of this milk is consumed and traditionally processed in households, and another part is delivered to dairies in order to be processed. In the market economy is particularly important the balance between the costs of production and sale price. As an economically efficient instrument, the genetic polymorphism of major proteins of milk can be used in animal' selection for traits related to milk quality, quantity and processability. Increasing the number of such type of researches conducted in herds in Romania and applying the obtained results in selection programs, could be an element of great importance in Romanian dairy industry, since many associations between various allelic variants and milk traits were demonstrated up to now in cattle breeds all over the world.

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