

On stabilization of acidified milk drinks – a review

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Abstract— Acidified milk drinks are popular drinks diversely consumed in many countries. Lowering of pH to less than 4.2 can be performed by direct addition of acids or activity of lactic acid bacteria. Separation of serum, due to aggregation of milk proteins, is the main industrial defect in these types of beverages. Hydrocolloids are very commonly used to prevent serum separation in acidified milk drinks by increasing the viscosity, electrostatic or/and steric repulsion. Viscosity increase can also be achieved by employing of exopolysaccharides-producing starter cultures. Casein-whey proteins ratio alteration, up to a certain level, is capable of stabilizing the system. However, homogenization pressure increase has no significant influence on the prevention of serum separation in acidified milk drinks.

Keywords—acidified milk drinks; stability; milk proteins; hydrocolloids; exopolysaccharides

I. INTRODUCTION

Acidified milk drinks (AMD) are popular in many countries. Acidified products can be obtained by microbial fermentation or direct addition of acid using fruit juice or acids such as citric, malic or glucono- δ -lactone. Most of the research has been conducted on drinkable yogurts which are made from milk fermented with lactic acid bacteria.

Fermented milk products are classified into the following categories on the basis of physical characteristics: (a) viscose products; (b) diluted or beverage products; (c) carbonated products [1]. From the microbial point of view, fermented drinking products may contain live starter culture bacteria as a fresh type or be extended shelf-life with no live micro-organisms by heating the final product [2]. Drinking type fermented milk products are known as Laban drink in most Arab countries, Lassi and Dahi in India, Viili, Tafil, filmjolk in Scandinavian countries, Ayran in Turkey and Doogh in Iran [1]. On the whole, they differ from each other in the amount of added water, fat and salt/sugar content, rheological properties and taste [3]. In Europe, for instance, consistency of the product is higher when compared with the low viscose drinking fermented milk produced in Asia and Middle East [1]. Low fat content in some drinking yoghurt products is appealing to diet-conscious consumers. In current commercial productions of AMDs, the reduction of fat content is normally achieved using skim milk to prepare the yoghurt, and

usually the final product is flavored with essential oils, such as those extracted from mint, oregano, thyme, etc. containing salt (in Ayran and Doogh) or fruit juice containing sugar [4]. Serum separation is the main textural defect in AMDs during storage, which is industrially called “*Whey*ing off”. It is the separation of product into a casein-rich lower layer and a clear upper layer of serum [4]. Stabilizers are commonly added during the manufacturing of these products in order to avoid sedimentation of milk solids and whey separations in the package [2].

In this study, we endeavored to review a wide range of articles reported in literature and interpret the given information on materials which are used to stabilization of AMDs products and their mechanisms. The aim was to generate some new interest in this field and to elaborate on some possible new direction for research.

II. WHEY SEPARATION MECHANISM

The caseins form the largest protein component in most milks of industrial significance representing 80% of the total milk protein. The major caseins are α_{S2} -, α_{S1} -, β - and κ -, and they are in a ratio of 1: 4: 4: 1, respectively and presenting predominantly in the form of hydrated association colloids called casein micelles [5]. The α - and β -caseins are clustered in the center of the micelle; the κ -casein molecules lie at the surface, with the glycosylated C-terminal sequences protruding to form an expanded “hairy layer” which acts as a barrier to aggregation [6]. Casein micelles have an average radius of ~ 100 nm containing approximately $3.4 \text{ g H}_2\text{O g}^{-1}$ dry matter which consist of, on a dry matter basis, $\sim 94\%$ protein and $\sim 6\%$ inorganic materials [7]. These inorganic materials are collectively referred to as micellar calcium phosphate (MCP) or colloidal calcium phosphate (CCP) including primarily calcium and phosphate, with lower levels of magnesium and citrate also present [8].

The stability of casein micelles can be divided into 2 categories: “inter-micellar” and “intra-micellar” stability. The inter-micellar, or colloidal, stability of casein micelles, denotes the stability of casein micelles against aggregation the three relevant types of interaction that play the most important roles in stability of casein micelles are van der Waals attraction, electrostatic repulsion, and polymer brush repulsion [9]. Such

stabilities are well characterized and, in some cases, form the basis of the conversion of milk into other products, for example rennet- or acid-induced coagulation of milk forms the basis of manufacture of cheese or yoghurt, respectively. The intra-micellar stability, that is, the ability of the casein micelle to maintain its internal structural integrity under the influence of environmental changes, is of considerable influence for properties of products derived from milk [9]. CCP which is dispersed within the casein micelle, can act as a linking agent between α - and β -caseins and is the main agent in maintaining micellar integrity [6].

