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ORIGINAL PAPER

Combination effect of sodium carboxymethyl cellulose and soybean soluble polysaccharides on stability of acidified skimmed milk drinks

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Abstract Sodium carboxymethyl cellulose (CMC) and soybean soluble polysaccharide (SSPS) are negatively charged polysaccharides that are used to prevent casein aggregation in acidified skimmed milk drinks (ASMDs). The objective of this study was to examine the effect of CMC and SSPS combination on the stability of acidified skimmed milk drinks in comparison to their individual effects. Stability was evaluated based on the changes in sedimentation ratio, viscosity, zeta potential, and particle size distribution of casein micelles. Increased CMC or SSPS concentration resulted in decreased sedimentation ratio and size diameter, while viscosity and zeta potential increased. The use of CMC-SSPS blend at the ratio of 1:3 showed better stability of ASMDs compared to when the two stabilizers were used individually. Sedimentation ratio and size diameter obtained from the ratio of 1:3 (CMC-SSPS) had no clear significant difference ($P<0.05$) with those stabilized by the ratio of 0:4, but CMC was crucial in controlling the blend viscosity and zeta potential, hence improving stability. Furthermore, SSPS showed a better stabilization behavior than CMC when they are used alone. It can therefore be suggested that a combination of CMC-SSPS is more effective than the individual use of the two stabilizers in stabilizing ASMDs, and thus, CMC can effectively supplement SSPS in the production of stable ASMDs.

Keywords Stability · Acidified milk · Casein · Sodium carboxymethyl cellulose · Soybean soluble polysaccharide

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1 Introduction

Milk is an emulsion of fat, water, lactose, and a mixture of proteins (casein and whey proteins) with important minerals (Le Tran et al. 2008). These components contribute immensely to its stability at neutral pH. Acidified skimmed milk drinks (ASMDs) are beverages and yogurt drinks generally composed of an acid dairy phase (fermented base) or a neutral base (fresh milk) with an acidic medium (Nakamura et al. 2006; Du et al. 2009; Wu et al. 2013). They usually have pH values in the range from 3.0 to 4.6.

Casein micelles in flesh milk (pH 6.7) are in stable state due to steric repulsive interactions between the micelles (Dalgleish 2011). However, when milk is acidified to pH below the isoelectric point (p*I*) either by lactic acid bacteria or direct acidification, the casein micelles lose their native stability and aggregate. Consequently, sediments and precipitates are condensed on the bottom of a container as a result of collapse of the extended conformation of κ-casein (de Kruif 1998; Tuinier et al. 2002).

High negatively charged polysaccharides such as high methoxyl pectin (HMP), propylene glycol alginate (PGA), soybean soluble polysaccharide (SSPS), and sodium carboxymethyl cellulose (CMC) can be used as stabilizers to prevent aggregation and sedimentation of casein and to control product properties like texture, viscosity, and mouth feel. The most popularly used polysaccharide is pectin (Jensen et al. 2010; Laurent and Boulenguer 2003). The interaction mechanism of pectin with casein micelles in ASMDs has been previously investigated in a number of research works (Sejersen et al. 2007; Jensen et al. 2010). However, CMC is often used because of its cost effectiveness compared to pectin and other polysaccharides (Du et al. 2007, 2009). CMC has been used as a stabilizer and thickening or gelling agent in dairy products, but its concentration levels have to be carefully controlled, since the properties of the final products depend on how it interacts with casein micelles in acidified milk systems (Yu et al. 2004).

SSPS is an acidic polysaccharide containing 18% galacturonic acid. Its main polysaccharide backbone is made of galacturonan and rhamnogalacturonan, organized in diglycosyl repeating units, (1-4)-α-D-Gal A-(1-2)-α-L-Rha-(1-4) (Nakamura et al. 2003), a structure pettern similar to pectin (Sejersen et al. 2007).

