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Production of caseins and their usages

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Abstract
Casein and caseinates are used in many industries. There are two types of casein. These are: rennet casein and acid casein. Acid caseins are produced with mineral acids and biological acids. Except for these two types, there are sodium, potassium, calcium caseinates and co-precipitates.

Keywords: casein, caseinates, rennet casein, acid casein, lactic casein, co-precipitates

1. Introduction
Casein is found only in the milk on nature. Casein constitutes approximately 80% of milk proteins. Casein and caseinates are widely used in many areas. In order to produce high quality casein, the raw material, skimmed milk, must be in good quality. If lactose is converted into lactic acid by bacteria, the quality of casein will be affected because of increasing of acidity. Excessive heating on milk before precipitation or manufacturing process is not only cause undesirable colour of casein but also reduce to yield (Rathour 2005) [32].

2. Casein
Milk proteins divided into two main categories in 20°C, 4, 6 pH which are separable and non-separable. The separable protein is known as casein, while the other part is known as whey (Fox 2001) [11]. Casein is found only in milk on nature and constitutes about 80% of milk proteins. Casein, the most important fraction of milk protein, is in colloidal dispersion form in milk. Casein is a component of the phosphoprotein structure found in the columns of mammals. Casein is found in form of particles called micelles. About 93% of casein micelles are casein and the rest is; salts such as calcium, magnesium, sodium, potassium, inorganic phosphate and citrate ions, and minerals. The distribution of elements constituting casein is as follows: C (52,96%), H (7,13%), O (22,47%), N (15,60%), P (0,86%) ve S (0,78%). Casein contains four basic constituents as the primary structure; α_{s1}-casein, α_{s2}-casein, β-casein ve κ-casein. Casein consist of 38% α_{s1}-casein, 10% α_{s2}-casein, 36% β- casein, 12% κ-casein. There are α_{s1}-casein 12-15 g/L α_{s2}-casein 3-4 g/L, β-casein 9-11 g/L, and κ-casein 3-4 g/L are found in milk. The casein constituents, α_{s1}-, α_{s2}-, β, and κ-casein, exist in proportions of approximately 3:0,8:3:1 by weight. Other caseins, except κ-casein, contain low levels of cysteine. Caseins contain varying amounts of phosphorus and they contain high amounts of proline, especially β-casein. α_{s1}-casein, α_{s2}-casein, β-casein are susceptible to calcium ions, they precipitate when the solution contains more than 6 mM in the solution. Even though the milk contains 30 mM calcium ion, caseins do not precipitate. Because κ-casein is not sensitive to calcium ions and can stabilize calcium-sensitive caseins up to ten times its own weight. Casein consists of miscellaneous subfractions. Casein micelles consist of sub-micelles aggregation. The ionic bonds between the sub-micelles are formed by calcium bridges. α_{s1}-casein, α_{s2}-casein and β-casein, which are susceptible to calsium, are located at the center of the sub-micelles, and κ-casein at the surface. The α-casein structure contains more charged part, less hydrophobic part and proline. The β-casein fraction is highly hydrophobic and 90% of κ-casein is found on the surface of the micelles (Diamante 1991, Smid *et al*. 1991, Chandan 1997, Fox 2001, Macej *et al*. 2002, de Kruif 2003, Guo *et al*. 2003, Metin 2005, Üçüncü 2005, Sarıkuş 2006, Horne 2006, 2009, Hallen 2008, Huppertz and de Kruif 2008, Livney 2010, Neha *et al*. 2012, Shima and Tanimoto 2015, Southward 2016) [6, 38, 3, 11, 25, 5, 14, 26, 44, 33, 18, 19, 17, 20, 23, 29, 37, 41].