In production of AMDs, when milk is progressively acidified, complex physico-chemical changes within the casein micelles occur. During acidification, the first step is a collapse of hairy brush (charge neutralization of κ -casein) and release of calcium phosphate. Then, molecules of casein, mostly β - and α -casein begin to leave the micelle, and as their isoelectric point is reached and then exceeded, they become positive, and try to reintegrate with the micelle (or casein particle) which is still overall negative: the electrostatic interactions begin to override the disaggregation forces. In the end, the altered micelles aggregate, a network begins to form, and a gel is obtained [10].

Whey protein fraction, which accounts for approximately 20% of total protein in bovine milk, comprises the non-casein proteins that remain soluble when caseins have been isoelectrically precipitated at pH 4.6 [11]. The principal whey protein is β -lactoglobulin (β -lg), which constitutes about 50% of the total content of whey protein in bovine milk [5]. In industrial preparation of fermented milks, the milk is normally pre-heated (generally for 10-20 min at 90 °C). Upon heating, a reactive thiol group is exposed in β -lg due to conformational changes (denaturation) of the molecule. This reactive thiol group can form disulfide links mostly with κ -casein at the surface of the micelles. Additionally, soluble disulphide linked whey aggregates are formed. So after heating, the hairy brush of the casein micelles contains casein associated whey proteins [12].

Acidification to pH values lower than the isoelectric point of protein particles results in the buildup of primary gel aggregates which are subsequently organized as self supportive *super-aggregates* structure. Stirring of yoghurt breaks down the gel network and produces the aggregate structures [13]. In the preparation process of AMDs, these super-aggregates still exist after adding water to yoghurt; however, they are more separated and free to sediment under gravity, causing massive loss of stability. Furthermore, the presence of salt in this type of beverage intensifies the serum separation [14].

III. USE OF HYDROCOLLOIDS

Different kinds of hydrocolloids are commonly used to improve consistency (increase viscosity) and reduce

syneresis. It was shown that both adsorbing and non-adsorbing hydrocolloids are capable to stabilize the system. The term “adsorbing” is related to charged polysaccharides, which can interact with proteins via electrostatic forces and the interaction is highly dependent on pH and ionic strength of solution. In case of using this type of hydrocolloids, stability of system is due to electrostatic repulsion, steric repulsion or both of them. On the other hand, “non-adsorbing” hydrocolloids can prevent serum separation by increasing the viscosity of continuous phase, entrapping water in a network and immobilizing the particles [15, 16]. In this review most commonly hydrocolloids used in AMDs have been studied.

A. Pectin

Pectin is found in the cell walls of most of higher land plants, contributing many cell wall functions. Pectin has a chain structure of places by a comparatively smaller amount of so-called rhamnogalacturonan with neutral polysaccharide side-chains. Some of the carboxyl groups are methyl esterified, and pectin with more than 50% of esterified carboxyl groups is termed high-methoxy pectin (HMP) [17]. The ester content can be reduced by pectin methyl-esterase enzymes, or by hydrolysis with alkali or acid, to give low-methoxy pectin (LMP), which typically has a degree of eseterification of ~ 30 -35%. HMP is widely used to stabilize acidified milk drinks, where it prevents the sedimentation problem.

In production of AMD, HMP is added following acidification, which can be done using lactic acid bacteria or by adding a chemical acidulant. This is due to HMP being most stable at pH values of 3.5 ± 1.0 and degradation occurring in milk [17]. In addition, HMP causes phase separation, with resulting casein precipitation, if added to milk at its natural pH [18]. Furthermore, lower concentrations can cause instability by attachment of individual pectin molecules to more than one particle, thus inducing formation of large flocks. This process of “bridging flocculation” has its maximum effect when the concentration of HMP reaches the value required to give 50% coverage of the surface of the particles [18]. LMP can be added prior to acidification as it is less prone to degradation at the natural pH of milk and causes less phase separation. LMP is, however, a much less efficient stabilizer in AMD [17].