The stabilizing behavior of SSPS in acidified milk drinks has been previously investigated by Nakamura et al. (2012) and Nobuhara et al. (2013) and reported that SSPS stabilizes acidified milk drinks by steric stabilization effect due to the adsorbed SSPS layer. However, the stability of acidified milk drinks induced by SSPS is different from that induced by other carbohydrates because of the thick layer of neutral sugar side chains of SSPS on the surface of the protein particles (Nakamura et al. 2003).

The combination of two or more carbohydrates in food formulation is made to bring new products on the market and to improve rheological characteristics of the product, which possibly may decrease manufacturing cost (BahramParvar and Razavi 2012). Several factors such as molecular weight, viscosity, mineral composition in the sample, polysaccharide-polysaccharide blending ratio, total polysaccharide concentration, temperature, and pH may affect the stability in mixed polysaccharides systems (Abedi et al. 2012).

Although acidified milk products are extensively produced, many research works have concentrated on the individual use of either CMC or SSPS, and research work where two or more polysaccharides were combined to stabilize skimmed acidified milk drinks is still limited. Therefore, the objective of this study was to examine the effect of combining CMS and SSPS on the stability of acidified skimmed milk drinks in comparison to their individual effects.

2 Material and methods

2.1 Materials

Skim milk powder with 54.1% lactose, 33.4% protein, 7.9% mineral, 3.8% moisture, and 0.8% fat was purchased from Fonterra Co. Ltd., New Zealand. SSPS (molecular weight 421 kg.mol^{-1}, radius of gyration 32.5) was purchased from Tianjing Plant Protein Co.. Ltd, Henan, China. CMC (molecular weight 700 kg.mol^{-1}, degree of substitution 0.8–0.95) was purchased from Sigma-Aldrich, Shanghai, China. Other analytical grade reagents were obtained from Shanghai Chemical Reagent Co. Ltd, China. Milli-Q water was used in the preparation of all solutions.

2.2 Production of acidified skimmed milk drinks

Samples were prepared on pilot plant as described by Du et al. (2007) with small modifications. Low heat skim milk powder was reconstituted with warm water at 45 °C to 200 g.L^{-1} total solids while agitating for 20 min. Meanwhile, CMC or SSPS concentrations in the range of 2–6 g.L^{-1} were mixed with 100 g.L^{-1} sucrose. The mixture of stabilizer and sucrose was dissolved into hot water (75 °C) and agitated with magnetic stirrer for 30 min. The reconstituted milk and stabilizer solution were then mixed and agitated for another 10 min. The final solution was adjusted to 85 g.L^{-1} milk solids nonfat (MSNF) and directly acidified with 100 g.L^{-1} citric acid solution to pH 4.0, homogenized at 10 MPa with first-stage and 20 MPa with second-stage value Rannie type 8.30H homogenizer (APV Rannie A/S, Denmark), pasteurized at 80 °C for 15 min and stored at 4 °C.

2.3 Stabilization of acidified milk drinks with a mixture of CMC and SSPS

The effect of CMC-SSPS mixture on the stability of acidified milk drinks was determined by combining CMC and SSPS with maximum concentration of 4 g.L^{-1}. To make 85 g.L^{-1} MSNF, polysaccharide ratios of 4:0, 3:1, 1:1, 1:3, and 0:4 (CMC-SSPS) were mixed with 200 g.L^{-1} of the skimmed milk solution. The samples were then adjusted to pH 4.0 homogenized with two passes at 20 MPa with two-stage value Rannie type 8.30H homogenizer (APV Rannie A/S, Denmark) and pasteurized at 80 °C for 15 min (Liu et al. 2006).

2.4 Measurement of sedimentation ratio

The effect of CMC or SSPS or a combination of CMC-SSPS concentration on sedimentation ratio was evaluated by weighing the sediments of the samples and expressed as percentage of the total sample weight. Milk samples were homogenized at 20 MPa and small portion of 15 g was put in plastic tubes of 50 mL and centrifuged at 4,500 rpm for 15 min at 4 °C (Hettich centrifuge, Werk, Germany). The clear supernatant was carefully poured from the sediments and the tubes were left upside down for 5 min. The sedimentation ratio (%) was calculated from the weight of the pellets to the sample weight times a hundred. All measurements were performed three times (Laurent and Boulenguer 2003).

