Effect of sodium hexametaphosphate on heat-induced changes in micellar casein isolate solutions

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Abstract

Micellar casein isolate (MCI) solutions (9%, w/w, casein; pH 6.7) containing 0, 12 or 24 mEq L^{-1} sodium hexametaphosphate (SHMP) were subject to three different thermal treatments: in-container sterilization (121°C for 8 min) and two different continuous flow sterilization treatments (124°C for 5 min and 140°C for 5.8 s). Samples were analyzed after 1-60 d storage at 20 and 40°C for pH, calcium ion activity, turbidity, particle size, viscosity and protein and mineral distribution. SHMP-induced casein micelle disruption in untreated samples was apparent from reductions in turbidity, particle size and increases in sedimentable caseins and calcium, and from strong increases in viscosity. After heating, the pH and viscosity decreased strongly due to the heat-induced hydrolysis of SHMP. SHMP altered casein micelle structure, but during heat treatment and storage, samples with SHMP showed good stability.

Keywords

Calcium sequestrants; heat-treatments; sodium hexametaphosphate; micellar casein

1. Introduction

Milk protein concentrates can be used to prepare protein beverages with specific functionalities and applications such as medical nutrition and sports performance drinks (Özer & Kirmaci, 2010). To ensure their microbial quality and extend the shelf-life, these beverages are often subjected to intense heat treatments such as ultra-high temperature (UHT) or retorting (Renhe, Indris & Corredig, 2018). However, it is key to ensure their stability during the heat treatment and also prevent destabilization during storage, e.g., in the form of gelation and sedimentation phenomena, e.g., through the interaction between calcium, caseins and whey proteins (Anema, 2019).

Due to their good functional properties such as heat stability and excellent nutritional quality, the use of micellar casein isolate (MCI) for high-protein beverage manufacture has increased (McSweeney, O´Mahony, & McCarthy, 2021; Sauer & Moraru, 2012). This is mainly due to the fact that casein micelles are more stable against thermal treatments compared to whey proteins, which denature at temperature >70°C. Nevertheless, depending on the intensity of the treatment, several changes can occur affecting the physicochemical properties of milk systems, mainly due to changes in the different equilibria that exist in milk solutions (Huppertz, 2016). For example, some heat-induced changes can be (partially) irreversible after intensive heat treatment; e.g., micellar calcium phosphate (MCP) becomes more insoluble (Pouliot, Boulet, & Paquin, 1989; Pouliot & Paquin, 1989). Also, during heating the phosphoserine residues can hydrolyze, lactose can be degraded and dissociation of κ-casein (κ-CN) can occur (Gaucheron, 2005).

Calcium sequestering agents are widely used in the dairy industry and play an important role in increasing the heat stability of milk systems as well as to modify certain parameters of milk solutions to improve their technological properties. For example, calcium sequestering salts are used in processed cheese manufacture to improve the emulsification of the cheese melt (Kapoor & Metzger, 2008). In addition, calcium sequestering agents can be added to milk systems to reduce fouling of heat exchangers surfaces during severe heat treatments such as UHT treatment (Prakash, Datta, Lewis & Deeth, 2007; Scudeller et al., 2021).

However, not all calcium sequestering agents act in the same way in their ability to bind calcium (De Kort et al., 2009) and their capacity to interact with calcium ions and proteins of the casein micelle (Mizuno & Lucey, 2007). Sodium hexametaphosphate (SHMP) is known as a good calcium chelating salt, but strongly affects the milk system; it can sequester calcium ions and also bind to positively charged amino acids of the casein micelle, altering the mineral equilibria and depending on the concentration, promoting disruption of the micelles (Anema, 2015; De Kort, Minor, Snoeren, Van Hooijdonk & Van der Linden, 2011; Mizuno & Lucey, 2005).

Some previous studies (De Kort, Minor, Snoeren, Van Hooijdonk, & Van der Linden, 2012; Pandalaneni, Amamcharla, Marella, & Metzger, 2018; Renhe et al., 2018) have evaluated the heat stability of milk systems with added calcium sequestering salts by the heat coagulation test, which, in general terms, is quite subjective (Dumpler, Huppertz, & Kulozik, 2020). However, no studies have been carried out with MCI solutions subjected to in-container or continuous sterilization, mimicking the industrial conditions. In addition, to our knowledge, the stability over time of these heated MCI

systems has not been yet studied. Thus, the objective of this research was to study the heat-induced changes of in-container and continues sterilization treatments on a micelle casein isolate solution at 9% of casein content with 0, 12, and 24 mEq L^{-1} of added sodium hexametaphosphate and to evaluate changes in physicochemical properties for up to 60 d at 20 and 40°C, including changes in pH, mineral and protein equilibria, turbidity, particle size and viscosity.

2. Material and Methods

2.1. Sample preparation

MCI retentate was obtained from FrieslandCampina (Lochem, The Netherlands). The composition was: total solids 18%, total protein 16%, casein content 14.5%, lactose content < 0.5%, fat content < 0.5%. A MCI solution of 9% of casein content was prepared by diluting the MCI retentate with the required amount of demineralized water at room temperature. Hereafter, different amounts of stock solution of sodium hexametaphosphate (Sigma-Aldrich GmbH, Germany) were added to reach the final SHMP concentration: 0, 12 and 24 mEq L^{-1}. Before the heat treatment, the pH was adjusted to 6.70 ± 0.05 using 0.1 M HCl or NaOH.

2.2. Heat treatments

Samples with and without added SHMP were thermally treated at three different conditions. For in-container sterilization, glass bottles of 100 mL were filled with the samples and closed with a tight screw cap. The bottles were introduced in the autoclave (Zirbus 160-5-170; Zirbus Technology GmbH, Bad Grund, Germany) and treated at 121°C for 8 minutes. For continuous-flow sterilization two different temperature-time combinations were selected: 124°C for 5 min and 140°C for 5.8 s. Both treatments were carried out in a HTST/UHT unit (OMVE HT220 HTST/UHT System; OMVE, Utrecht, The Netherlands) equipped with a tubular heat exchanger system. The treated-samples were rapidly chilled to room temperature in the heat exchanger and then outflow was filled into sterile 180 mL PP containers (Gosselin Corning, France) leaving a headspace under a positive laminar flow hood to avoid environmental contamination. Subsequently, all heated samples were stored at 20 and

40°C and were analyzed after 1, 7, 14, 30 and 60 d of storage. At each day of storage, a separate sample container was used.

2.3. pH and calcium ion activity

pH was measured at 25°C using a pH meter calibrated with buffer solutions at pH 4.0, 7.0 and 9.0. The calcium ion activity was measured using a calcium-ion-selective electrode (Sension +9660C; Hach, Loveland, CO, USA) as described by Crowley et al. (2014).

2.4. Mineral and protein distributions

To separate the non-sedimentable and sedimentable fractions of the samples, the samples were first diluted 3-fold with demineralized water and subsequently centrifuged at 100.000 x g for 1 h at 20°C using an Avanti JXN-30 ultracentrifuge (Beckman Coulter, Indianapolis, IN, USA) with a swinging-bucket rotor (JS-24.38; Beckman Coulter). Pellet and supernatant were separated by decanting.

The content of Ca in the whole samples and the ultracentrifugal supernatants were determined by ICP-AES as described by Cruijsen, Poitevin & Brunelle (2019). The protein composition of the whole sample and the ultracentrifugal supernatants was determined by RP-HPLC using a method adapted from Visser, Slangen and Rollema (1991). Values for Ca and individual caseins (κ-casein: κ-CN; β-casein: β-CN; α_{s1}- + α_{s2}-casein, hereafter denoted as α_s-CN) in the ultracentrifugal were expressed as a percentage of their concentration in the whole sample.