3. Production of Casein
In order to take advantage of the functional properties of casein, there are two basic types named according to clotting agents. These are called rennet casein and acid caseins. Acid caseins are divided into mineral acidified and biological acidified. Apart from these two main types of production, Casein and caseinate products are also produced, which are called caseinates (sodium, potassium, calcium, etc.) and co-precipitates (casein and whey protein complexes) (Southward and Aird 1978, Joshi *et al*. 2013, Southward 2016) [40, 22, 41]. Caseins are also classified as edible casein and industrial casein according to their using area (Joshi *et al*. 2013, Shiksha 2016) [22, 36].
Casein is commercially produced from skim milk. The casein begins to precipitate with diluted acid added gradually reduce to pH 4,0. Sulfuric, hydrochloric or lactic acid is used as precipitated acid. According to the preparation, curds are named as repressed curd, baked curd, granular curd, continuous process curd. Baked curd from these methods have a less soluble property and more ash content than the others. If granular curd and continuous process curd are produced at an appropriate temperature using as much acid as possible, better quality casein with a fast dissolving, low ash content can be obtained. Milk temperature is obtained with an undesirable softness and shredded curd at a temperature below 35°C. Curd occurs larger particles at 37,7°C and washing process is performed more easily. A baked curd with heating at a temperature of 55°C or above is obtained, resulted in a product that has difficult to dissolve, hard, high acid and ash content. Lactose, whey proteins, free amino acids and salts found in curd are removed with whey. Remaining components in curd affect quality of casein, it should be removed as much as possible. Low lactose and ash content improves casein quality. Remaining components are washed several times with cold water. Receding of the particles from the curd depends on amount of particles, depth of penetration, temperature and movement of water. Excess water

can be taken at maximum level by pressing, which is followed by completion of the process with minimum energy requirement in next stage of drying. Dewatered casein is dried to a moisture content of 8-12% by spray or roller dryer method. With this method, casein becomes uniform and gains a good wettability and dispersibility. Casein size with irregular cavities occurs up to 100 μm. Then, the desired granular size is milled and packed in mills. There are also new methods in caseine production such as cryo-precipitation, precipitation with ethanol, ultrafiltration and centrifugation at high speed. Acid casein from casein types contains low ash (2%), while rennet casein contains high ash (8%) (Stathopoulos 2008, Fox 2009) [42, 12].

The manufacture of casein and caseinates are given in Figure 1.