Different theories have been proposed for the specific stabilizing mechanism. It has been shown that pectin adsorbs via electrostatic interactions in diluted acidified milk systems where adsorption takes place at or below pH 5.0 [19]. High negative charge (low ester content) regions of the pectin binds tightly to the surface of casein particles and regions of lower charge (higher ester content) protrudes from the surface as loops and dangling tails. The entropy of the protruding pectin chains confer stability due to introducing of steric repulsion between the

casein aggregates. Stabilization of acid-casein dispersions by HMP, however, requires enough pectin to cover the surface of the particles [4, 20]. It turns out that in acidified milk systems of practical concentration less than 20% of the pectin added is directly interacting with casein micelles. The remaining 80% is involved in a network with casein/pectin complexes but plays no role in stabilizing the final product. This excess fraction is, however, crucial in effecting sufficient pectin adsorption during the mixing process of yoghurt and pectin solution [21]. The concentration of HMP required to ensure stability in acid milk drinks is around 0.25 wt% [22].

Recently, another theory suggests that long-term stability is achieved by the presence of a weak gel network of pectin preventing sedimentation of casein aggregates [21]. This proposed weak gel formed in the serum phase is assumed to restrict the suspended casein particles from colliding, and the weak gel is considered to be strong enough to overcome gravity. Thus, the stability of AMD depends on the concentration and the type of pectin used the concentration of the casein and the ionic strength and pH [23].

B. Soybean Soluble Polysaccharides

Soybean soluble polysaccharides (SSPS), extracted from soybean cotyledons, has recently been used as stabilizer in acidified milk drinks [24]. SSPS has a pectin like structure, that is, composed of D-galactose, L-arabinose, D-galacturonic acid and L-rhamnose. However, the content of neutral monosaccharides in SSPS is much higher than that in pectin [25].

The stabilizing ability and mechanism of SSPS have been investigated in comparison with pectin [26]. SSPS, just like pectin, stabilizes acidified milk drinks by steric stabilizing effect due to adsorbed SSPS layer. However, because of the thick layer of neutral sugar side chains of SSPS on the surface of the protein particles, the stability of acidified milk drinks induced by SSPS is different from that induced by pectin. Less SSPS is more effective than pectin at stabilizing and dispersing protein particles SSPS at concentrations of less than 0.2% stabilized acidic beverages prepared with 8.0% of milk solid non-fat at pH 3.4-4.4, in contrast with pectin which needed at least 0.4% concentration [25]. SSPS could give rise to better stability at pH lower than 4.2 and the stability was not affected by pH between pH 4.2 and 3.2, while acidified milk drinks homogenized with pectin showed a particle size distribution that depended on pH.

C. Gum tragacanth

Gum tragacanth (GT) is a dried exudate which is obtained from slitting the stem of Asiatic species of *Astragalus*. This gum has potentially utilities as a stabilizer, emulsifier and thickener in food industry, pharmaceuticals and cosmetics [27]. GT is composed of at least two components: a water-soluble part, tragacanthin, (40-30% of GT) and water-insoluble part, bassorin, (60-

70% of GT) [15, 28]. Bassorin fraction is a complex structure of polymethoxylated acids and responsible for capability of gel formation in GT owing to its high molecular weight. In contrast, tragacanthin, a highly branched arabinogalactan, dominantly composed of L-arabinose are linked to C-3 site of galactose in the backbone [28].

Stabilization mechanism of Doogh incorporated with GT and tragacanthin was investigated by Azarikia and Abbasi (2010). Based on their performed rheological experiments, Dooghs containing tragacanthin and GT exhibited dominant viscose and elastic behaviours, respectively [15]. In Fig. 1 a proposed model for the explanation of the stabilization of Doogh is illustrated. This model suggests that soluble tragacanthin adsorbs on the caseins and its side chains induce steric and/or electrostatic repulsion by which the casein particles are stabilized. In addition, side chains overlapping may cause an increase in viscosity as well as inducing shear thinning behaviour. On the other hand, in case of GT which contains soluble tragacanthin as well as insoluble bassorin, the latter might sterically prevent the overlapping of coils and side chains of tragacanthin but increases the viscosity considerably and contributes to a higher stability of the Doogh samples. It was reported that Doogh, with approximately 6 wt% dry matter and 1 wt% fat, can be stabilized by soluble tragacanthin and GT at concentration of 0.10% and 0.20%, respectively [15].