2.5 Measurement of viscosity

The apparent viscosity of acidified milk drinks stabilized by CMC or SSPS and a combination of the two polysaccharides was measured immediately after sample preparation by a Brookfield viscometer (Brookfield DV-II+, Brookfield Inc., Middleboro, MA, USA) equipped with LV spindles. The rotation speed was set at 60 rpm and 20 °C of temperature. The viscosity measurement was expressed in millipascal per second. An average of three individual measurements was taken for data analysis (Jensen et al. 2010).

2.6 Measurement of particle size distribution

The particle size distribution and size diameter of the casein micelles in ASMDs stabilized by CMC or SSPS and a combination of the two carbohydrates were measured using Zetasizer Nano ZS, ZEN 3600 (Malvern Instruments, Worcestershire, UK) with a relative refractive index of particles of 0.01 and a refractive index of dispersant of 1.56. The changes in particle size were evaluated by checking either the shape of the particle size distribution or the average droplet size reported as equivalent volume mean particle diameter $D(4,3)=\sum_i n_i d_i^4/\sum_i n_i d_i^3$, where n_i is the number of droplets of diameter d_i. Droplet size distribution measurements were performed after 24 h of storage at refrigeration temperature (4 °C). All measurements were performed three times (Liu et al. 2006).

2.7 Measurement of zeta potential

The zeta potential of casein micelles was determined using Zetasizer Nano ZS, ZEN 3600 (Malvern Instruments, Worcestershire, UK). Measurements were taken after diluting the milk sample 100 times in citrate phosphate buffer at pH 4.0. The zeta potential was determined by measuring the direction and velocity of droplet movement in a well-defined electric field. All measurements were performed three times with freshly prepared samples, and the zeta potential measurements were reported as the mean and standard deviation of three separate samples (Du et al. 2009).

2.8 Data analysis

Data were analyzed using Statistical Package for Social Sciences for Windows 19 (SPSS, Inc. Chicago, USA). All experiments were conducted three times and presented as mean ± standard deviation ($n=3$). Analysis of variance was used to compare means with Duncan multiple range tests for post hoc analysis. Mean values were considered statistically significant at $P<0.05$ and $P<0.01$.

3 Results and discussion

3.1 Effect of stabilizer concentration on sedimentation ratio

Measurements of sedimentation as a function of CMC and SSPS concentration (2–6 g.L^{-1}) were taken at pH 4.0. As shown in Fig. 1a, the sedimentation ratio changed between treatments and decreased significantly ($P<0.01$) as the concentration of the stabilizer increased. Higher amounts of sediments were found in samples containing 2 and 3 g.L^{-1} CMC as well as in SSPS containing samples. This showed that there were not enough stabilizers to cover all the micelle particles which resulted in increased sediments. There was no significant difference ($P<0.05$) in sedimentation ratio among samples with concentrations from 4 to 6 g.L^{-1} in both CMC and SSPS, but there was a clear significant difference ($P<0.05$) in the samples stabilized with CMC compared to those stabilized with SSPS. The reason could be explained by the stickiness of the suspended material causing the dispersed particles to adhere to each other during mixing of the sample. It could be also due to the original positive charge of casein in contact with the adsorbed CMC or SSPS to form a balance charge after interaction (Du et al. 2007). These results are in agreement with the findings of Wu et al. (2013) who reported a sedimentation ratio of 2.71±0.14% when stabilizing whole milk drinks with 4 g.L^{-1} CMC concentration.