2.5. Turbidity and particle size

The absorbance of the samples was measured at 600 nm at room temperature following 10 or 100-fold dilution with demineralized water to be within the linear range of the spectrophotometer. Reported values are corrected for the dilution. Particle size of samples diluted 100-fold with demineralized water was analyzed in triplicate by dynamic light scattering using a Zetasizer Nano (Malvern Instruments, Malvern, UK) at 25°C at a scattering angle of 173º. Values are expressed as a Z-average hydrodynamic diameter (in nm).

2.6. Viscosity

The viscosity of the undiluted samples was measured at 20°C with a Discovery hybrid rheometer HR-2 (TA Instruments, New Castle, DE, USA) using a cup and bob geometry. Samples were conditioned at 20°C for 120 s, followed by 0.1 s^{-1} for 60 s 0.1 to 1000 s^{-1} over 300 s, 1000 to 0.1 over 300 s, and finally at 0.1 s^{-1} for 60 s. Data points were collected each 5 s. Viscosity results presented are at a shear rate of 100 s^{-1} in the upward curve.

151 ## 3. Results

152 ### *3.1. Influence of sodium hexametaphosphate addition on the heat-induced*
153 ### *changes in pH and mineral and protein equilibria in micellar casein isolate*
154 ### *solutions*

155 The effect of heating and subsequent storage on the pH of MCI solutions with 0, 12 or
156 24 mEq L^{-1} SHMP is shown in **Fig. 1**. The pH of the samples without SHMP were
157 largely unaffected by heat treatments and also showed little change during subsequent
158 storage at 20 or 40°C. In contrast, for samples with added SHMP notable reductions
159 in pH were observed after the three different heat treatments. The in-container
160 sterilization (8 min at 121°C) caused the largest reductions in pH, by ~0.2 and ~0.3 pH
161 units for samples containing 12 and 24 mEq SHMP L^{-1}, respectively (**Fig. 1**). This is
162 consistent with results from De Kort et al. (2012), who observed an even stronger
163 decreases in pH (by ~0.5 pH units) in reconstituted MCI with added SHMP (15 mEq
164 L^{-1}) after subjecting the samples to a more extensive retort treatment, at 126°C for 15
165 min. In addition, the larger heat-induced decrease in pH in samples with higher
166 concentrations of added SHMP (**Fig. 1**) was in agreement with Tsioulpas, Koliandris,
167 Grandison and Lewis (2010). This strong heat-induced reduction of pH in samples with
168 added SMHP may be attributed to heat-induced hydrolysis of SHMP at high
169 temperatures. Rulliere, Perenes, Senocq, Dodi and Marchesseau (2012) reported that
170 the hydrolysis of SHMP occurred at temperatures above 120°C, an effect which was
171 enhanced by the presence of calcium. De Kort et al. (2012), indicated that SHMP was
172 hydrolyzed during heating into sodium trimetaphosphate and sodium orthophosphate,
173 and H^+ ions are liberated, decreasing the pH of the solution, which is consistent with
174 results in **Fig. 1**. In our studies, seems that the drop in pH was more dependent on the
175 duration of the treatment as the in-container treatment was the one that showed the

biggest changes. The continuous treatments were done at higher temperatures, but their shorter holding times and faster heating and cooling rates, compared to in-container sterilization were likely not enough to promote the same level of hydrolysis of SHMP.

The heat-treated samples showed only limited changes in pH (typically no larger than 0.1 pH unit) during storage for up to 60 d 20 and 40°C (**Fig. 1**). Samples stored at 40°C showed a slightly lower pH than samples stored at 20°C (**Fig. 1**), which is coincident with other studies (Gaucher, Mollé, Gagnaire & Gaucheron, 2008) that evaluated the influence of the storage temperature on the evolution of the pH in UHT-treated milk. The small changes in pH during storage may be attributable to some (re-)equilibration of the salt balance in samples.

The addition of SHMP to unheated samples strongly reduced the calcium ion activity (data not shown), in line with previous studies (De Kort et al., 2011), presumably due to the calcium-sequestering effect of the SHMP. The heat treatments and subsequent storage had little further effect on the calcium ion activity compared to the effect of SHMP addition with only small changes in Ca-ion activity observed (data not shown).

Non-sedimentable calcium in the unheated samples without SHMP was ~21% of total Ca. After heating, a small decrease in non-sedimentable Ca was observed for the in-container sterilized samples and the samples treated at 124°C for 5 min, but no notable changes were observed for the samples heated at 140°C for 5.8 s (**Fig. 2**). These reductions in non-sedimentable Ca are likely due to the heat-induced precipitation of calcium phosphate, thus, increasing the amount of sedimentable Ca

(Nieuwenhuijse & Huppertz, 2022). This is agreement with the results obtained for the slight decrease in pH observed for the in-container sterilized samples without SHMP (**Fig. 1A**).

For the unheated samples, the non-sedimentable calcium increased with increasing concentration of added SHMP salt, from 21% (0 mEq L^{-1}) to 33 (12 mEq L^{-1}) and 67% (24 mEq L^{-1}) of total Ca. The heat-treatments promoted only small changes in the amount of non-sedimentable calcium, except from the in-container sterilized sample with 24 mEq L^{-1}, which showed a reduction from 67% to ~47%. This result is coincident with the results obtained by Hardy, Muir, Sweetsur and West (1984). It seems that the duration of the treatment influenced again the changes in the mineral distribution, which could explain the differences observed between in-container and the continuous sterilization treatments. The amount of non-sedimentable calcium in stored samples (**Fig. 2**) at 20°C practically did not vary during the storage time for all the treatments and concentrations, even in the samples without SHMP. At 40°C, a decrease was observed in the samples with 24 mEq L^{-1}, which showed a notable reduction of non-sedimentable calcium (**Fig. 2B, 2D** and **2F**).

κ-CN (**Fig. 3**), β-CN (**Fig. 4**) and α_s-CN (**Fig. 5**) were solubilized as a result of the addition of SHMP, with κ-CN the most affected one followed by β-CN and α_s-CN. This is consistent with the results of Anema (2015), who pointed out that the level of the dissociated caseins upon the addition of SHMP was inversely related to their content in phosphoserine residues, suggesting that the dissociation may be caused by the solubilization of or changes in the MCP induced by SHMP. On the other hand, the thermal treatments decreased the levels of non-sedimentable caseins (**Fig. 3-5**). This

effect was stronger for the in-container treated samples, showing considerable reductions in non-sedimentable casein, especially for the solutions with 24 mEq L^{-1}. The continuous-flow treatment for 5.8 s at 140°C induced notably smaller changes in non-sedimentable caseins (**Fig. 3-5**). The results suggested that new aggregates were likely formed, observed by the decrease of non-sedimentable caseins and increase in turbidity (**Fig. 6**) and particle size (**Fig. 7**), results that will be discussed further in Section 3.2. The casein distributions showed different variations during storage. κ-CN (**Fig. 3)** which was the protein most affected by SHMP and the heat treatments, solubilized during the eight weeks of the study, for all the heat treatments, SHMP concentrations, and storage temperatures. For β-CN and α_s-CN (**Fig. 4** and **5**, respectively) the samples treated by continuous sterilization showed more instability, concretely for the samples with SHMP stored at 40°C, in which important reductions in the amount of non-sedimentable protein was observed, as well as lower values of non-sedimentable CNs when compared with their counterparts stored at 20°C.