D. Carboxy Methyl Cellulose

As one of the most important derivatives of cellulose, carboxy methyl cellulose (CMC) is a typical anionic polysaccharide and has been widely used as stabilizer in food. CMC is commonly chosen as a stabilizing agent for its low cost in acidified milk drinks instead of pectin in Asia [29]. CMC chains are linear $\beta(1 \rightarrow 4)$ -linked glucopyranose residues. The average degree of substitution (DS) of CMC is defined as the average

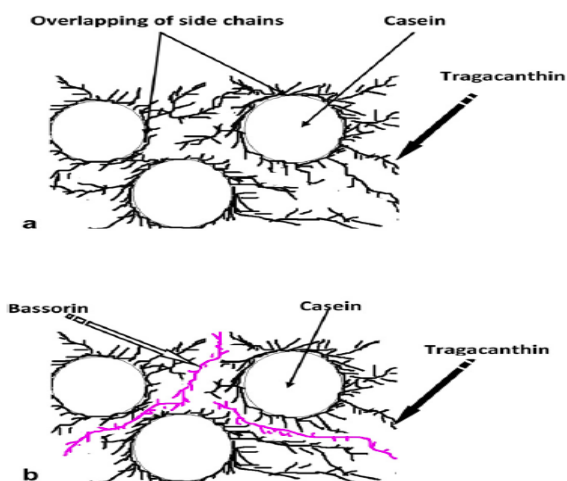


Figure 1. Proposed model for stabilization mechanisms of Doogh by a) T and b) G [15]

number of carboxymethyle groups per repeating unit and is usually in the range of 0.4-1.5.

CMC is generally found under sodium salt form, a water-soluble product for $DS > 0.5$. A maximum degree of substitution of 1.5 is permitted, but more typically DS is in the range of 0.6-0.95 for food applications [30]. One of the important characteristics of the CMC is that it can be dissolved in both hot and cold water and has certain viscosity. CMC is a polar adhesive and as such may allow the formation of complex with proteins such as caseins at, or around, the isoelectric region of the protein.

It was indicated by Du et al. (2007) that electro-sorption of CMC onto casein micelles took place below pH 5.2. At pH 6.7, there was no interaction between caseins and CMC due to charge repulsion and mixtures of casein and CMC were stable at low CMC concentrations. Above a certain CMC concentration, flocculation occurred leading to phase separation. Below pH 5.2 CMC adsorbed onto casein micelles. In the case of low CMC concentrations, CMC/casein micelles mixture was phase separated via bridging flocculation. With increasing CMC concentrations, the casein micelles were effectively coated and consequently sterically stabilized. In addition, the non-adsorbed CMC increases the viscosity of serum and slows down the sedimentation of casein particles. The adsorbed CMC layer causes a repulsive interaction between the casein micelles at low pH in the same way as κ -caseins do at neutral pH. This phenomenon is related to the interaction between protein (mainly casein micelles) and CMC.

The influence of CMC's molecular weight (M_w) and DS on the stability of AMD has been also investigated by Du et al. (2009). Both M_w and DS of CMC affected the interaction between CMC and casein micelles and thus the stability of acidified milk drinks. The amount of CMC needed for effective coverage onto casein micelles increased with increasing the M_w of CMC. It was found that the high M_w CMC or low DS CMC led to a thick adsorbed layer onto casein micelles. However, this thick layer was not directly related to the long-term stability of acidified milk drinks, because the stability of acidified milk drinks was also related to the net charges of casein micelles, the particle size of casein micelles, and the viscosity of the system. Acidified milk drinks with high M_w CMC showed good stability due to both the thick adsorbed layer and the high viscosity of the system. The absolute value of charge of CMC-coated casein micelles increased when the DS of CMC was high enough to 1.2 compared with CMC with DS of 0.7 and 0.9. The high net charge contributed to the stability of the system by electrostatic repulsion.

The best results to stabilize AMD with approximately 4% MSNF were achieved by application of 0.4% CMC which was of 700.000 Dalton M_w and DS of 1.2 [29, 30].

