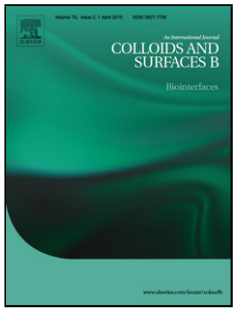

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Synergistic effect of casein glycomacropeptide on sodium caseinate foaming properties

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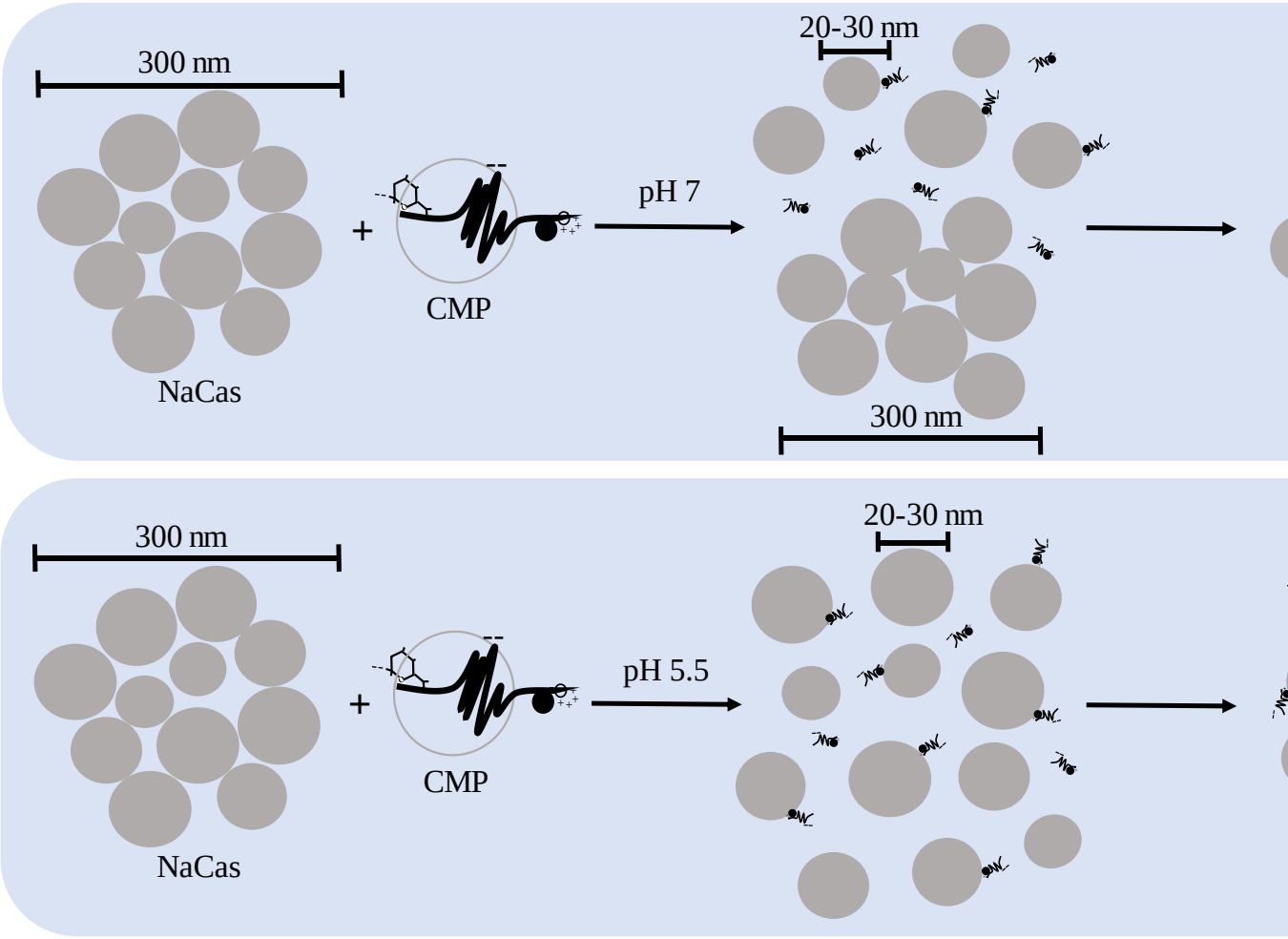
Graphical Abstract

Scheme of the model proposed to explain the improvement of foaming properties by mixing NaCas and CMP.



Highlights

- CMP induce a decrease in the aggregation state of NaCas.
- A reduction of foam drainage by mixing CMP and NaCas is observed.
- A model to explain the synergistic effect observed in mixed foams is proposed.

Abstract

Several strategies to improve the interfacial properties and foaming properties of proteins may be developed; among them, the use of mixtures of biopolymers that exhibit synergistic interactions. The aim of the present work was to evaluate the effect of casein glycomacropeptide (CMP) on foaming and surface properties of sodium caseinate

(NaCas) and to establish the role of protein interactions in the aqueous phase. To this end particles size, interfacial and foaming properties of CMP, NaCas and NaCas-CMP mixtures at pH 5.5 and 7 were determined. At both pH, the interaction between CMP and NaCas induced a decrease in the aggregation state of NaCas. Single CMP foams showed the highest and NaCas the lowest foam overrun (FO) and the mixture exhibited intermediate values. CMP foam quickly drained. The drainage profile of mixed foams was closer to NaCas foams; at pH 5.5, mixed foams drained even slower than NaCas foam, exhibiting a synergistic performance. Additionally, a strong synergism was observed on the collapse of mixed foams at pH 5.5. Finally, a model to explain the synergistic effect observed on foaming properties in CMP-NaCas mixtures has been proposed; the reduced aggregation state of NaCas in the presence of CMP, made it more efficient for foam stabilization.

Keywords: casein glycomacropeptide; sodium caseinate; interactions; interfacial properties; foams

Statistical summary

Total number of words (without references): 4942

Total number of figures/tables: 8

1. Introduction

Most of the food owe their characteristic texture to the existence of a foamed structure stabilized by the presence of adsorbed proteins at the air/water interface[1]. The interfacial behaviour of proteins determines the formation and stability of interfacial films, which contribute to the structure and the sensory qualities and the perceived by the consumer.

Milk proteins are excellent foaming and emulsifying agents commonly used in food products. Some studies indicate that casein micelles, which account for by 80% of milk

proteins, play an important role in the stability of milk foams[2,3]. Caseinates, the water-soluble form of casein, consist of a mixture of four individual fractions, the α_{s1}, α_{s2}, β and κ caseins. β and α_{s1} are the most abundant fractions and so they govern the functionality of caseinates; in particular, sodium caseinate (NaCas) is absorbed to the air-water interface similarly to the β fraction, which shows the highest surface activity among the four fractions [4,5]. NaCas is widely used in the food industry as functional ingredient in a wide variety of food products due to their simple production, excellent nutritional value, versatility and functional properties, including thermal stability, water holding capacity, rheological properties (viscosity increase and gel formation), emulsifying properties[6–8].

The casein glycomacropeptide (CMP) derived from κ-casein has great commercial interest for numerous bioactive properties and does not contain phenylalanine, making it suitable for foods for patients suffering phenylketonuria. CMP is also an interesting peptide because of its ability to bind positive charged molecules, such as minerals [9,10] due to the negative charges provided by the acidic amino acids and the sialic acid residues in glycosylated form of CMP [11].