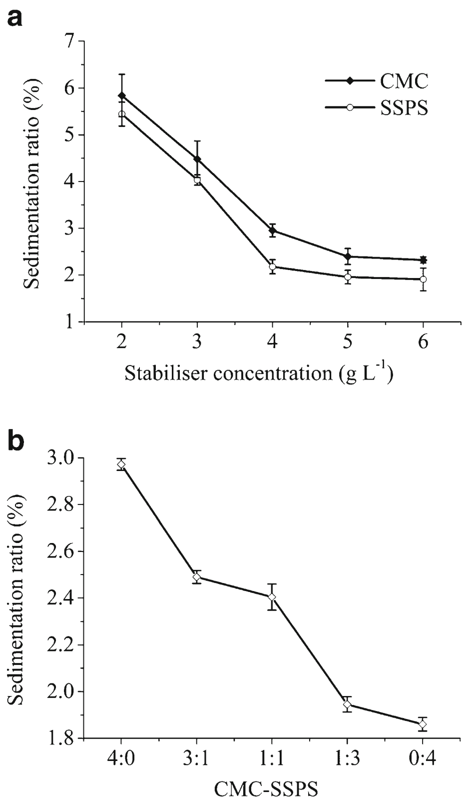

Fig. 1 Sedimentation ratio of acidified skimmed milk drinks at pH=4.0 as a function of CMC or SSPS concentration (**a**) and combination of CMC and SSPS (**b**)

The sedimentation ratio resulting from acidified milk drinks stabilized by a combination of CMC and SSPS is presented in Fig. 1b. The results showed that sedimentation ratio ranged between 1.86±0.28 and 2.97±0.15%. There were no clear aggregates in all the samples because the stabilizer was sufficient to cover casein micelles at all surfaces. Additionally, the stability of acidified milk drinks was related not only to the stability of casein micelles themselves, but also to the viscosity of the serum as reported by Du et al. (2009). Contrary, these results oppose the findings of Liu et al. (2006) who reported that when SSPS is added to pectin, the samples compete with each other for the adsorption on the surface of casein micelles, and they concluded that the samples cannot be mixed in ASMDs.

3.2 Effect of stabilizer concentration on viscosity

At ambient temperature of 20 °C, milk and skim milk drink exhibit Newtonian behavior where the viscosity is not dependent on the shear rate (Raikos 2010). The viscosity of ASMDs stabilized with different concentrations of CMC or SSPS measured in a shear rate of 60 rpm is presented in Fig. 2a. When the concentration of CMC or SSPS increased from 3 to 6 g.L^{-1}, the viscosity of the samples increased from 2.57±0.28 to 5.53±0.37 for CMC and 1.67±0.08 to 1.91±0.01 for SSPS. From Fig. 2a, ASMDs showed a higher viscosity (3.73±0.17 mPa.s) on the sample with SSPS concentration of 2 g.L^{-1}. This abnormal increase is related to the small aggregation

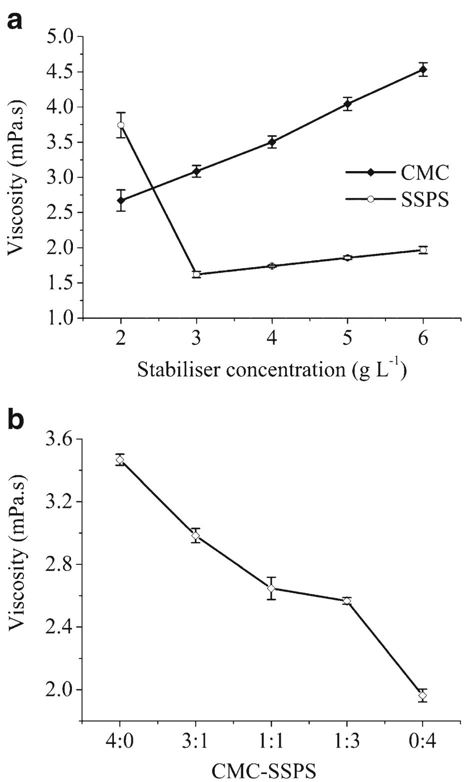

Fig. 2 Apparent viscosity of acidified skimmed milk drinks at pH=4.0 as a function of CMC or SSPS concentration (**a**) and combination of CMC and SSPS (**b**)

of casein micelles within the sample as this concentration was not enough to fully adsorb on casein micelles (Tuinier et al. 2002; Ion Titapiccolo et al. 2011). The viscosity of the samples stabilized by SSPS showed no significant increase ($P<0.05$) in all treatments contrary to the viscosity of the samples stabilized with CMC where the viscosity increased with increased concentration of CMC in the system.