E. Gellan

Gum Gellan is an extracellular polysaccharide produced by *Sphingomonas elodea* (previously identified as *Pseudomonas elodea*, but later reclassified). In structure of this microbial stabilizer, a linear tetrasaccharide sequence of $\rightarrow 3)\text{-}\beta\text{-D-Glcp-(1} \rightarrow 4)\text{-}\beta\text{-D-GlcpA-(1} \rightarrow 4)\text{-}\beta\text{-DGlc-(1} \rightarrow 4)\text{-}\alpha\text{-L-Rhap-(1} \rightarrow$, is repeated containing one carboxyl side group. In aqueous solution at high temperatures gellan polymers are in a disordered single-coiled state. By cooling of gellan solutions, the polysaccharide chains form threefold left-handed double helices (ordered state) stabilized by internal hydrogen bonding [31]. Coil-helix conformational transition occurs in a temperature range from 30 to 50 °C, depending on the ionic strength of the solution. The gelation process depends on the type of cation, ionic strength, temperature and polymer concentration [32].

Application of gellan in order to Doogh stabilization was investigated by Kiani et al. (2010). Serum separation in sample containing highest gellan concentration (0.05 wt %) was 12.5 % after 15 days storage in 5 °C. Rapid development of syneresis was observed, with little further separation at longer times up to 1 month. Furthermore, incorporation of gellan resulted in dramatic increase in particle size more than 10-fold which can be attributed to increase in the number of stable electrostatic associations between gellan and acid-casein fragments. Ultimately, three types of junction in samples of Doogh with added gellan were envisaged: a) association of acid-casein particles, b) gellan-casein associations, giving bridging between the casein particles, and c) tenuous association of gellan helices, as in weak gels of gellan, allowing the coupled network to flow in response to small stresses, with overall resistance to flow (viscosity) and network concentration (syneresis) increasing with increasing concentration of gellan up to 0.05 wt %.

IV. EXOPOLYSACCHARIDES

Exopolysaccharides or extracellular polysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented dairy products such as yoghurt and drinking yoghurt. They are produced in situ by the LAB-starters that have General Recognized As Safe status (GRAS). EPSs may act both as texturizers and stabilizers, firstly increasing the viscosity of a final product, and secondly by binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network. As a consequence EPS can decrease syneresis and improve product stability [33].

Produced exopolysaccharides by LAB bacteria can be divided into two groups: a) homopolysaccharides composed of one monosaccharide moiety such as dextran produced by *Leuconostoc mesenteroids* (6.2 to 7.1×10^6 Da), levan produced by several strains of *Streptococcus mutans* (2.7 - 21.6×10^6 Da) and b) heteropolysaccharides

composed of different sugar moieties, e.g. glucose, galactose, rhamnose, mannose, N-acetyl glucosamine, N-acetylgalactosamine, glucuronic acid. EPS often differ by monosaccharide composition, charge, linkage between units (and consequently the rigidity of the molecule), presence of repeated side chains [34].

EPS producing cultures are also known as ropy bacteria and have been successfully used for the manufacture of Nordic ropy milk. Scandinavian fermented milk drinks display a firm thick, slimy consistency and these rely on the souring capacity of mesophilic ropy strains of *Lactococcus lactis* subsp. *Lactis* and ssp. *cremoris* and concomitant production of heterotype EPS for texture [35]. It has been determined that Dahi prepared using EPS culture had better body and texture and exhibited little syneresis accompanied by significantly improving in rheological properties [36]. However, it should be noted that not all types of EPS-producing starter cultures increase viscosity or decrease serum separation. The amount, chemical structure, and degree of interaction with milk proteins of EPS play important roles. In Ayran (Turkish fermented milk drink containing salt), EPS producing bacteria have not given satisfactory results as they do in other fermented products. This can be explained by the reduction in protein contents and interactions arising from dilutions, mechanical applications (pumping, etc) after fermentation and salt addition which reduce protein-water interactions [37].

EPS can either be attached to the bacterial cells as capsules or excreted as unattached materials into the growth medium. Both types of EPS can be produced by the same bacterial cell. The presence of associative interactions between the EPS and milk proteins is still under debate. Part of the problem was the unavailability of a suitable microscopy technique to observe fully hydrated samples. With conventional SEM, the EPS appeared as thin filaments attached to the protein network and bacterial cells. In most of the published images, EPS unattached to bacterial cells was not observed [38]. The study of EPS during structure formation of a fermented product is particularly challenging because of the dynamics of change of polymer concentration, casein micelles charge, destabilization conditions and ionic equilibrium. Therefore, while at pH near neutral phase separation may be induced by EPS, at acidic pH interactions may occur with negatively charged polysaccharides [39]. Microstructural studies on fermented milk gels have shown networks with large interstitial cavities filled with bacterial colonies surrounded by polysaccharide [40].