A growing number of studies have shown that CMP has biological activities which deserve a great interest as a food ingredient [12,13]. In addition, this peptide has good surface and foaming properties; due to its molecular structure is capable of rapidly covering the interface at low concentrations[14,15]. Nevertheless the interfacial films are weak[16]thus exhibiting a low stability[17].Kreuß et al. [18]evaluated the interfacial and emulsifying properties of the aglyco and glyco fractions of CMP (aCMP and gCMP, respectively) and reported a better behavior for aCMP. Finally, they proposed a model for surface adsorption of CMP as a function of the glycosylation degree and pH.

Several strategies to improve the interfacial properties and the foaming properties of proteins may be found; among them the use of synergistic mixtures of biopolymers [14,19] and the variation of pH that can modify their charge and accordingly their foamability [20,21].

In a previous work, it was demonstrated that NaCas and CMP strongly interact in solution forming assembled structures that formed a firmer protein network thus improving gelling properties [22]. As this interaction could be useful to improve the surface/foaming properties, in the present contribution was undertaken to evaluate the effect of CMP on foaming and surface properties of NaCas and to establish the role of protein interactions in the aqueous phase.

2. Materials and methods

2.1. Sample preparation

BioPURE caseinglycomacropeptide (CMP) was supplied by DAVISCO Foods International, Inc. (Le Sueur, MN, USA). Its composition was: 86.3 % CMP (N x 7.07), fat 0.6%, ash 6.3% and moisture 6.4%. The extent of glycosylation is about 50%. The pH value of the CMP after dissolving in Milli-Q water was 6.7.

Sodium caseinate (NaCas) powder was provided by MP Biomedical, whose composition was: 91.7 % protein, 3.6% ash and 4.6% moisture. The pH value after NaCasdissolution in Milli-Q water was 6.8.

CMP, NaCasand NaCas/CMP systems were studied at1% (w/w), concentration that ensure the formation of foams by the whipping method, at pH 5.5 and 7.The pH was adjusted by using 1 or 0.1 N HCl or NaOH. Mixed systems NaCas/CMP at ratios 1/1 and 3/1 (v/v)were prepared by mixing the appropriate volume of each protein solution to achieve the required final concentration.

2.2.Particle size

Dynamic light scattering experiments were carried out in a Dynamic Laser Light Scattering (DLS) (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire, United Kingdom). Measurements were made at a scattering angle of 173 °. The instrument's measurement range is from 0.6 to 6000 nm. The results were obtained by CONTIN method, which is used to analyze the data for percentile distribution of particle/aggregate sizes. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and it is therefore known as an intensity size distribution. Through Mie theory, with the use of the input parameter of sample refractive index, it is possible to convert the intensity distribution to volume distribution. As such, volume distributions derived from dynamic light scattering measurements are best used for comparison purposes or for estimating the relative amounts of multimodal (multiple size peaks) samples, and should never be considered absolute[23,24]. The assay was performed in duplicate on two individual samples.

2.3. Viscosity

The viscosity of protein solutions was determined at 25ºC in a Brookfield LVT viscometer with cone and plate system (CP-40 cone spindle) at increasing and decreasing shear rate from 250 to 1500 s^{-1}. Viscosity (Pa.s) was reported as a mean and standard deviation of triplicates.

2.4. Foaming properties

2.4.1. Foam formation

Solutions at 1% w/w (20 mL) were foamed at room temperature in a graduated tube (3 cm diameter) for 3 min with Griffin & George stirrer at 200 rpm. Foam overrun (FO) was calculated by Eq. (1):

FO (%) = [(foam volume (mL) – 20 (mL))/20 (mL)] x 100 (1)

The data reported are means of at least two replicates. The error was less than 10%.

2.4.2. Foam drainage and collapse

The volume of liquid drained to the bottom of the graduated tubes and the foam height decrease (foam collapse) after foam formation, were recorded over time in duplicate samples.

Foam collapse (FC) was calculated as:

$FC = (V_t - 20)/ (V_f - 20)$ (2)

Where V_t is the foam volume at time t, V_f is the foam volume immediately after foam formation and 20 ml is the initial volume of liquid used to make the foam. Therefore, FC ranges within 1 and 0.

On the other hand, liquid drainage ranges within 0 and 20 ml which is the initial volume of liquid used to make the foam.

2.5. Interfacial properties

The interfacial tension and dilatational rheologyof adsorbed protein films at the air-water interface was determined with an interfacial tensiometer PAT-1 (SINTERFACE Technologies, Berlin, Germany) using the pendant drop method. A drop of the protein solution is formed at the tip of a capillary, which is in a cuvette that is filled with water saturated atmosphere to avoid droplet evaporation, covered by a compartment, which is maintained at constant temperature (20.0 ± 0.2 ºC) by circulating water from a thermostat. Then, the silhouette of this drop is cast onto a CCD camera and digitized. The digital images of the drop are recorded over time and fit to the Young-Laplace equation to accurately (± 0.1 mN/m) determine surface tension using drop profile analysis tensiometry.The surface pressure is $\pi = \gamma_0 - \gamma$, where γ_0 is the surface tension of pure water in the absence of any surface-active component and $\gamma$the time-dependent surface tension of protein solutions.

The dilatational rheology was determined with the same equipment. The method involves a periodic automatically controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at the desired amplitude (3 %) and angular frequency (0.05 Hz). These conditionsguarantee that the rheological parameters are independent of the amplitude. The sinusoidal compressions and expansions were achieved with six oscillations cycles followed by 10 min constant interfacial area recording. The surface area perturbations lead to a respective harmonic surface tension response. The data obtained were analyzed by using the Fourier transformation, obtaining the dilatational parameters of the interfacial layer, namely the interfacial elasticity and viscosity.

The surface dilatational modulus is a complex term, first derived by Gibbs, as the change in surface tension induced by a small change in surface area. In general, any perturbation of the interfacial area results in a response of the surface tension. The Gibbs dilatational modulus is built up by a storage part *E'*, representing the real part of the term and a loss part *E''*, describing the imaginary part of the modulus (Eq. 3):

$$E(i\omega) = E'(\omega) + iE''(\omega) \quad (3)$$

where *E'* is the interfacial elasticity and $E''/\omega = \eta$ is the interfacial viscosity ($\omega = 2\pi f$, which *f* is the angular frequency of the generated area variations).

All experiments were performed in duplicate in two individual samples.

2.6. Statistical analysis

The analysis of variance (ANOVA) ($p<0.05$) using the statistical program Statgraphic Plus 5.1 was done.

3. Results

3.1. Characterization of protein solutions

3.1.1. Particle size

Particle size distributions for CMP, NaCas and their mixtures at pH 7 and 5.5 are shown in Figure 1 A and B, respectively. As previously reported [25], at neutral pH (Figure 1 A), CMP is present in a monomeric form with a predominant size peak close to 2 nm. NaCas presented two populations at pH 7, one between 20 and 70 nm with a maximum peak value at 35 nm, corresponding to small casein aggregates, and a wide higher size population between 70 and 1000 nm, corresponding to larger casein aggregates. These results are in agreement with previous reports[26–28]. In a recent work, we found a similar size particle distribution for another sample of sodium caseinate; although most of the protein was associated forming large particles in the range of 100 and 800 nm, the lower size population was not observed [22].The occurrence of monomodal [29] or polymodal [26,27] size distributions for NaCas solutions has been attributed to the samples origin.