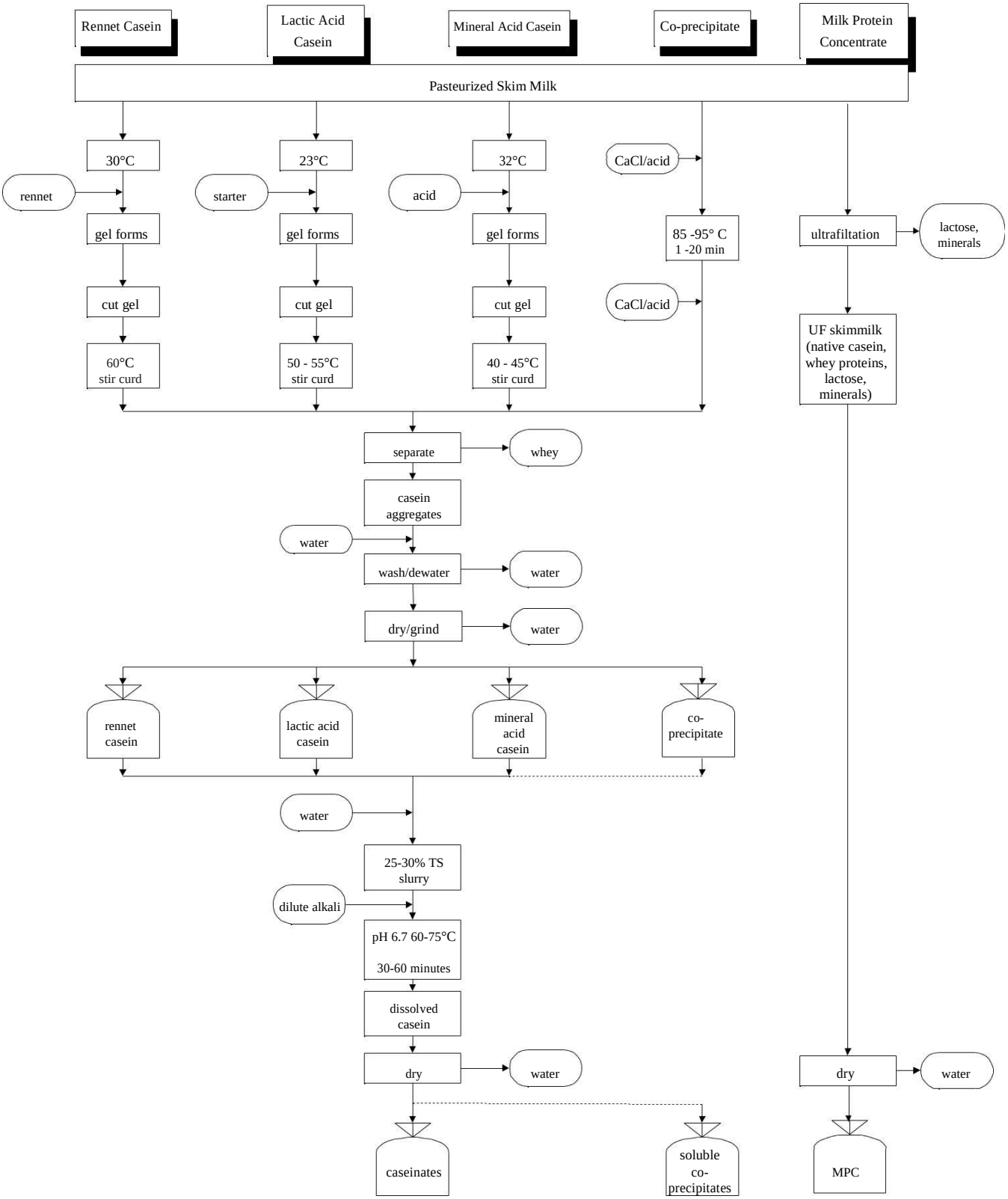

Fig 1: The manufacture of casein and caseinates (quoted from Smith 2001)

3.1 Rennet Casein

Rennet casein production is obtained by coagulation of rennet at 30°C with skim milk. First, as in the case of cheese production, the casein micelles are converted into gel, and whey is separated from casein, washed with water, after the washing, water is removed then dried and ground. When casein is treated with rennet, casein is completely rennet-converted and casein becomes rennet casein. For this reason, casein obtained by rennet or acid are different from each other. Therefore, proteolytic coagulation is described as two stages. In first step, rennin enzyme affects casein between the Phe_{105}-Met_{106} chain by hydrolysis and casein breaks to κ-casein and macropeptides. In second stage, casein micelles are oxidized with Ca^{2+} ions and become rennet casein. To obtain rennet casein for used food production, skim milk pasteurized at 72 ° C is cooled to 30°C. Rennet and calcium chloride are added, for coagulation in 15-20 minutes. Rennet is diluted with 200 ml of water before adding skim milk. Then, formed curd is cut and heated to 60-65°C. The enzyme is deactivated by heating. Heating takes about 30 minutes. After separating whey, curd is washed with water at a temperature of 45-60°C (washing process is done in 2 or 3 stages), drained, pressed, crushed and dried with hot air to 12% moisture content. Dried casein is milled and packed to form granules at a size of 0,5-1,0 mm. Produced rennet casein must be white or slightly yellowish in colour. A darker color is a sign of poor quality and is caused by the high lactose content of the product (Southward and Aird 1978, Tomasula *et al*. 1997, Güneş 1998, Ennis and Mulvihill 1999, O-sullivan *et al*. 2002, Stathopoulos 2008, Üçüncü 2008, Yetişemiyen and Yıldız 2008, Fox 2009, Jacob *et al*. 2010, Schuck 2013, Shima and Tanimoto 2015) [40, 43, 30, 42, 16, 9, 12, 45, 42, 46, 21, 35, 37].

Rennet casein consists of an insoluble structure of calcium paracaseinate. Soluble form, in other words sodium phosphate paracaseinate is converted by emulsifying-calcium-releasing salts such as disodium phosphate (DSP) or trisodium citrate (TSC) and by heating (Ennis and Mulvihill 1999) [9].

Typical aroma composition of two commercial rennet caseins was tested. According to results, typical flavor of rennet casein consists of hexanoic acid, indole, guaiasol and p-cresol. Casein has its own character; an unpleasant stale flavor and animal / wet dog smell (Yüceer *et al*. 2003) [47].