The interactions between milk proteins and EPS have been studied using scanning electron microscopy [41]. Fig. 1 is a micrograph taken at pH 4.6 for a whey protein suspension fermented with *Lactococcus lactis*. The EPS is clearly present with the proteins and appears as filament strands attached to the protein aggregates as well as to the

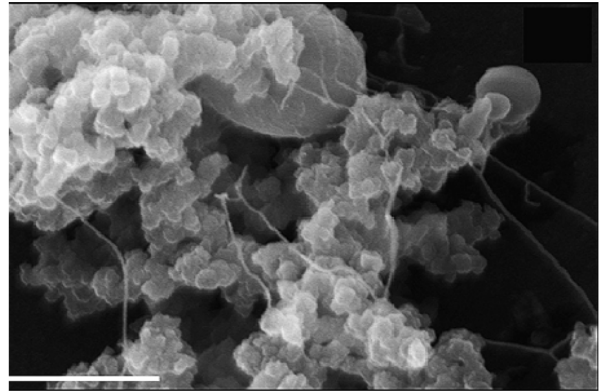


Figure 2. Field emission scanning electron micrograph prepared using a self assembled monolayer technique to covalently bind the proteins to the gold support, and then wash any material not attached to the proteins. Image was fixed at pH 4.5, after fermentation of milk serum permeate containing 6% whey protein isolate. Scale bar is 600 nm [41].

bacteria cells. Therefore, although it is possible to hypothesize that although once the protein network has formed around the bacteria, the EPS will reside in separated pockets within the gel, interactions with the milk proteins may also play a major role in the structuring behaviour of EPS.

V. PROTEIN COMPOSITION

Whey protein concentrate (WPC) are used in order to improve the physical properties and enhance nutritional value of fermented milk products [42]. Physical improvement of these types of beverages by WPC has been examined recently [43]. Reconstituted skim milk samples with 6 wt% and additional WPC ranged from 0.5 to 3 % were prepared followed by heat treatment. WPC addition caused an increase in the consistency coefficient and thixotropy and decrease in the particle size of samples. There was no serum separation in the sample with 2% WPC. Highest instability was observed in the sample with 3% WPC. Caseins are found essential for structure formation and whey proteins are found to support this structure up to a level of 2% WPC addition which can be attributed to more casein–whey proteins and whey proteins–whey proteins interactions. However, above this level, whey proteins weaken the structure due to a reduction in casein–whey protein ratio and increase the aggregation of denatured whey proteins with casein and with each other.

VI. HOMOGENIZATION

Size reduction of milk fat globules is performed by homogenization to a diameter of less than 1 μm and 4-10 fold increase in surface area of globules is occurred. But for acidified milk drinks it is done to reduce the size of the clusters, make the particles homogenous and improve the organoleptic properties of the product. Industrial homogenization pressure is about 100 bars. It could be prospected that higher pressure may affect the stability,

viscosity and particle characteristics. This hypothesis has been examined by Kiani et al. (2008) at applied pressures more than 100 bars for homogenization of Doogh samples.

There were no significant changes in apparent viscosity by raising the pressure up to 300. However, size of particle decreased a little. Furthermore, obtained data from volumetric measurement of phase separation indicated that homogenization did not have significant effects on the serum volume of Doogh. Ultimately, increasing of homogenization pressure up to 100 has no significant influence on the rheological properties and so quality improvement of Doogh samples with 6 wt % total solids [3].

VII. CONCLUSION

Main textural defect in acidified milk drinks is serum separation during storage. When the pH lowers below 5 irreversible aggregation of casein micelles occurs and creates a three-dimensional network constituted by the cluster of aggregates strands. Using hydrocolloids is one of the most powerful techniques to prevent serum separation in acidified milk beverages. It was shown that both adsorbing and non-adsorbing hydrocolloids are capable of stabilizing the system.

Exopolysaccharides synthesized by lactic acid bacteria may act both as texturizers and stabilizers through increasing the viscosity of a final product and binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network resulting in improving product stability. Whey protein concentration addition to fermented milk drink was found beneficial for improving physical properties. However, homogenization did not affect the viscosity by raising the pressure.

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