At pH 5.5 (Figure 1B) CMP showed a predominant peak close to 3 nm that, according with the Mw estimation by the software of Zetasizer Nano-Zs instrument, would correspond to a dimeric form of CMP [25]. NaCas size distribution at this pH presented three populations (30-100 nm, 100-1000 nm and >3000 nm) showing a higher protein association than at pH 7. The increase in size of NaCas aggregates can be explained by the decrease of the electrostatic repulsion approaching the isoelectric point (4.6).

CMP and NaCas interacted in the aqueous phase at 25 °C (NaCas/CMP ratio: 3/1 and 1/1) forming assembled structures at pH 7 and 5.5. At pH 7, both mixed systems (NaCas/CMP ratio: 3/1 and 1/1) showed similar size distributions with two populations: one with a maximum value at about 25 nm (which was predominant in both volume size distributions) and another at larger sizes (maximum values close to 300 nm) (Figure 1 A). At pH 5.5, both mixed systems showed a maximum peak value between 20-30 nm (Figure

1 B). At both pH, the peak corresponding to CMP disappeared indicating an interaction between CMP and NaCas that induced a decrease in the aggregation state of the NaCas due to increasing electrostatic repulsion arising from the negative charge of bound CMP.At pH 5.5 most of NaCas and CMP formed particles of 20-30 nm. Nevertheless, at pH 7, a significant amount of the higher size NaCas aggregates were still present.

The changes in the aggregation state of the NaCas, even more at low pH, can be attributed to the role of CMP in sequestering Ca, making it less available for the stabilization of NaCas aggregates, or to the interaction between CMP and NaCas via hydrophobic interactions as it was previously reported [30]. In this previous work [22] it was shown that this interaction promoted the gelation of NaCas at higher pH values as well a decrease of the size of particles forming the gel which affected its microstructure and texture.

3.1.2. Viscosity

The flow curves for each system (results not shown) showed that all the solutions exhibited Newtonian behaviour. The viscosity, determined from the slope of the flow curves, is reported in Figure 2. The viscosity of NaCas solutions at pH 7 was significantly higher than that of CMP, but at pH 5.5 they exhibited similar viscosities. If no interactions would occur between NaCas and CMP, a viscosity proportional to the content of each component would be expected for the mixed solutions (dotted lines in Figure 2). However, as compared to the single components, the mixtures showed a significant higher viscosity at pH 5.5 and a slightly lower viscosity at pH 7. It is known that protein concentration, protein aggregation and the aggregates size and shape may modify the viscosity of solutions[31]. Moreover, water absorption capacity, which is pH dependent may also influence the viscosity of solutions. Anyway, since the total protein concentration was the same in all the systems, the results for the mixed solutions, mainly

at pH 5.5, may be attributed to interactions between CMP and NaCas, as also revealed by particle size determinations (Figure1).

3.2.Interfacial properties

Figure 3 A shows the change of the surface pressure (π) over adsorption time at pH 5.5 and 7. The increment of the surface pressure with time is related to the protein adsorption at the air-water interface [32]. At both pH, NaCas exhibited greater interfacial activity than CMP, while NaCas/CMP mixed solutions showed a behavior closer to NaCas indicating that the last dominated the surface activity of the mixtures. During competitive adsorption between proteins at high bulk concentrations, the surface pressure would be controlled by the component which adsorbs more rapidly (in this case NaCas). Moreover, the competitive adsorption of biopolymer mixed systems, may be affected by interactions in the bulk phase or even by specific interactions at the interface that may affect film rheology [33].

Figure 3 B shows dilatational elasticity of surface films at pH 5.5 and 7. CMP exhibited the highest elastic modulus at pH 5.5 (~ 25 mN/m) which was almost twice that of NaCas and the mixtures. Thus,at pH 5.5, the elasticity of mixed films, as well as the surface pressure (Figure 3 A), were dominated by NaCas.

At pH 7, CMP film showed a lower elastic modulus than at pH 5.5 and more similar to NaCas. The mixed films exhibited a slightly lower dilatational modulus than each single protein film (Figure 3 B), that indicates an antagonistic behavior between NaCas and CMP at the air-water interface.

The interfacial properties of proteins are affected by intrinsic molecular factors, such as size, charge distribution, hydrophobicity, hydrophilicity, molecular flexibility and steric properties[34]. In the present work, regarding the effect of pH, only CMP showed differences in dilatational elasticity. As it was previously described, at pH 5.5 CMP self-

assembles to form dimers[25]which could form a more elastic film than the monomeric form of CMP observed at pH 7. Kreuß et al.[18] determined interfacial and foaming properties of both fractions of CMP and they observed a higher adsorption of CMP at the air-water interface at pH 5.5 than at pH 7, which was attributed to the loss of electrical repulsion by decreasing pH.

3.3. Foaming properties

3.3.1. Foam overrun

Figure 4 shows the foam overrun (FO) of CMP, NaCas and NaCas/CMP mixed solutions at pH 7 and pH 5.5. At both pH, single CMP foams showed the highest and NaCas the lowest FO. The high FO of CMP may be attributed to its lower size that allows it to diffuse rapidly to the nascent interface and form an elastic film (Figure 3 B). In fact, the diffusion coefficient for CMP (88 ± 3 $\mu m^2/s$ and 66.7 ± 0.8 $\mu m^2/s$ at pH 7 and 5.5 respectively) in solution was approximately 30 times higher than that of NaCas (2.70 ± 0.03 $\mu m^2/s$ and2.57 ± 0.02 $\mu m^2/s$ at pH 7 and 5.5 respectively). It has been previously reported the high foaming capacity of CMP as compared to other proteins, for example β-lactoglobulin [14,17], whey protein isolate and gelatin [35].The FO was similar at both pH values indicating that the decrease in pH does not influence the overall process of foaming, except at NaCas/CMP 1/1 ratio in which the FO at pH 5.5 was significantly higher than at pH 7. Zhang et al.[5] reported an important influence of pH on the foaming capacity of whey proteins and caseins. They found a lower foamability for skim milk proteins (where caseins are the main components) at pH 4-5 than at pH 7 that was attributed to the precipitation of caseins near their isoelectric point. However, in the present study the pH (5.5) is still above the isoelectric point of caseinate.

The FO was increased by increasing CMP in the mixtures (Figure 4). Additionally, the mixture NaCas/CMP 1/1, showed a FO higher than that expected according to its

composition (dotted line in Figure 4), pointing out a synergistic performance of this mixture during foam formation at both pH values.

3.3.2. Foam stability

Although CMP exhibited an outstanding foamability (Figure 4), the foams were very unstable at both pH values, as shown in Figure 5 A. Liquid quickly drained, reaching the maximum drained volume (20 ml) in 5 or 10 minutes for foams at pH 5.5 and 7 respectively. During the first minutes foam volume slowly decreased, but when almost all liquid was drained out (4-5 min), foam volume sharply decayed (mainly at pH 7). Thus, CMP foams totally collapsed after 10 minutes. Overall, CMP foams were slightly more stable at pH 7 than at pH 5.5.

Contrarily, NaCas that foamed less than CMP, showed a much higher stability (Figure 5 B). Drainage was not affected by pH. Initially NaCas foams drained about 14 ml of liquid within the first two minutes,that could reveal that not all the liquid was incorporated to the foam. Then drainage proceeded slowly reaching the maximum volume (20 ml) after 180 minutes. Foam volume at pH 5.5 almost persisted after 180 min but at pH 7 continuously decreased until disappearance of the foam at 180 min. Overall, NaCas foams were more stable at pH 5.5 than at pH 7.