With the stabilizer concentration higher than 4 g.L^{-1}, CMC resulted in higher viscous products that may not be used for commercial ASMDs. At the same concentration, samples stabilized by SSPS showed better viscosity and thus better stability. However, SSPS is expensive and need to be supplemented with CMC. The findings were supported by Du et al. (2009) who reported that when CMC is enough to cover the casein micelles, the nonadsorbed CMC increased the viscosity of acidified milk drinks and thus contributed to the stability of acidified milk drinks.

Figure 2b shows the change in the viscosity of ASMDs stabilized with different ratios of CMC-SSPS combination. The ASMDs stabilized with SSPS alone resulted in the lowest viscosity (1.96±0.1 mPa.s), while the highest was 3.5±0.14 mPa.s for sample CMC alone (4:0). This shows that the viscosity of ASMDs stabilized by CMC was higher ($P<0.05$) than that of ASMDs stabilized with SSPS. Furthermore, results for the ASMDs stabilized by the CMC-SSPS blend 1:3 (2.56±0.13 mPa.s) were not significantly different ($P<0.05$) from the results obtained from CMC-SSPS in the ratio of 0:4 (Fig. 2b). Since the viscosity of CMC is higher than that of SSPS, samples stabilized with CMC-SSPS significantly ($P<0.05$) showed difference among all treatments, with increased viscosity in the samples containing a high amount of CMC in the ratio. These results have shown that the viscosity of CMC-stabilized ASMDs was higher ($P<0.05$) than that of SSPS-stabilized ASMDs. Furthermore, results for the ASMDs stabilized by the CMC-SSPS mixture in the ratio of 1:3 (2.56±0.13 mPa.s) were not significantly different ($P<0.05$) from the results obtained when SPSS-CMC was in the ratio of 0:4, which concurred with data from other variables such as sedimentation ratio. The viscosity at this ratio was also close to the viscosity of normal fresh milk (2.64±0.21 mPa.s) reported by Fava et al. (2013). Similar results were reported by Nilsson et al. (2007) and Burgardt et al. (2013) when they were studying the viscosity of milk as a function of temperature and stabilizers. Therefore, the results have shown that the combination of 1 g.L^{-1} CMC and 3 g.L^{-1} SSPS can be used in acidified skimmed milk drinks to effectively prevent aggregation and increase the viscosity of the product.

3.3 Effect of stabilizer concentration on zeta potential

Zeta potential has been used as an indicator of the electrical charge of milk fat globules (Wade and Beattie 1997) and of casein micelle particles (Anema et al. 2004; Considine et al. 2011). Since the electric repulsive force plays an important role in preventing the aggregation of protein, the zeta potential of casein micelles stabilized with CMC, SSPS, and a combination of CMC-SSPS was determined as shown in Fig. 3a, b. The data obtained revealed that the zeta potential of particles stabilized by CMC concentration in the range of 2–6 g.L^{-1} was from −22.1±0.4 to −33.8±0.4 mV, indicating the adsorption of CMC molecules on the surface of the positively charged casein micelles and from −3.4±0.2 to −12.5±0.3 mV for particles stabilized with SSPS with the same concentration. As these polysaccharides are negatively charged, the negative zeta

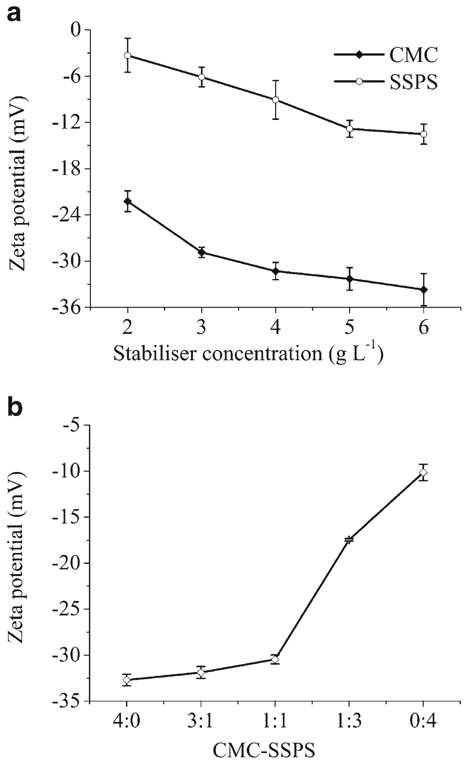

Fig. 3 Zeta potential of acidified skimmed milk drinks at pH=4.0 as a function of CMC or SSPS concentration (**a**) and combination of CMC and SSPS (**b**)