3.2. Effect of heat treatment and sodium hexametaphosphate addition on physicochemical properties of micellar casein isolate

The changes in turbidity and particle size in the samples are shown in **Fig. 6** and **7**, respectively. The turbidity of casein micelle suspensions is usually a reliable indicator of the integrity of the micelles, as they are responsible for the light scattering (Anema & Klostermeyer, 1997; Kaliappan & Lucey, 2011). In-container sterilization reduced the absorbance of samples without SHMP by ~15% (**Fig. 6A**). However, the continuous-flow sterilization induced smaller reductions in the turbidity of the solutions without added SHMP. SHMP addition reduced turbidity by ~30% at 12 mEq L^{-1}, and almost 90% for 24 mEq L^{-1}, in line with previous reports by others (McCarthy et al.,

2017; Pandalaneni et al., 2018), and in concordance with the other results reported in this study, which likely indicate that the micelle has been (partially) disrupted in presence of SHMP. For the samples with added SHMP, some heat-induced changes in turbidity were observed, which were most noticeable at 24 mEq L^{-1}. At this concentration of SHMP, an increase in turbidity was observed after the heat treatments, especially for the in-container sterilization, which may be attributed to calcium-induced protein aggregation (De Kort et al., 2012), but also to the transfer of minerals from the continuous phase to the colloidal state, that has been previously suggested (Hardy et al., 1984). The turbidity tended to decrease slightly for the heated samples when stored at 20°C (**Fig. 6A, C** and **E**), whereas at 40°C, small increases were observed (**Fig. 6B, D** and **F**).

The particle size of the solutions was affected by the addition of SHMP and also by the treatments (**Fig. 7**). Adding 12 mEq L^{-1} SHMP did not affect particle size, but adding 24 mEq L^{-1} reduced it by ~20 nm. For samples without added SHMP, a small reduction in particle size was observed after heating, most notably for the sample treated at 140°C for 5.8 s. For samples with added SHMP, heat-induced reductions in particle size were notably larger, particularly at the higher concentration of added SHMP. Such effects were particularly noticeable for the continuous-flow treatments, indicating that a shear during continuous flow sterilization may be a contributing factor to particle size reductions. Particle size remained constant during storage (**Fig. 7)**.

3.3. Heat-induced changes of SHMP on the viscosity of MCI

The viscosity was strongly affected by the thermal treatments and by the addition of SHMP. The initial viscosity of the untreated sample without SHMP was ~7 mPa.s.

When the SHMP was added, a strong increase was observed, being 28 mPa.s at 12 mEq L^{-1}, and 105 mPa.s at 24 mEq L^{-1} (**Fig. 8**). This effect was also reported by other authors (De Kort et al., 2012), who stated that probably an effect of swelling of the micelles in addition to an effect of cross-linking of SHMP was produced, increasing the viscosity to this high extent. In contrast, the heat treatments reduced the viscosity for all the samples and all the concentrations and nearly completely reversed the initial SHMP-induced increase in viscosity (**Fig. 8**). The strongest reduction was observed in the thermal processed samples with added SHMP. The viscosity was reduced by 75% and 90% of the initial value of the untreated samples with 12 and 24 mEq L^{-1} SHMP, respectively. The heat-induced hydrolysis of SHMP was probably the main cause of this reduction in viscosity, as suggested for the reductions in pH (**Fig. 1**). It is likely that the formed network was broken down and the linked-SHMP was released from the caseins. This effect was contrary to that observed in studies by Renhe et al. (2018), carried out with citrate, orthophosphate and a mixture thereof, who observed that the viscosity increased after heat treatment. However, this difference can be attributed to the type of salts used in their study, which differ from the polyphosphate used in our research.

Concerning the evolution of the viscosity during storage, very small changes were observed (**Fig. 8**), showing high stability after the heat treatments, even in the samples with SHMP. Without SHMP, the viscosity of the samples did not vary, which was around 5 mPa at both temperatures of storage. In presence of the sequestering salt, only small variations in the viscosity were detected, with practically no storage temperature effect observed.

4. Discussion

Previous studies (De Kort et al., 2009; Kaliappan & Lucey, 2011; Mizuno & Lucey, 2007) have demonstrated that calcium sequestrants such as citrate, EDTA, pyrophosphates, polyphosphates have an important impact in the structure of the micelle, which effect differ considerably depending on their composition, concentration of the added salt, and the ionic environment, the pH, as well as the protein concentration of the solution. SHMP is a polyphosphate that has a strong capacity to bind calcium. When it is added to milk, which is already supersaturated with calcium phosphate (Lucey & Horne, 2009), it binds ionic calcium but also has the ability to interact with the caseins in the micelle.

In addition, it has been established that SHMP alters the mineral equilibria of the milk solutions, and induces the micelle disruption, promoting several physicochemical changes in the solution where it was added (Mekmene, Le Graët & Gaucheron, 2009). SHMP sequesters free calcium ions from the serum phase, reducing its concentration, and therefore, reducing the calcium ion activity of the solution. As a consequence, the MCP dissolves and the micellar structure is disrupted, also releasing caseins from the micelle. This promotes the modification of the turbidity (**Fig. 6**) and particle size parameters (**Fig. 7**). When the micellar structure is disrupted, the micelles dissociate into smaller structures, reducing their particle size (McCarthy et al., 2017) and decreasing the turbidity of the solutions (**Fig. 6**). These changes are directly linked to increasing amounts of Ca (**Fig. 2**), and caseins (**Fig. 3, 4, 5**) in the non-sedimentable fraction, and parallel to a strong increase in viscosity (**Fig. 8**). Power, Fenelon, O´Mahony & McCarthy (2019) demonstrated that the phosphoserine residues of the caseins played an important role in the increase of the viscosity in milk solutions in

presence of SHMP. This can indicate that SHMP bound to caseins involving the phosphoserine residues of the phosphorylated caseins, forming a kind of network between proteins and SHMP. Is important to point out that caseins link with MCP via their SerP present in their sequences, so it is likely that calcium is involved in the SHMP formed network. However, despite of the extensive research carried out by other authors (Anema, 2015; Vujicic, DeMan & Woodrow, 1968), this mechanism of how SHMP binds to caseins remains unclear.

Heating the MCI solutions with added SHMP promoted numerous changes that were mainly attributed to the heat-induced hydrolysis of SHMP. The hydrolysis of SHMP induced to a liberation of H^+, reducing the pH (**Fig. 1**), and the transformation of the polyphosphate into orthophosphate and trimetaphosphate (De Kort et al., 2012). In addition, the formed network between SHMP and caseins was broken down due to the hydrolysis, which is reflected in the strong decrease of the viscosity after the heat treatment (**Fig. 8**). The differences observed between the heat treatments are mainly attributed to the duration of the heating. Thus, in-container sterilized samples showed the biggest changes in pH, calcium ion activity, turbidity, non-sedimentable Ca and viscosity, and the samples treated at 140°C for 5.8 s were the least affected, except for the particle size. In addition, the amount of added SHMP also influenced the heat-induced changes. With 24 mEq L^{-1} the modifications observed in the studied physicochemical properties were markedly higher in comparison with 12 mEq L^{-1}. The biggest changes were observed one day after the heat treatments and then, in general terms, the samples were quite stable over time at both storage temperatures: 20 and 40°C during the eight weeks of this study, and small variations were observed in the physicochemical properties of the studied solutions.