The liquid drainage of the mixed foams is compared to the drainage of CMP or NaCas foams in Figure 6A (pH 7) and Figure 6B (pH 5.5). At both pHs the drainage profile for mixed foams was closer to NaCas foams, thus showing a slower drainage dominated by NaCas (Figure 6). In fact, at pH 7 (Figure 6 A) the maximum drained volume (20 ml) was reached within 60 min for NaCas/CMP ratio 1/1 and 3/1. At pH 5.5 mixed foams were even more stable than at pH 7, as after 180 min they still retained some of the liquid (Figure 6 B). Moreover, at pH 5.5, mixed foams drained slower than NaCas foam, exhibiting a synergistic performance.

When analyzing the initial drainage at pH 7, up to 12 min. in the inner plot in Figure 6A, it can be observed that NaCas foam rapidly drained during the first 2 minutes and then leveled off as possibly not all the liquid could be incorporated in the foam. By increasing the CMP in the mixture, the initial drainage slowed down close to the single CMP foam, as related to the improved foamability of the mixtures as CMP concentration increases (Figure 4).

The drainage of NaCas foam was similar at both pHs (Figure 5 B) but CMP drained more rapidly at pH 5.5 than at pH 7 (Figure 5 A). Therefore, increasing NaCas in the mixture at pH 5.5, greatly delayed the initial liquid drainage (inner plot in Figure 6 B).

Foams were more stable against collapse at pH 5.5 than at pH 7 (Figure 7). As shown above, a quick collapse of CMP foams at both pH values may be observed, as after 10 minutes the initial foams totally collapsed. In contrast, NaCas foams were much more stable, even more at pH 5.5, as 80 % of initial foam volume was still observed at 100 min after foam formation.

The collapse profile of mixed foams at pH 7 (Figure 7 A) was closer to CMP foam. The time to collapse 80 % of the initial foam volumes was 10 min, 20-25 min and 90 min for CMP, the mixtures and NaCas foams respectively. Thus, the positive effect arising from mixing NaCas and CMP on drainage at pH 7 (Figure 6 A) was not observed in the collapse behavior. Moreover, CMP was detrimental for NaCas foam collapse, as was also detrimental in the elasticity of mixed films (Figure 3 B).

However, at pH 5.5 the collapse behavior of mixed foams was initially very similar to NaCas foam (Figure 7 B), in agreement to film elasticity (Figure 3 B). In particular, NaCas/CMP 3/1 mixed foam was even more stable than NaCas foam as after 120 min. only collapsed 20 % of the initial foam volume. However, the foam NaCas/CMP 1/1, sharply collapsed after 80 min, with a profile similar to CMP foam. Therefore, a strong

synergism is observed on the foam collapse at pH 5.5, by mixing NaCas and CMP (mainly for the mixture NaCas/CMP 3/1), in agreement with the synergism observed on drainage behavior (Figure 6 B).

A synergistic performance of CMP on the stability of mixed foams has been also observed in foams of CMP with β-lactoglobulin [17] and gelatin [35] in which the mixed foams at acidic conditions were much more stable than the single protein foams. This behaviour was attributed to the existence of interactions between these biopolymers at the air-water interface and also in the aqueous solutions [16,35,36].

4. Discussion

It is generally accepted that foam stability is controlled by the rheological properties of interfacial films (i.e. elasticity), as strong films could avoid bubbles collapse[37–39]. Although several articles have reported a strong relationship between the stability of foams and the interfacial properties [40–46], other authors reported that the correlation may be more complex and that the interfacial rheological properties in some cases do not influence foams stability. In fact CMP that exhibited a higher film dilatational elastic modulus than NaCas, produced very unstable foams. Actually there is a big amount of evidence that the presence of protein aggregates could be responsible of the improvement of foaming properties [37,47]. In fact, in a recent work Dombrowski et al. [48] reported an interesting correlation between particle characteristics (particle size, surface hydrophobicity, zeta potential) and surface and foaming properties of aggregated β-lactoglobulin.

In the present work, the higher stability of NaCas foam may be attributed to the existence of submicelles of 20-30 nm (Figure 1) that would retard drainage and bubbles collapse.Mechanistically speaking, there appear to be two main ways whereby particles (i.e micelles) can function as stabilizing agents in foams [37]. When present at a

sufficiently high concentration in the bulk continuous phase, the particles may generate a structural barrier in the form of a network of particles within the spaces amongst the dispersed gas bubbles. Alternatively, the particles may become directly attached to the air-water interface, generating a particle-loaded surface layer which acts to protect individual bubbles against instability events such as disproportionation or bubble shrinkage.

Drainage and stability mechanisms of thin foam films containing aqueous sodium caseinate solutions were investigated by Koczo et al.[28].They demonstrated that microlayering takes place in the film formed by 2% NaCas. The heights of the film step-transitions were in the same range as the effective size of the casein-aggregates, the so-called submicelles (about 20 nm).These particles (submicelles) were present also in a small proportion in the NaCas solution used in the present work (Figure 1) and could explain the stability of NaCas foam. In the CMP solution particles of such sizes were not present, so the interface could not be stabilized by this mechanism and thus prone to destabilization.

Based on our results a scheme to explain the improvement of foaming properties by mixing NaCas and CMP is proposed (Figure 8). In the present work, the interaction in the aqueous phase between NaCas and CMP as determined by DLS (Figure 1) reduced the particle size of NaCas aggregates from ≥300 nm to about 30 nm, which could explain the decreased drainage and collapse of mixed foams, mainly at pH 5.5 where all NaCas dissociated to submicelles. As reported by Koczo et al.[28], the number of layers and thickness increases with increasing caseinate concentration. At 4 wt% caseinate four steps could be observed, corresponding to the formation of four layers of submicelles. This model of foam films containing caseinate submicelles ordered layers matches well with the observed collapse profile for NaCas/CMP 1/1 (Figure7 B). Initially this mixture

collapsed as NaCas for which submicelles layers in the film makes possible to retard foam collapse. After 80 min, it seems that the collapse is not inhibited any more (possibly because total detachment of submicelles), thus remaining adsorbed CMP that is prone to rapidly collapse.

Additionally, the increased viscosity of mixed solutions at pH 5.5 (Figure 2), can further contribute to the foam stability at this pH as a higher viscosity will slow down plateau border drainage and drainage from the thin film to the plateau border.

In a recent work[47] foam stability was strongly related to the particle size of casein micelle dispersions and no correlation between foams stability and interfacial rheological properties could be observed.They concluded that aggregates are not adsorbed at the interface, and were either attached to it as a sublayer or remain in the bulk phase. Both of these options can lead to a pinning of the thin film, and a slowing down of the drainage of liquid from the film (and foam).

Several studies demonstrated that the size of protein aggregates affects the drainage stability of foams. Schmidt et al. [49]reported the effect of the size of complexes between pectin and a globular protein from rapeseed on foam stability. They found that smaller aggregates formed more stable foams than non-aggregated proteins, while larger aggregates provoked a fast drainage. In a similar way, Rullier et al.[50] reported that larger aggregates of β-lactoglobulin (200-400 nm) had a negative influence on the foaming properties acting as an anti-foam agent, while smaller aggregates (70-140 nm) seem to stabilize foams because of steric effects. They concluded that the main factor influencing the stability of foams was the amount and the size of aggregates in the samples which prevented foam destabilization by reducing the drainage rate. Similarly,Kim et al.[51], reported that enhanced stability of β-lactoglobulin foams subjected to thermal treatments at different pH could not be attributed to the interfacial rheological properties,

but to differences in steric interactions. In an interesting review, an extensive discussion about the processes and mechanisms related to foaming properties was developed by Wierenga & Gruppen [53]. They evaluated proteins that were modified by the Maillard reaction and observed, for certain protein solutions, significant changes on foaming properties, while the interfacial properties remained unchanged. They suggested that the presence of protein aggregates could be the responsible of the improvement in foaming properties. Wierenga & Gruppen [53] suggested that researchers should reconsiderthe relationship between foam stability and interfacial properties,especially for systems with protein aggregates or surfactant and particle mixtures.