potential increased with an increase of their concentration in the system. There was no significant difference ($P<0.05$) in zeta potential within the sample stabilized with 4–6 g.L^{-1} CMC or SSPS concentration. However, there is a significant difference ($P<0.05$) between samples stabilized with concentrations of 3 and 4 g.L^{-1} in both CMC and SSPS.

The zeta potential of samples stabilized with CMC was higher ($P<0.05$) compared to those of SSPS, an indication that the electrostatic repulsive forces of protein particles stabilized with CMC are stronger compared to those covered with SSPS. The reason of this decrease could be that the negative zeta potential of SSPS is neutralized by the positive highly charged casein micelles. The small zeta potential of ASMDs containing SSPS indicates that their steric repulsive forces are weak compared to those ASMDs containing CMC.

A similar comparative study was conducted by Du et al. (2009) for acidified milk drinks stabilized by two types of carboxymethylcellulose with high and low molecular weights and the authors concluded that higher molecular weight contributed to the stability of acidified milk drinks. Furthermore, Nakamura et al. (2012) reported that the zeta potentials were significantly different in SSPS-HMW molecules suspended in aqueous solution and those of SSPS-LMW molecules, indicating that the molecular weight contributed to the stabilization effect of polysaccharides.

The results from the combination of the two stabilizers as shown in Fig. 3b demonstrated that the addition of CMC to SSPS increased the zeta potential of casein micelles. The zeta potential obtained from the combination of CMC-SSPS showed significant differences ($P<0.05$) among treatments. However, the zeta potential corresponding to the ratio 1:3 (CMC-SSPS) was −17.46±0.126, which was higher compared to that of SSPS alone. This observation clearly showed that the CMC-SSPS ratio of 1:3 was the best combination as evidenced by more stable products compared to the results when the stabilizers were used individually.

3.4 Effect of stabilizer concentration on particle size distribution

The results of size distribution of casein micelles of ASMDs coated with CMC or SSPS are presented in Fig. 4a–e. ASMDs without stabilizer or with a concentration of 1 $g.L^{-1}$ resulted in the formation of aggregates before the required pH 4.0 and showed multimodal distribution of particle size (data not presented as the samples were not suitable for analysis with Zetasizer Nano ZS machine). The small protein aggregation was observed in the sample with a concentration of 2 $g.L^{-1}$ of CMC and SSPS and