3.2 Acid Casein

Acidification can be achieved by mineral acid (hydrocloric -HCl, sulfiric -H_2SO_4, nitric -HNO_3 acid etc.), organic acids (citric, lactic acid etc.), by ion-exchange and by addition of CO_2 at high pressure (de Kruif 2003) [5].

3.2.1 Biological Acidification – Lactic Acid Casein

For the production of lactic acid casein, skim milk at pH 6,6 is pasteurized at 72°C for 15 s. Mesophilic, non-gas starter bacteria (eg *Streptococcus lactis* 0,5%, *Streptococcus cremoris* 0,5%, *Lactococcus lactis sub-species cremoris* 0,1-0,2%) are inoculated at 22-27ºC to pasteurized milk. Lactic acid bacteria ferment lactose during the incubation period (14-16 h) to reduce pH 4,6 and casein coagulate. Very rapid incubation affects quality, accelerates proteolysis and reduces the amount of product obtained. This clot-curd is called "soft gel-coagulum". In addition to lactic acid, compounds such as diacetyl ($CH_3COCOCH_3$), acetone ($CH_3CHOHCOCH_3$) and benzoin ($C_6H_5COCHOHC_6H_5$) are formed with lactose fermentation. These compounds do not cause serious problems because of the small amount. Then the lactic clot is washed and dried by heating in plate heat exchangers at 50-55ºC, removing whey. Occasionally, the presence of gas-producing *Leuconostoc spp* or *Streptococcus diacetylactis* bacteria in starter cultures lead to gas-curd, which makes it easier to heat, wash and dry. In addition, it has been stated that the presence of excess gas have a negative effect on casein production (Southward and Aird 1978, Mulvihill 1992, Tomasula *et al*. 1997, Yüceer *et al*. 2003, Schuck 2013, Gupta 2008, Stathopoulos 2008, Shiksha 2016, Southward 2016) [40, 28, 43, 41, 36, 35, 42, 15, 47].

3.2.2 Mineral Acidification-Acid Casein

In the case of acid casein production, skim milk's at 25-32°C pH value is reduced to 4,3-4,6 by the addition of dilute hydrochloric or sulfuric acid (0,5-1,4N) and it is waited for about 2 minutes until a smooth casein clot is obtained. All the remaining processes are carried out as in production of lactic casein. Acid caseins are insoluble form in the water, but the caseinates obtained in the roll-to-spray drier provide water-soluble conditions in the alkaline environment (Southward and Aird 1978, Gupta 2008, Stathopoulos 2008, Fox 2009, Shiksha 2016, Southward 2016) [12, 42, 40, 41, 15, 36].

3.3 Caseinates

Caseinates are produced to the acid casein coagulant by adding sodium hydroxide (NaOH), potassium hydroxide (KOH) or calcium hydroxide $Ca(OH)_2$. The aim is to convert and dissolve casein fractions to appropriate salt form at pH 6-7. After salt addition, heat treatment is applied then viscosity and pH of solution are controlled. Control of viscosity is extremely important before drying. Dry-matter of caseinate solution in viscous structure is dried appropriate form (20%) to obtain caseinate. Sodium caseinate (Na-caseinate) prepared by adding NaOH to casein curd is widely used in the food industry due to its water-solubility. Sodium hydroxide is added until the final product reaches pH 6, 7. Sodium hydroxide is usually required 1, 7-2,2% of solid casein for this exercise. In addition to, sodium bicarbonate or sodium phosphates can also be used, but they are not used except for special purposes because of increasing cost. In production of ammonium caseinate; NH_4OH or KOH is used instead of NaOH and in production of citrate caseinate; all but using trisodium citrate or tripotassium citrate instead of NaOH, production process is carried out as Na-caseinate. Calcium caseinate (Ca-caseinate) production is carried out like Na-caseinate production except for a few details. Calcium caseinate solution destabilizes especially heated below pH 6. It also dissolves more slowly than sodium caseinate. In order to accelerate the reaction between casein and $Ca(OH)_2$ it is necessary to completely dissolve casein in ammonium. Besides, calcium, ammonium, potassium, magnesium, aluminum and citrate caseinates are also produced. Aluminum casein is produced for medical purposes, use as emulsifier in production of meat products, heavy metal caseins production (silver, mercury, iron, bismuth, copper etc.), use baby and dietary products (Southward and Aird 1978, Mulvihill 1992, Güneş 1998, de Kruif 2003, Stathopoulos 2008, Fox 2009, Joshi *et al*. 2013, Shiksha 2016, Southward 2016) [40, 28, 16, 12, 5, 22, 42, 41, 36].