5. Conclusions

The results of this study show that synergistic interactions take place between CMP and NaCas on foaming at pH 5.5 that enhances foamability and stability as well. The synergism may be mainly attributed to interactions in the aqueous phase that induces the decrease of the size of NaCas micelles from 300-400 nm to 20-30 nm. These small aggregates are able to form adsorption layers at the film surfaces (Figure 8) that arrest drainage and foam collapse. However, the improved stability may be also dueto particles entrapment in foam lamellae which subsequently slow down drainage of liquid from the foam collapse.

The role of CMP in the mixed system is to reduce the aggregation state of NaCas, thus making it more efficient for stabilization of foam films, and to contribute to enhanced foamability and liquid incorporation to the foam.

Therefore, the incorporation of CMP on NaCas solutions not only has a potential benefit related to the bioactive properties provided by CMP, but also provides enhances foamability and stability as well, which could contribute to extend the use of NaCas as a foaming agent, mainly in acid foods.

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Figure captions

Figure 1. Volume size distribution at pH 7 (A) and pH 5.5 (B) of CMP (■), NaCas (●), NaCas/CMP 3/1 (★), NaCas/CMP 1/1 (△) solutions. Total protein concentration: 1% w/w. Temperature 25°C.

Figure 2. Viscosity of CMP, NaCas, NaCas/CMP 1/1 and NaCas/CMP 3/1 solutions at pH 5.5 (grey bars) and pH 7 (white bars). Dotted lines indicate the behaviour expected

for the mixed foams by the NaCas/CMP ratio at each pH. Total protein concentration: 1% w/w. Temperature 25°C. Error bars mean SD, n=3. Different letters indicate significant differences ($P<0.05$).

Figure 3. Interfacial pressure (π) versus time (A) and elastic modulus (E) versus time (B) at pH 5.5 (open symbol) and pH 7 (close symbol) of solution of CMP (□, ■), NaCas (○, ●), NaCas/CMP 3/1 (☆, ★), NaCas/CMP 1/1 (△, ▲). Total protein concentration: 1% w/w. Temperature 25°C.

Figure 4. Foaming capacity (FO) of CMP, NaCas, NaCas/CMP 1/1 and NaCas/CMP 3/1 solutions at pH 5.5 (grey bars) and pH 7 (white bars). Dotted lines indicate the behaviour expected for the mixed foams by the NaCas/CMP ratio at each pH. Total protein concentration: 1% w/w. Temperature 25°C. Error bars mean SD, n=2. Different letters indicate significant differences ($P<0.05$).

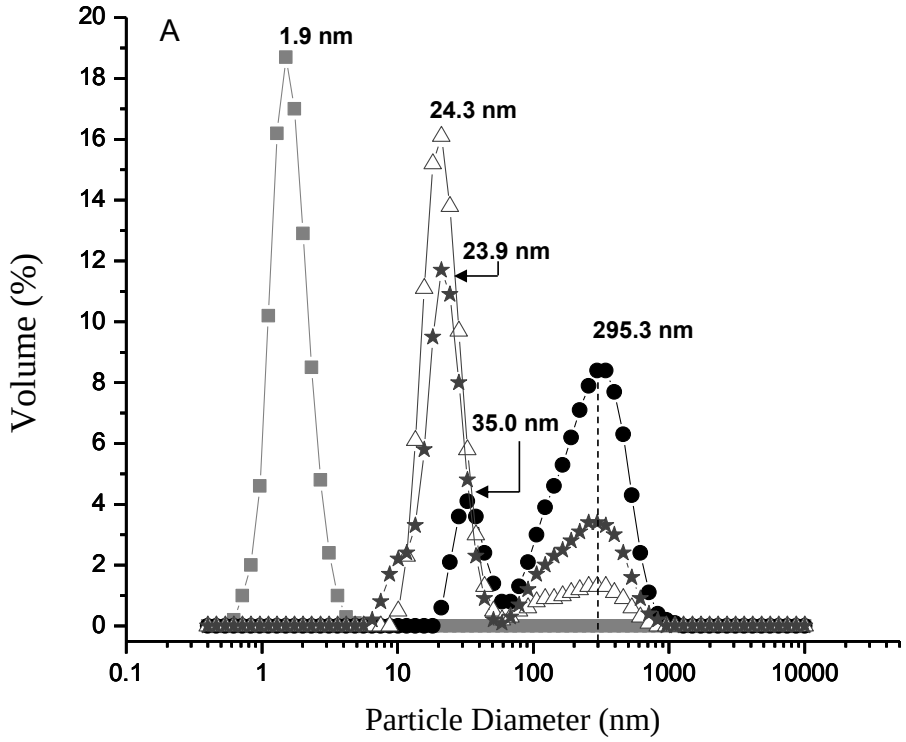
Figure 5. Volume collapse and drained versus time of CMP (A) and NaCas (B) foams at pH 7 (open symbol) and pH 5.5 (solid symbol). Total protein concentration: 1% w/w. Temperature 25°C. Error bars mean SD, n=2.

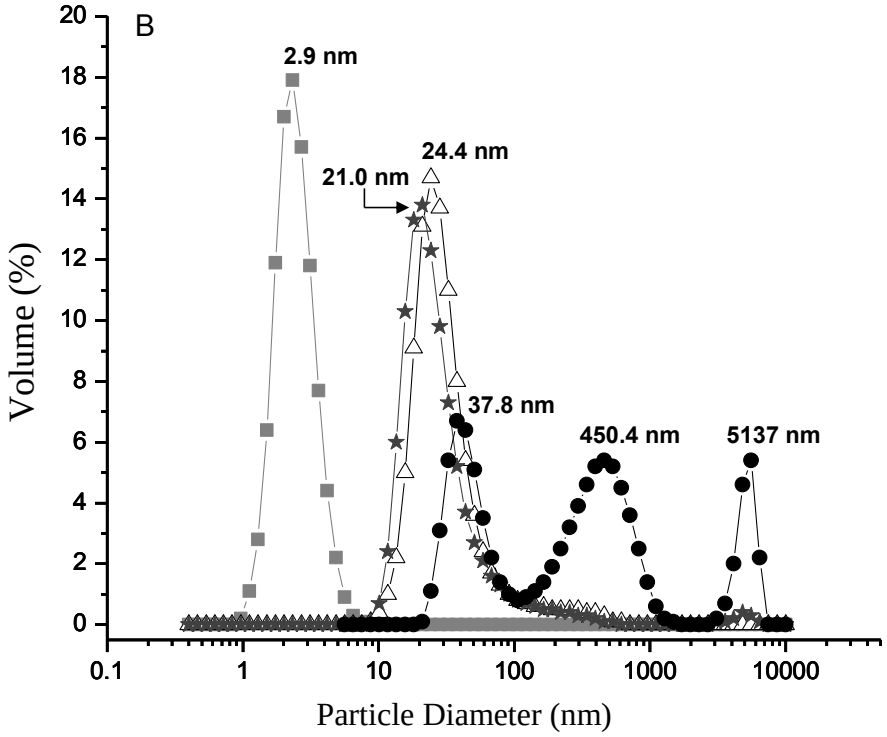
Figure 6. Volume drained versus time of CMP (■), NaCas (●), NaCas/CMP 3/1 (★) and NaCas/CMP 1/1 (△) foams at pH 7 (A) and pH 5.5 (B). Total protein concentration: 1% w/w. Temperature 25°C.