5. Conclusions

SHMP promoted important changes in the physicochemical parameters of MCI solutions, essentially due to the shifts in the mineral equilibria and casein micelle disruption. Heat treatment also affected the studied parameters: the pH dropped under heating as well as a strong reduction in viscosity. When SHMP was added to the solutions a network was created between SHMP and caseins, but during heating, SHMP is hydrolyzed and the network is broken down, dropping the pH due to the liberation of H^+ to the solution and sharply reducing the viscosity. Throughout eight weeks of storage, limited variations in the studied parameters were observed in MCI solutions with SHMP, but in general terms, samples showed good stability when stored at 20 but also at 40°C, with no gelation of sedimentation phenomena observed during the storage time. Overall, SHMP strongly affected the micelle structure, but the induced changes did not show a detriment to the stability when compared with the samples without the salt. These results indicated that the use of SHMP in dairy solutions allow control of stability during processing and storage. These findings provide the dairy industry with a better understanding of the effect of SHMP on the MCI solutions that are subjected to severe heat treatments like continuous-flow or in-container sterilization, which are commonly used in the manufacture of numerous dairy beverages.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Anema, S. G. (2015). The effect of hexametaphosphate addition during milk powder manufacture on the properties of reconstituted skim milk. *International Dairy Journal*, *50*, 58–65. https://doi.org/10.1016/j.idairyj.2015.06.006

Anema, S. G. (2019). Age Gelation, Sedimentation, and Creaming in UHT Milk: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 140–166. https://doi.org/10.1111/1541-4337.12407

Anema, S. G., & Klostermeyer, H. (1997). Heat-Induced, pH-Dependent Dissociation of Casein Micelles on Heating Reconstituted Skim Milk at Temperatures below 100°C. *Journal of Agricultural and Food Chemistry*, *45*(4), 1108–1115. https://doi.org/10.1021/jf960507m

Crowley, S. V., Megemont, M., Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J. A. (2014). Heat stability of reconstituted milk protein concentrate powders. *International Dairy Journal*, *37*(2), 104–110. https://doi.org/10.1016/j.idairyj.2014.03.005

Cruijsen, H., Poitevin, E., & Brunelle, S. L. (2019). Determination of Minerals and Trace Elements in Milk, Milk Products, Infant Formula, and Adult Nutrition: Collaborative Study 2011.14 Method Modification. *Journal of AOAC International*, *102*(6), 1845–1863. https://doi.org/10.5740/jaoacint.19-0073

De Kort, E., Minor, M., Snoeren, T., van Hooijdonk, T., & van der Linden, E. (2011). Effect of calcium chelators on physical changes in casein micelles in concentrated micellar casein solutions. *International Dairy Journal*, *21*(12), 907–913. https://doi.org/10.1016/j.idairyj.2011.06.007

De Kort, E., Minor, M., Snoeren, T., Van Hooijdonk, T., & Van der Linden, E. (2012).

405 Effect of calcium chelators on heat coagulation and heat-induced changes of
406 concentrated micellar casein solutions: The role of calcium-ion activity and
407 micellar integrity. *International Dairy Journal*, *26*(2), 112–119.
408 https://doi.org/10.1016/j.idairyj.2012.03.014

409 De Kort, E., Minor, M., Snoeren, T., Van Hooijdonk, T., & Van der Linden, E. Van
410 Der. (2009). Calcium-binding capacity of organic and inorganic ortho- and
411 polyphosphates. *Dairy Science and Technology*, *89*(3–4), 283–299.
412 https://doi.org/10.1051/dst/2009008

413 Dumpler, J., Huppertz, T., & Kulozik, U. (2020). Invited review: Heat stability of milk
414 and concentrated milk: Past, present, and future research objectives. *Journal of*
415 *Dairy Science*, *103*(12), 10986–11007. https://doi.org/10.3168/jds.2020-18605

416 Gaucher, I., Mollé, D., Gagnaire, V., & Gaucheron, F. (2008). Effects of storage
417 temperature on physico-chemical characteristics of semi-skimmed UHT milk.
418 *Food Hydrocolloids*, *22*(1), 130–143.
419 https://doi.org/10.1016/j.foodhyd.2007.04.007

420 Gaucheron, F. (2005). The minerals of milk. *Reproduction Nutrition Development*,
421 *45*(4), 473–483. https://doi.org/10.1051/rnd:2005030

422 Hardy, E. E., Muir, D. D., Sweetsur, A. W. M., & West, I. G. (1984). Changes of
423 Calcium Phosphate Partition and Heat Stability During Manufacture of Sterilized
424 Concentrated Milk. *Journal of Dairy Science*, *67*(8), 1666–1673.
425 https://doi.org/10.3168/jds.S0022-0302(84)81490-3

426 Huppertz, T. (2016). Heat stability of milk. In *Advanced Dairy Chemistry: Volume 1B:*
427 *Proteins: Applied Aspects: Fourth Edition*. https://doi.org/10.1007/978-1-4939-
428 2800-2_7

Kaliappan, S., & Lucey, J. A. (2011). Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science*, *94*(9), 4255–4263. https://doi.org/10.3168/jds.2010-3343

Kapoor, R., & Metzger, L. E. (2008). Process Cheese: Scientific and Technological Aspects—A Review. *Comprehensive Reviews in Food Science and Food Safety*, *7*(2), 194–214. https://doi.org/10.1111/j.1541-4337.2008.00040.x

Lucey, J. A., & Horne, D. S. (2009). *Milk Salts: Technological Significance BT - Advanced Dairy Chemistry: Volume 3: Lactose, Water, Salts and Minor Constituents* (P. McSweeney & P. F. Fox (eds.); pp. 351–389). Springer New York. https://doi.org/10.1007/978-0-387-84865-5_9

McCarthy, N. A., Power, O., Wijayanti, H. B., Kelly, P. M., Mao, L., & Fenelon, M. A. (2017). Effects of calcium chelating agents on the solubility of milk protein concentrate. *International Journal of Dairy Technology*, *70*(3), 415–423. https://doi.org/10.1111/1471-0307.12408

McSweeney, D. J., O'Mahony, J. A., & McCarthy, N. A. (2021). Strategies to enhance the rehydration performance of micellar casein-dominant dairy powders. *International Dairy Journal*, *122*, 105116. https://doi.org/10.1016/j.idairyj.2021.105116

Mekmene, O., Le Graët, Y., & Gaucheron, F. (2009). A model for predicting salt equilibria in milk and mineral-enriched milks. *Food Chemistry*, *116*(1), 233–239. https://doi.org/10.1016/j.foodchem.2009.02.039

Mizuno, R., & Lucey, J. A. (2005). Effects of emulsifying salts on the turbidity and calcium-phosphate-protein interactions in casein micelles. *Journal of Dairy Science*, *88*(9), 3070–3078. https://doi.org/10.3168/jds.S0022-0302(05)72988-X

Mizuno, R., & Lucey, J. A. (2007). Properties of milk protein gels formed by phosphates. *Journal of Dairy Science*, *90*(10), 4524–4531. https://doi.org/10.3168/jds.2007-0229

Nieuwenhuijse, H., & Huppertz, T. (2022). Heat-induced changes in milk salts: A review. *International Dairy Journal*, *126*, 105220. https://doi.org/10.1016/j.idairyj.2021.105220

Özer, B. H., & Kirmaci, H. A. (2010). Functional milks and dairy beverages. *International Journal of Dairy Technology*, *63*(1), 1–15. https://doi.org/10.1111/j.1471-0307.2009.00547.x

Pandalaneni, K., Amamcharla, J. K., Marella, C., & Metzger, L. E. (2018). Influence of milk protein concentrates with modified calcium content on enteral dairy beverage formulations: Physicochemical properties. *Journal of Dairy Science*, *101*(11), 9714–9724. https://doi.org/10.3168/jds.2018-14781

Pouliot, Y., Boulet, M., & Paquin, P. (1989). Observations on the heat-induced salt balance changes in milk II. Reversibility on cooling. *Journal of Dairy Research*, *56*(2), 193–199. https://doi.org/10.1017/S0022029900026418

Pouliot, Y., & Paquin, P. (1989). Observations on the heat-induced salt balance changes in milk I. Effect of heating time between 4 and 90 °C. *Journal of Dairy Research*, *56*(2), 185–192. https://doi.org/10.1017/S0022029900026406

Power, O. M., Fenelon, M. A., O'Mahony, J. A., & McCarthy, N. A. (2019). Dephosphorylation of caseins in milk protein concentrate alters their interactions with sodium hexametaphosphate. *Food Chemistry*, *271*, 136–141. https://doi.org/10.1016/j.foodchem.2018.07.086

476 Prakash, S., Datta, N., Lewis, M. J., & Deeth, H. C. (2007). Reducing fouling during
477 UHT treatment of goat's milk. *Milchwissenschaft*, *62*(1), 16–19.