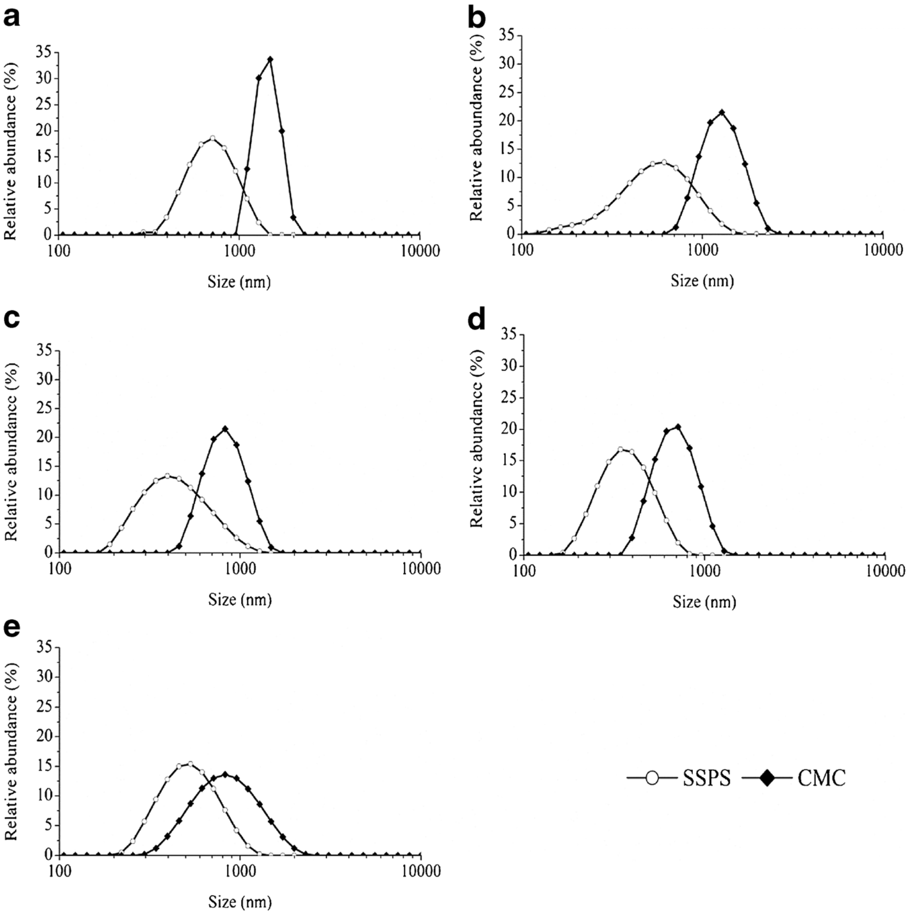

Fig. 4 Particle size distribution of acidified skimmed milk drinks as a function of stabilizers concentration at pH 4.0. **a** 2 $g.L^{-1}$, **b** 3 $g.L^{-1}$, **c** 4 $g.L^{-1}$, **d** 5 $g.L^{-1}$, and **e** 6 $g.L^{-1}$

resulted in increased size diameter where the maximum peak appeared at 1,581 nm for CMC and 946 nm for SSPS (Fig. 4a). On the hand, samples stabilized with 4–6 g.L^{-1} of CMC or SSPS concentration showed a stable system characterized by monomodal distribution (Fig. 4c, d). The stabilizing behavior of CMC is quite different to that of SSPS as the size diameter of CMC was larger than that of SSPS (Fig. 4a–e). This difference could be caused by the weak electrostatic interaction of CMC compared to SSPS at low pH (Du et al. 2007; Nakamura et al. 2006). From these results, it is evident that ASMDs needed a high concentration of CMC or SSPS to produce a stable system with monomodal distribution and minimum size diameter (<1 μm). In a similar study, it was reported that 4 g.L^{-1} CMC added to 85 g.L^{-1} of milk solid resulted in a stable system when acidified at pH 4.0 (Du et al. 2009).

It was further observed that below the 4-g.L^{-1} concentration, samples showed small aggregates which might be the cause of increased size diameter because the stabilizer concentration was not enough to cover all positive charges of casein micelles (Maroziene and de Kruif 2000). Similar results were reported by Roudsari et al. (2006) when stabilizing soy protein isolate emulsion with HMP and SSPS. These results suggested that ASMDs with 4–6 g.L^{-1} CMC or SSPS resulted in complete coverage of the positively charged casein micelles with negatively charged CMC or SSPS molecules and, therefore, prevented casein aggregation.

3.5 Size distribution of milk dispersion with the combination of CMC and SSPS

The size distribution in ASMDs stabilized by CMC or SSPS alone showed that 4 g.L^{-1} concentration was enough to stabilize ASMDs. This concentration was therefore used in the combination of CMC and SSPS as the maximum total concentration of the two stabilizer blend.

The sample prepared with CMC-SSPS combination showed a monomodal distribution of size and smaller average particle size compared with dispersions containing CMC alone (Fig. 5). In addition, these results indicated that size diameter and distribution were smaller (≤1 μm) in all formulations and decreased with the increase of SSPS concentration in the mixture. The size diameter significantly decreased (P<0.05) as the concentration of SSPS decreased in the CMC-SSPS blend. The stabilization difference of the two polysaccharides could be attributed to their differences in charge, neutral sugar side chains (Nakamura et al. 2004; Roudsari et al. 2006).