Figure 7. Relation between foam volume and initial foam volume $(V_t-20)/(V_f-20)$ versus time of CMP (■), NaCas (●), NaCas/CMP 3/1 (★) and NaCas/CMP 1/1 (△) foams at pH 7 (A) and pH 5.5 (B). Total protein concentration: 1% w/w. Temperature 25°C.

Figure 8. Scheme of the model proposed to explain the improvement of foaming properties by mixing NaCas and CMP.

Figr-1Figure 1





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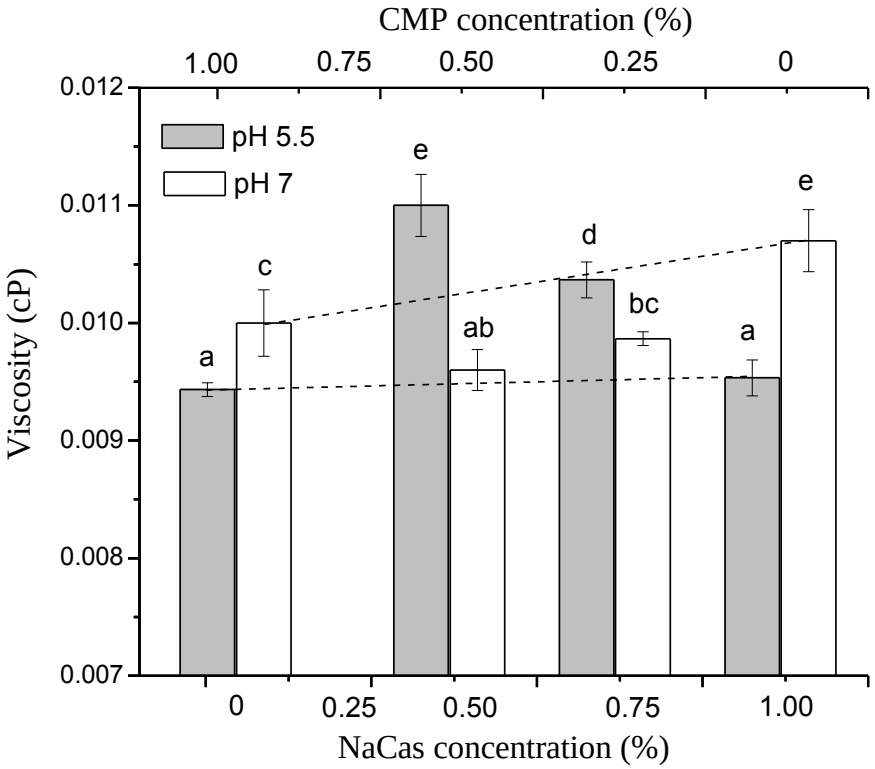
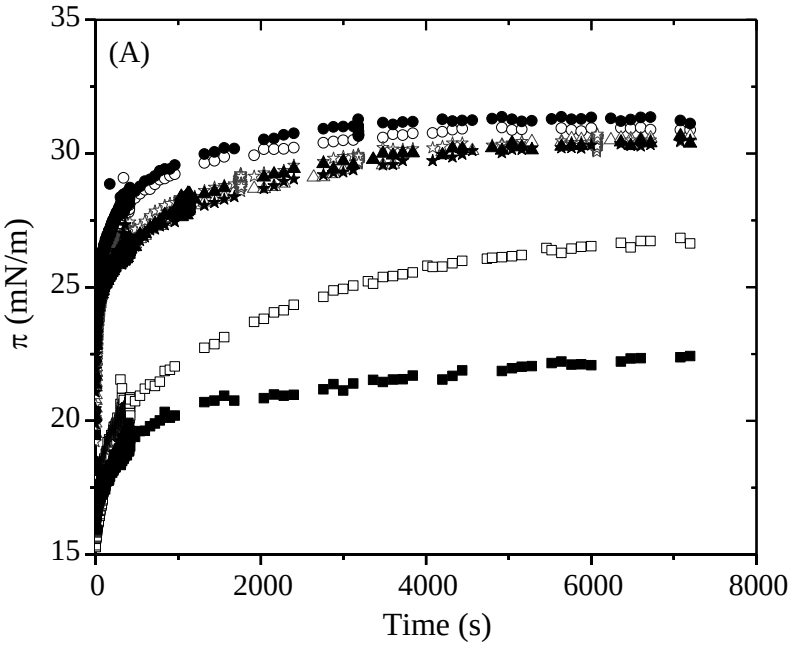
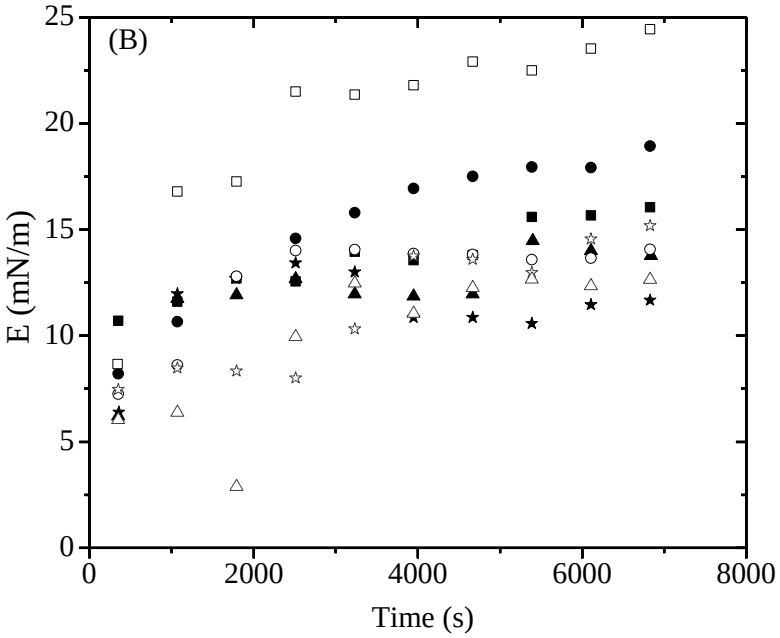