478 Renhe, I. R. T., Indris, L. M., & Corredig, M. (2018). Effect of calcium chelators on
479 heat stability and heat-induced changes of milk microfiltered concentrates.
480 *International Dairy Journal*, *82*, 4–10.
481 https://doi.org/10.1016/j.idairyj.2018.02.009

482 Rulliere, C., Perenes, L., Senocq, D., Dodi, A., & Marchesseau, S. (2012). Heat
483 treatment effect on polyphosphate chain length in aqueous and calcium
484 solutions. *Food Chemistry*, *134*(2), 712–716.
485 https://doi.org/10.1016/j.foodchem.2012.02.164

486 Sauer, A., & Moraru, C. I. (2012). Heat stability of micellar casein concentrates as
487 affected by temperature and pH. *Journal of Dairy Science*, *95*(11), 6339–6350.
488 https://doi.org/10.3168/jds.2012-5706

489 Scudeller, L. A., Blanpain-Avet, P., Six, T., Bellayer, S., Jimenez, M., Croguennec,
490 T., André, C., & Delaplace, G. (2021). Calcium chelation by phosphate ions and
491 its influence on fouling mechanisms of whey protein solutions in a plate heat
492 exchanger. *Foods*, *10*(2). https://doi.org/10.3390/foods10020259

493 Tsioulpas, A., Koliandris, A., Grandison, A. S., & Lewis, M. J. (2010). Effects of
494 stabiliser addition and in-container sterilisation on selected properties of milk
495 related to casein micelle stability. *Food Chemistry*, *122*(4), 1027–1034.
496 https://doi.org/10.1016/j.foodchem.2010.03.063

497 Visser, S., Slangen, C. J., & Rollema, H. S. (1991). Phenotyping of bovine milk
498 proteins by reversed-phase high-performance liquid chromatography. *Journal of*
499 *Chromatography A*, *548*(C), 361–370. https://doi.org/10.1016/S0021-

9673(01)88619-2

Vujicic, I., DeMan, J. M., & Woodrow, I. L. (1968). Interaction of polyphosphates and citrate with skim milk proteins. *Canadian Institute of Food Technology Journal*, *1*(1), 17–21.

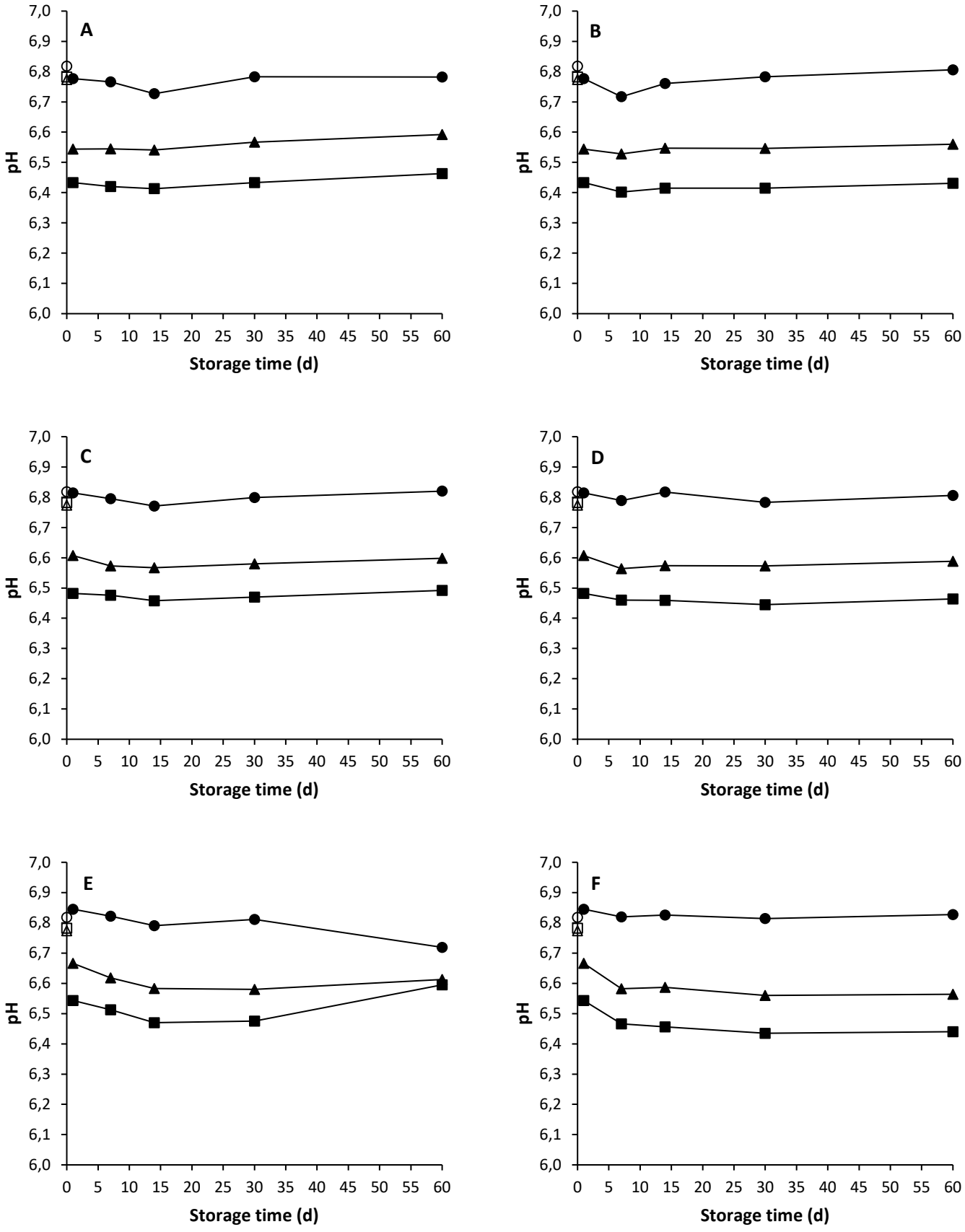

Fig. 1. Influence of in-container sterilization (121 °C for 8 min; A, B) or continuous-flow sterilization treatment at 124 °C for 5 min (C, D) or 140 °C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20°C (A, C, E) or 40°C (B, D, F) on the pH of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to pH before heat treatments.