Although acid casein and dried casein can be used in caseinate production, fresh acid casein is usually preferred because of cost. Furthermore, casein obtained from curd is more soft-flavour (Üçüncü 2008) [45]. Caseinates contain high-proline and low-sulfur sulphated amino acids. As a result, caseinates can not be

easily gelation with heat and not readily denature (Güneş 1998) [16].

Sodium caseinate has widespread use due to steric stabilization mechanism in fat / water emulsion, strong and long-lasting electrostatic combination and water absorption. In emulsion prepared with Na-caseinate at neutral pH, colloidal stability is provided by α_{s1}-casein and β-casein (Dickinson 1999, 2006) [7, 8].

Acid casein and rennet caseinate are insoluable in water. Sodium, potassium and ammonium caseinats exhibit a high solubility in water. The functional properties of caseinates are based on their physicochemical properties. Due to their particular primary structure, all casein components have amphiphilic character (de Kruif 2003) [5]. Besides, all caseinate powders are white color (Southward 2016) [41].

Casein compositions obtained by different methods are given in Table 1.

Table 1: Comparison of casein compositions* (Tomasula *et al.* 1997) [43].

Compound	Acid	Rennet	Sodium	Calcium
Protein, %	96,4	90,2	95,0	94,8
Ash, %	2,0	8,8	3,7	4,0
Lactose, %	0,1	0,1	0,1	0,1
Fat, %	1,5	0,9	1,1	1,1
Calcium, %	-	3,0	0,1	1,3-1,6
pH	4,9	7,5	6,5-6,9	6,8-7,0

*All values were corrected on the basis of dry matter.

3.4 Co-precipitates

Co-precipitates are obtained by heating or by precipitating treatment with rennet. Protein complex occurs precipitation of proteins with heating and $CaCl_2$ addition via rennet enzyme. Production yield of rennet casein is up to 80% in acid casein production, while almost all of milk proteins are precipitated as co-precipitates production yield reachs up to 92-95%. Structure and character of co-precipitates develop depending on production process. The amount of calcium found in co-precipitate has major effects on functional properties of product. Duration of incubation at 90°C, how much $CaCl_2$ is added and production pH affect calcium content of co-precipitate. Casein-whey ratio in co-precipitates affects such as flexibility, solubility, foaming, water binding, moisture content, and viscosity on casein (Southward and Aird 1978, Mulvihill 1992, Smith 2001, Stathopoulos 2008, Shiksha 2016) [40, 28, 42, 36].

Table 2: Classified co-precipitates varieties (quoted from Shiksha 2016).

	Acid (%)	Low-Ca (%)	Medium Ca (%)	High Ca (%)
Muller *et al.*, (1967)		0,5-0,8	1,5	2,5-3,0
Kozhev *et al.*, (1970)	0,8-1,0	1,2-1,5	2,0-2,5	