Figr-3

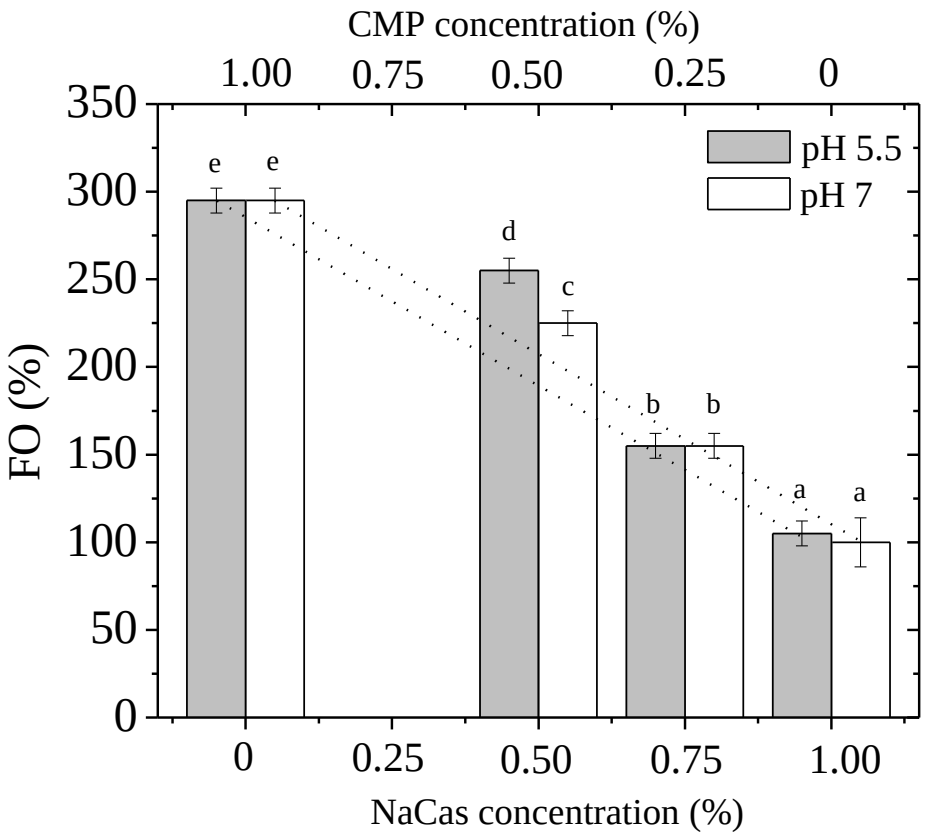
Figure 3



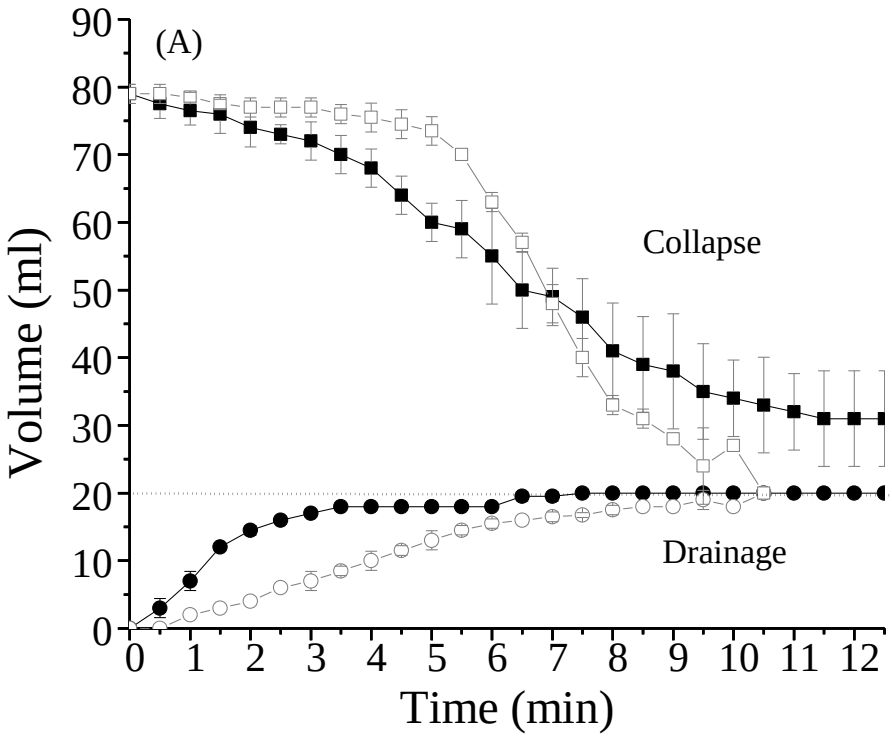


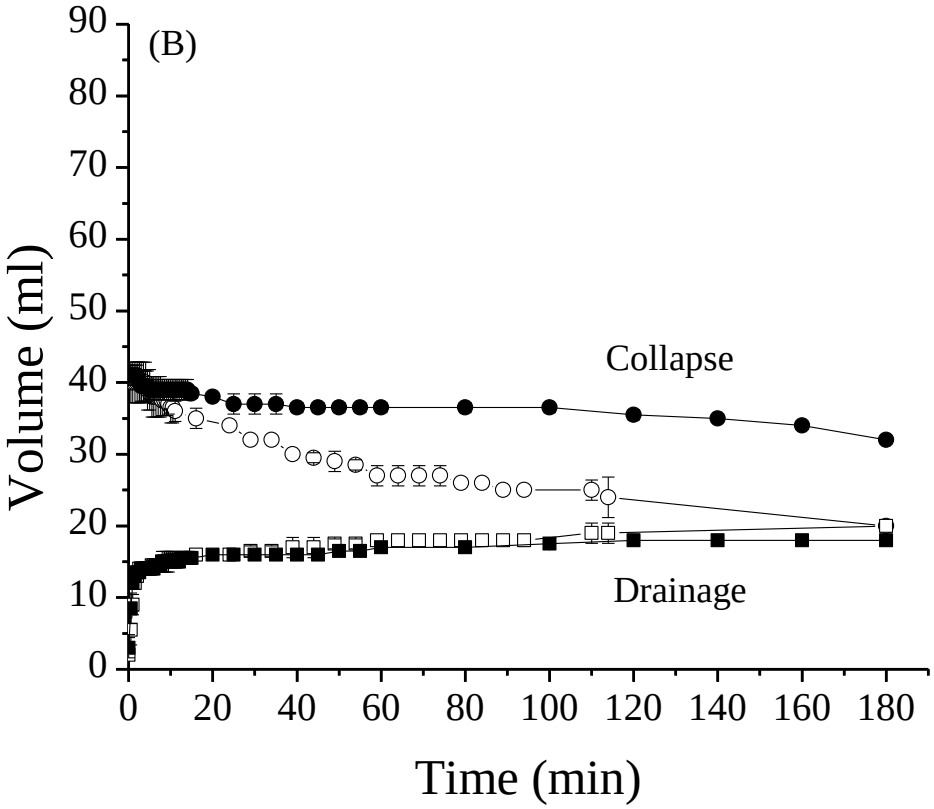
Figr-4

Figure 4

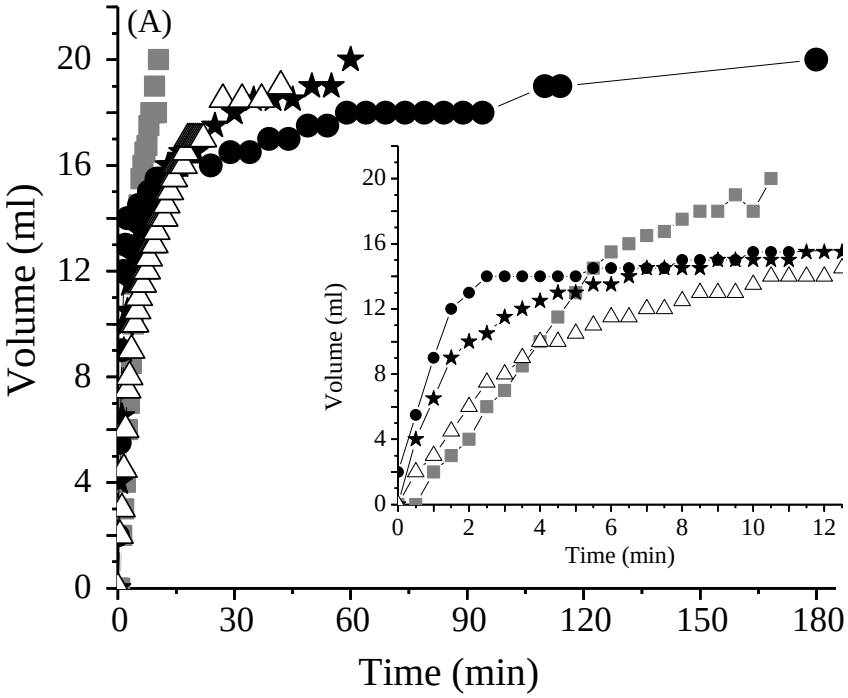