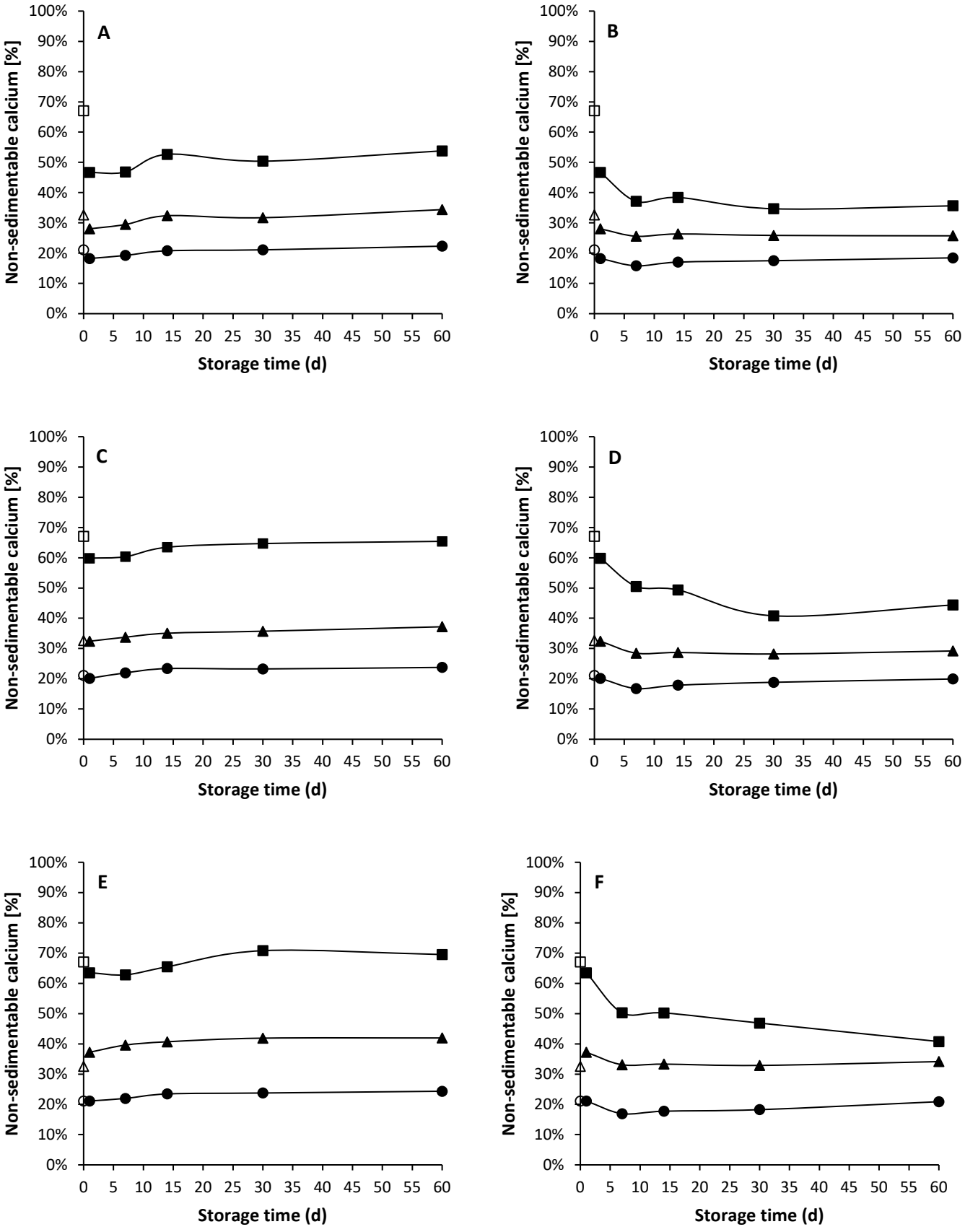

Fig. 2. Influence of in-container sterilization (121°C for 8 min; A, B) or continuous-flow sterilization treatment at 124 °C for 5 min (C, D) or 140 °C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20 °C (A, C, E) or 40 °C (B, D, F) on non-sedimentable calcium (expressed as percentage of total calcium) of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to non-sedimentable calcium before heat treatments.

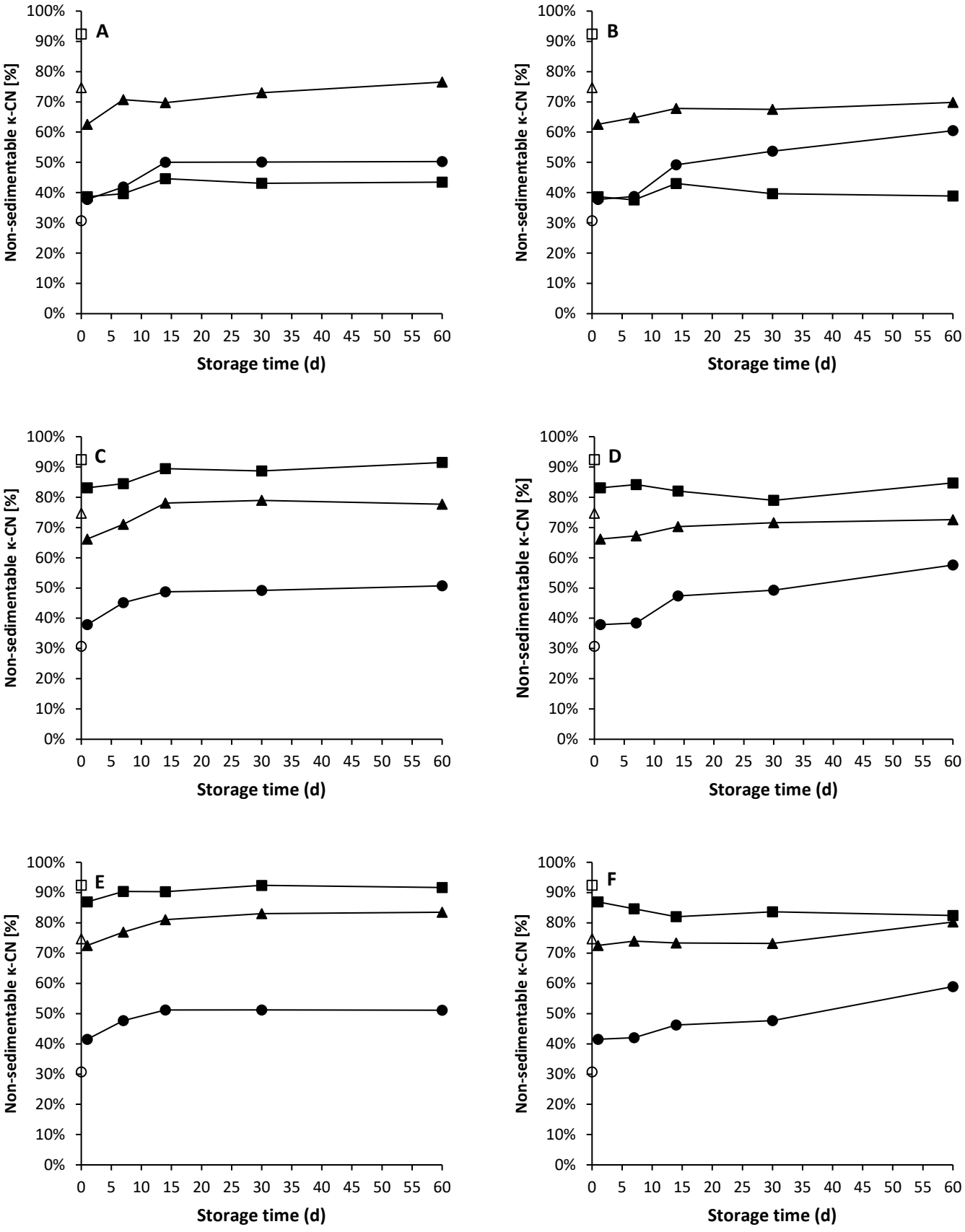

Fig. 3. Influence of in-container sterilization (121 °C for 8 min; A, B) or continuous-flow sterilization treatment at 124 °C for 5 min (C, D) or 140 °C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20 °C (A, C, E) or 40 °C (B, D, F) on the level non-sedimentable κ-casein (expressed as percentage of total κ-casein: κ-CN) of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to non-sedimentable κ-casein before heat treatments.