4. Usage Areas of Casein and Caseinates

Casein and caseinates are biopolymer powders obtained from milk and used in food, furniture, paper, textile, plastic, ink, fiber, paint, cement, photography, adhesive and leather sectors. Caseins are commonly used as a food additive in food industry. Use of casein in production of dairy products improves texture, viscosity, water binding, emulsifying and foaming properties of product. Caseins are also used in production of pastry, desserts, bakery products, pasta, confectionery products, sauces, pancakes, cakes, yogurt, cheeses, imitation cheese, energy drinks, edible coating materials and snack coats, bars, cereals, breads, snacks and meat products. Calcium caseinate is added as a nutritious and dietary supplement in instant sausages-soups, coffee creams and milk drinks. Except for food industry, it is also used in production of pharmaceuticals, health products, pet food, label adhesives, paper coating, plastic material adhesives, paint and coating agents and biodegradable fibers. Nano-gel casein particles are used to bind vitamins and minerals to network via covalent bonds in the drug in field of pharmacy. Function and use of casein products are given in Table 3 (de Kruif 2003, Yüceer *et al*. 2003, Purevsuren and Davaajav 2001, Monaci *et al*. 2006, Abu Diak *et al*. 2007, Stathopoulos 2008, Üçüncü 2008, Zegota and Malolepszy 2008, Neha *et al*. 2012, Schuck 2013, Cabot 2016) [5]. In recent years, casein and caseinate derivatives (Na-caseinate etc.) are also the subject of research as edible film coating and food coating material (Purevsuren and Davaajav 2001, Cho *et al*. 2002, Longares *et al*. 2005, Schou *et al*. 2005, Fabra *et al*. 2008, Gaygadzhiev *et al*. 2012) [45, 42, 31, 1, 27, 29, 48, 35, 47].

Co-precipitates are used in food industry to develop functional properties and increase nutritional value such as milk, meat, oven products (Southward and Aird 1978, Mulvihill 1992, Stathopoulos 2008, Shiksha 2016) [40, 28, 42, 36].

Table 3: Function and use level of casein products (modified and unified from de Kruif 2003 [5] and Southward 2016) [41]

Food Category	Casein product	Use level* %	Function
Baked Products	Casein, caseinates	1-25	Nutrition, water binding
Cheese products	Rennet casein, acid casein, caseinates	2-25	Fat and water binding, texture, matrix formation
Fermented milk Products	Caseinate, co-precipitate	-	Emulsifier, stabilizer, water binding, viscosity building
Coffee whiteners	Sodium caseinate	1-10	Fat emulsification
Beverages	Caseinate	-	Nutrition, stabilizer, emulsifier, stabilizer,
Confectionery	Caseinates, co-precipitate, acid casein	1-25	Texture, foam stabilizer
Dietary preparations	Caseinate	-	Nutritive value
Cultured products	Sodium caseinate	2-3	Fat emulsifier, stabiliser
High fat powders	Sodium caseinate	Up to 10	Fat emulsifier
Ice cream	Sodium caseinate	1-5	Texture, Stabiliser
Infant foods	Rennet casein, acid casein	1-25	Nutrition
Instants breakfasts	Sodium caseinate	2-30	Nutrition
Meat products	Sodium caseinate, co-precipitate	3-20	Nutrition, fat emulsifier, water binding, texture, structure formation
Nutritional food bars	Casein, caseinates	10-20	Nutrition, texture
Pasta and snacks	Casein, caseinates	5-20	Nutrition, texture

Pharmaceuticals	Casein, caseinates, hydrolysed casein	5-95	Nutrition
Soups and gravies	Sodium caseinate	5-20	Nutrition, thickener
Sports drinks	Sodium caseinate	2-10	Nutrition
Whipped toppings	Sodium caseinate	5-10	Film former, fat emulsifier, stabiliser, bodying agent
Desserts	Caseinate	-	Emulsifier, stabilizer, foam stabilizer
Extruded foods	Caseinate, rennet casein, acid casein	-	Texture, nutritive value, emulsifier
Photoresist for Photo-engraving Processes	Caseinate	-	Light-induced cross-linking by Cr^{3+}
Glues	Caseinate	-	Thickener, structure formation; water resistance of the glue
Paints	Caseinate, Acid Casein	-	Thickener, emulsifier, structure formation; to keep the pigment particles
Plastics	Rennet casein	-	structure formation; plastics in the form of buttons, buckles, imitation tortoiseshell (cobs and hairclips), imitation ivory (knife handles and piano keys), fountain pen barrels, shoehorns, dominoes, novelties
Cements	Acid casein	-	Joint in wallbord

* Typical or estimated values.

Different acids using and applied different process steps are used production of caseins usually cause casein mineral contents to be different. For example, casein products obtained with enzymes contain higher amounts of calcium and phosphorus than those obtained with acids (Stathopoulos 2008) [42].

5. Conclusion

Caseins are used in many sectors, especially in food sector. Caseins are produced in different properties for different use purposes. It is likely to continue to be a subject of researchs because caseins and caseinates have an important value.

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