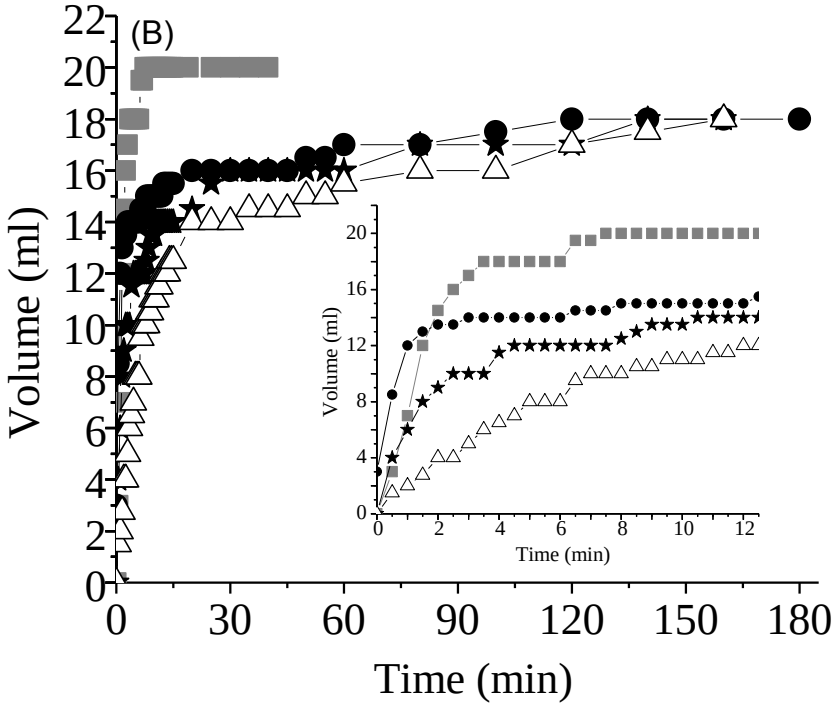
Figr-5Figure 5



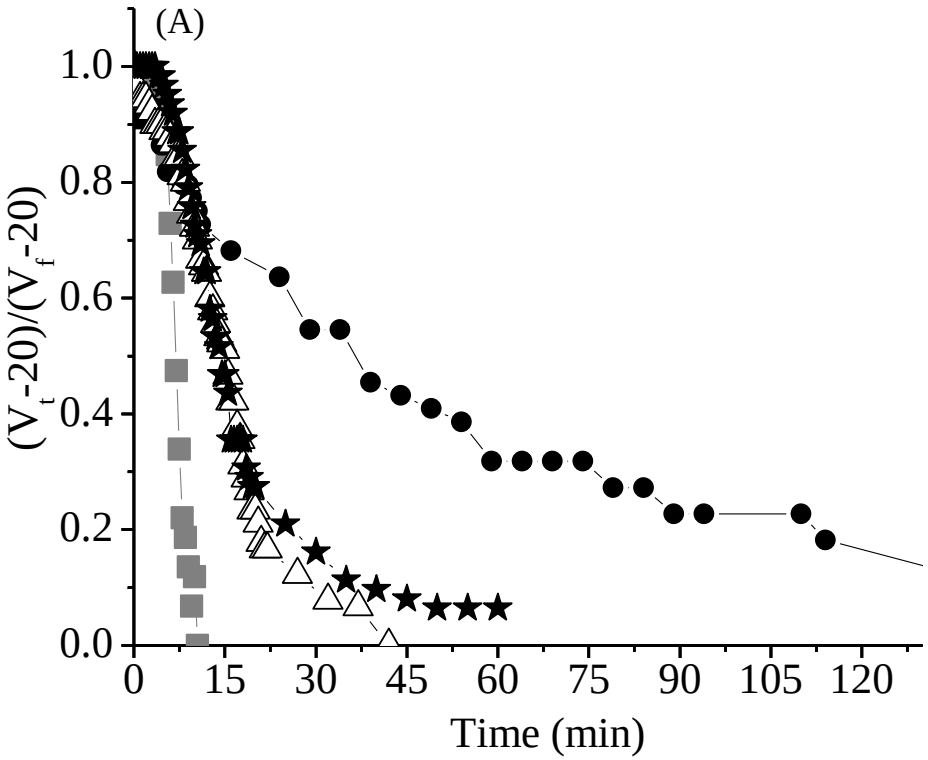


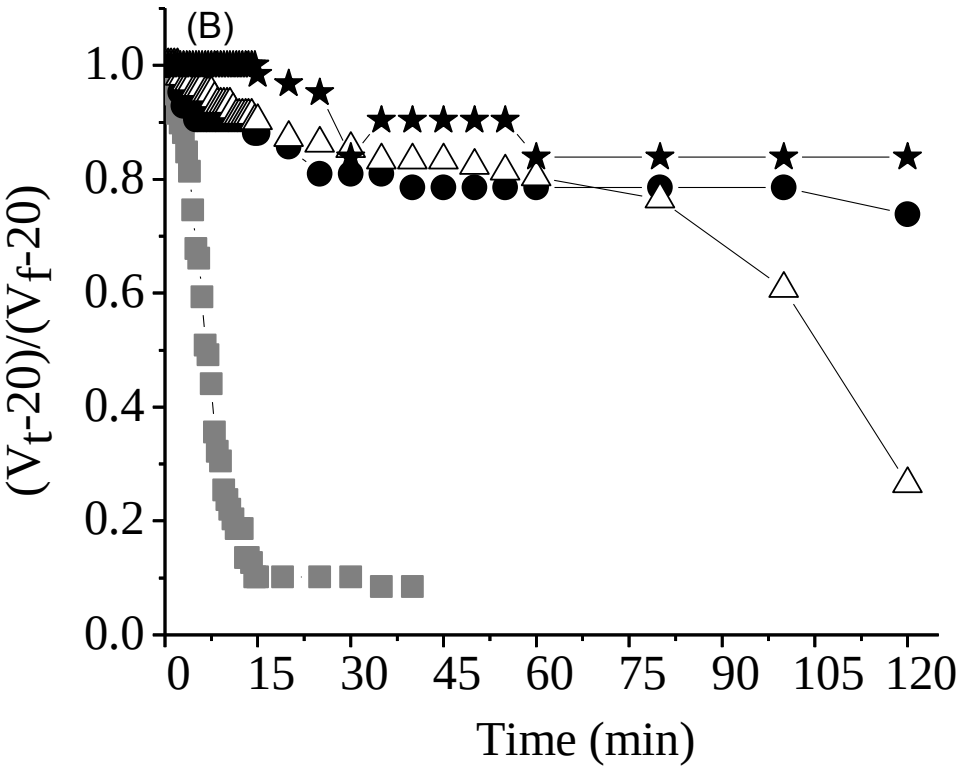
Figr-6Figure 6





Figr-7 Figure 7





Figr-8Figure 8