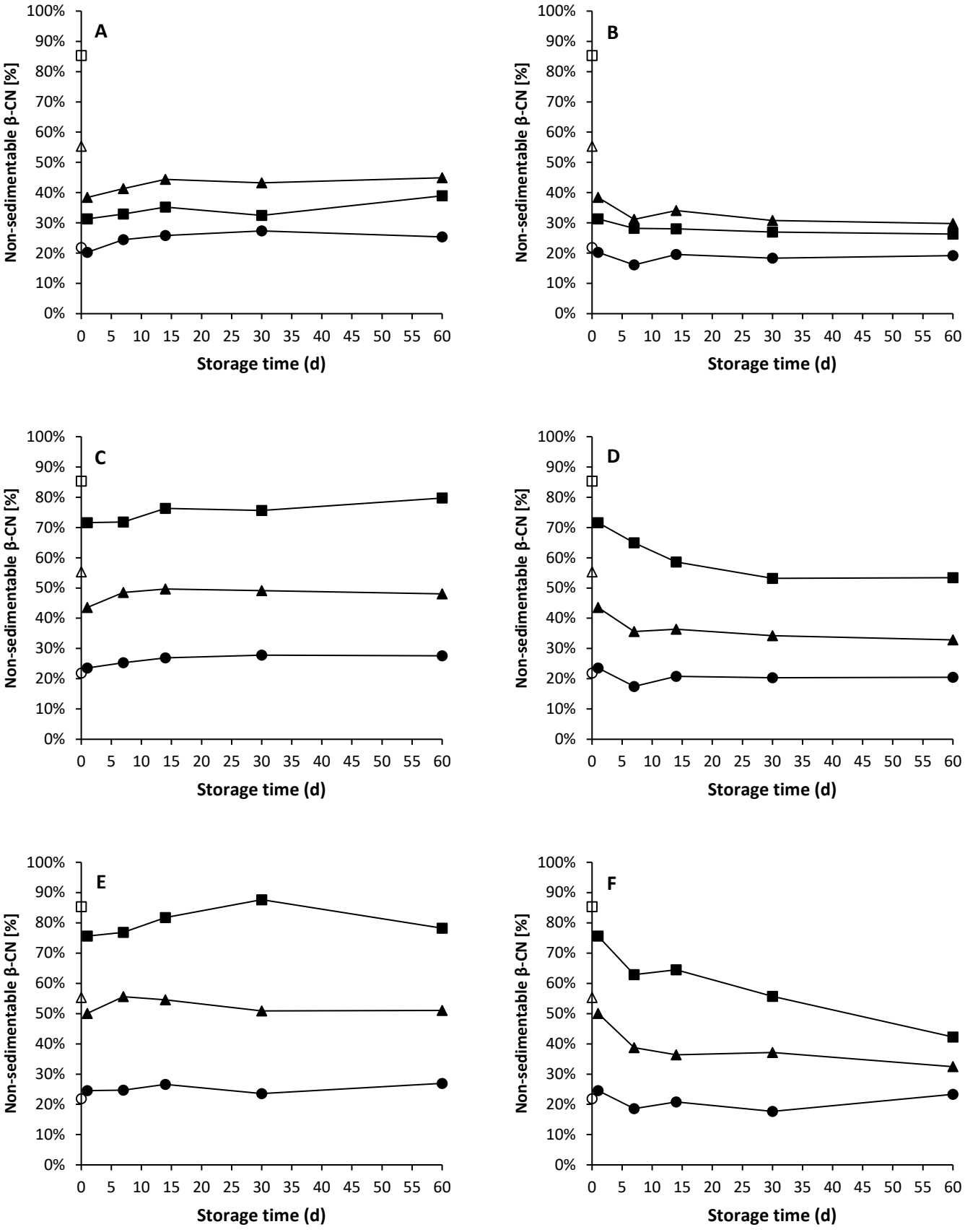

Fig. 4. Influence of in-container sterilization (121°C for 8 min; A, B) or continuous-flow sterilization treatment at 124°C for 5 min (C, D) or 140°C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20°C (A, C, E) or 40°C (B, D, F) on the level non-sedimentable β-casein (expressed as percentage of total β-casein: β-CN) of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to non-sedimentable β-casein before heat treatments.

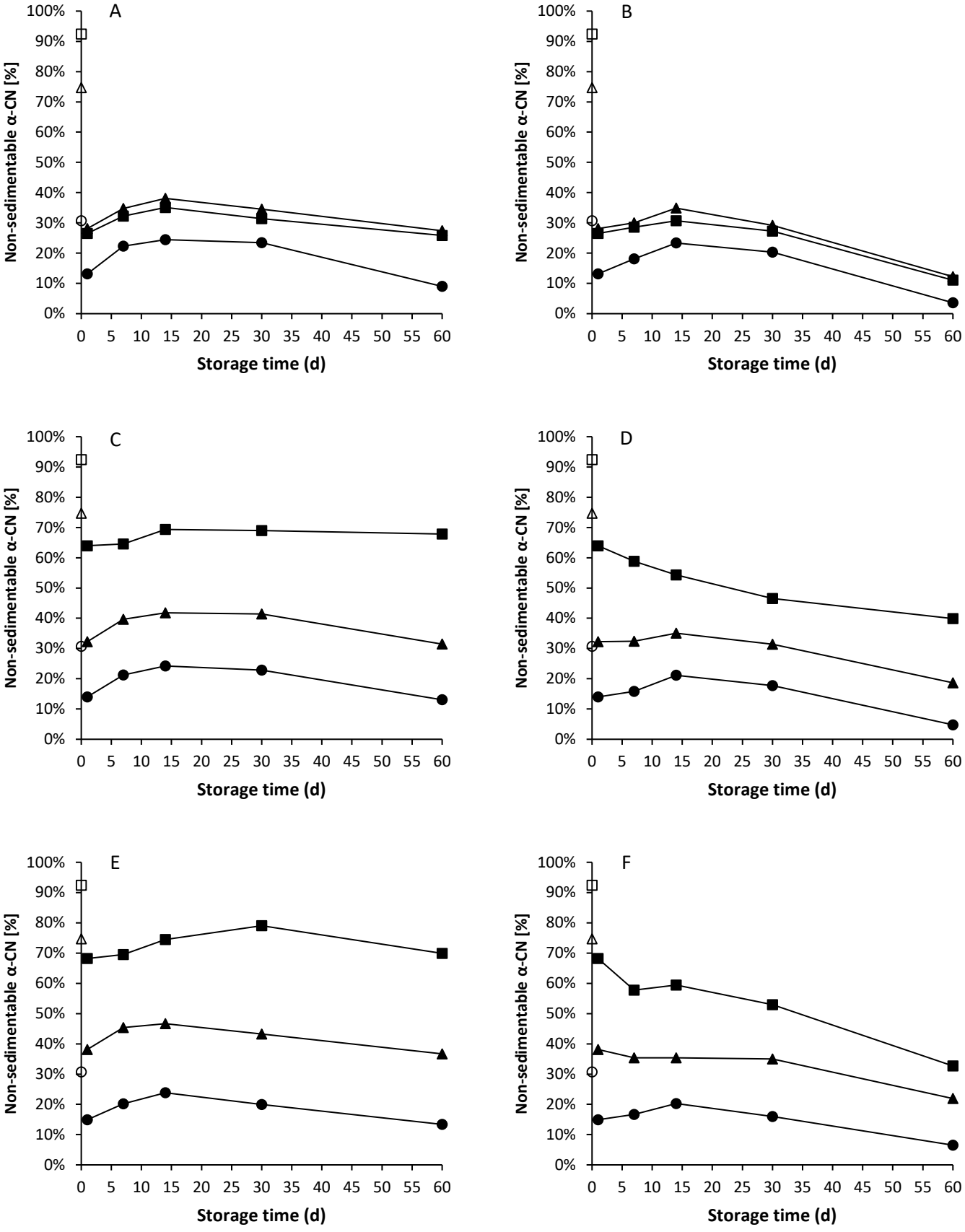

Fig. 5. Influence of in-container sterilization (121°C for 8 min; A, B) or continuous-flow sterilization treatment at 124°C for 5 min (C, D) or 140°C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20°C (A, C, E) or 40°C (B, D, F) on the level non-sedimentable α_s-casein (expressed as percentage of total α_s-casein: α_s-CN) of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to non-sedimentable α_s-casein before heat treatments.