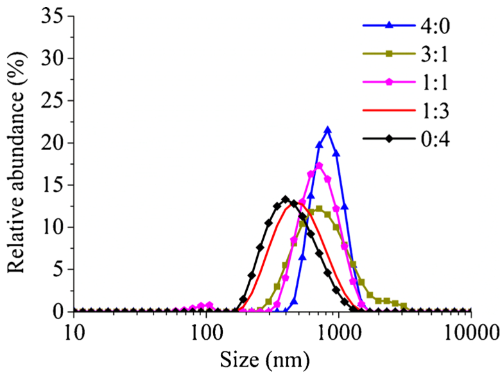

Fig. 5 Particle size distribution of acidified skimmed milk drinks at pH=4.0 as a function CMC-SSPS combination. The total concentration of stabilizer was 4 g.L^{-1}

Only the sample with the ratio of 1:1 showed double distribution where one has 98% with a particle diameter of 789.1±12.6 and the other 2% with 98.7 nm. As large particles scatter more lightly than small particles, thus the small peak could be caused by the small particles of casein which were not reacted with stabilizers. These results were in good agreement with the findings of Abedi et al. (2012) who found that different hydrocolloids can be mixed to stabilize juice milk drinks, but are contrary to the findings of Liu et al. (2006) who reported that SSPS and pectin cannot be mixed in acidified milk dispersion because one can compete with the other. This behavior difference of the two polysaccharides might be attributed to the difference in the balance between electrostatic interactions of CMC and casein micelles to that of SSPS and casein micelles (Roudsari et al. 2006; Nakamura et al. 2004). These results were supported by sedimentation and viscosity as shown in Figs. 1b and 2b. The mixture of 1 $g.L^{-1}$ CMC and 3 $g.L^{-1}$ SSPS produced the best stable milk dispersion with monomodal size distribution and small particle size diameter.

In the production of ASMDs, sedimentation ratio, viscosity, zeta potential, and particle size are the key characteristics as they contribute to the physical stability and rheological properties of the finished products. The perceived quality of these beverages is strongly influenced by their stability, rheology, and appearance. Thus, sedimentation ratio plays a predominant role in deciding the stability of ASMDs as it contributes to the physical stability and organoleptic properties of the final products. Production of ASMDs with a uniform small droplet size was achieved, confirming the results from other variables like sedimentation ratio and viscosity.

The increased concentration of stabilizers resulted in decreased sedimentation ratio and particle size diameter, while on the other hand, zeta potential and viscosity increased significantly. Furthermore, the increased combined use of the stabilizers has shown to significantly improve viscosity and zeta potential as compared to when they are used individually which consequently implies better stability. Furthermore, the results have shown that beyond certain concentrations of the stabilizers, stability would not be greatly achieved and the combined ratio of 1:3 for CMC-SSPS optimally produced better stability through the improvement of sedimentation ratio, viscosity, zeta potential, and particle size. This interaction is beneficial to dairy processors who want to formulate ASMD products with desirable appearances and have milk-like characteristic. More considerations should be taken on the relationship of their organoleptic properties and microstructure properties.

4 Conclusion

The combination of CMC and SSPS (CMC-SSPS) improved the stability of acidified skimmed milk drinks as compared with CMC and SSPS alone. The stability of acidified skimmed milk drinks can be influenced by the type as well as the concentration of the stabilizers used. The average particle size of ASMDs from CMC-SSPS was smaller than the size of dispersions containing CMC alone and higher than the size of dispersions with SSPS alone. These results were confirmed by reduced sedimentation ratio, increased viscosity, and high zeta potential in CMC-SSPS. A combination of CMC and SSPS in the ratio of 1:3 (CMC-SSPS) improved stability by reducing the sedimentation ratio and particle size with improved viscosity and zeta potential as compared to a single stabilizer and, hence, could potentially be used to acidify skimmed milk drinks.

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