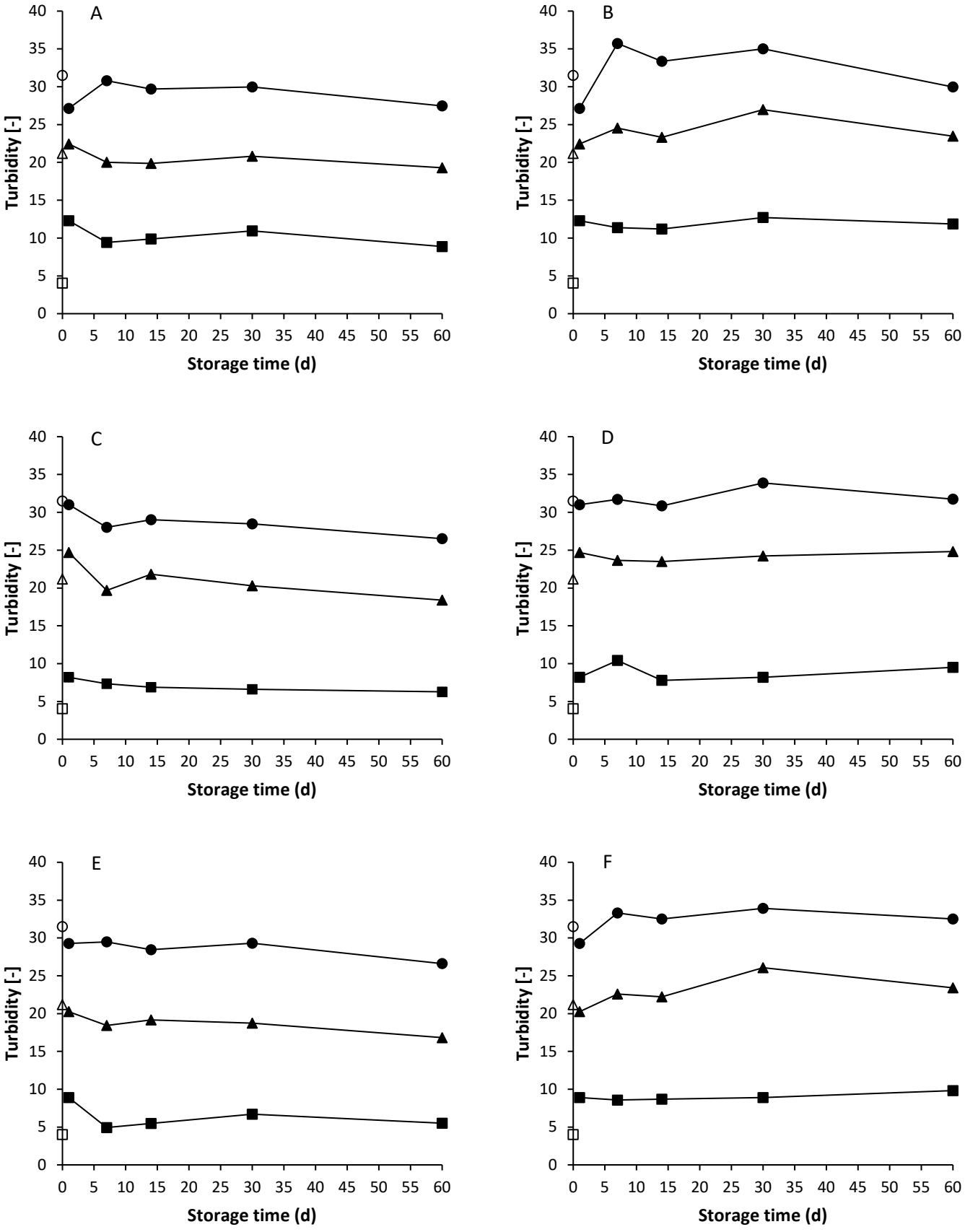

Fig. 6 Influence of in-container sterilization (121°C for 8 min; A, B) or continuous-flow sterilization treatment at 124°C for 5 min (C, D) or 140°C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20°C (A, C, E) or 40°C (B, D, F) on the turbidity at 600 nm of 9% MCI containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to turbidity before heat treatments.

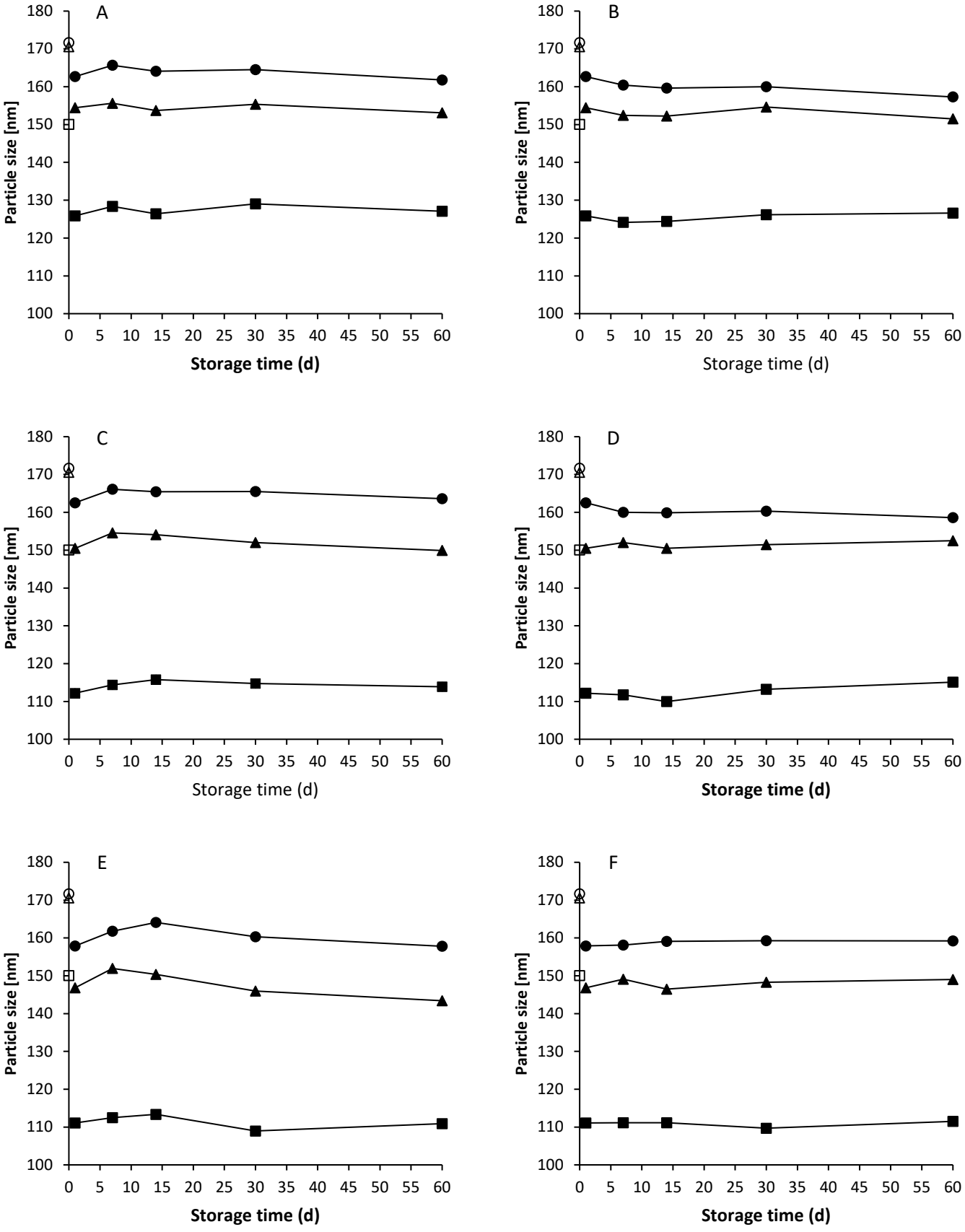

Fig. 7. Influence of in-container sterilization (121°C for 8 min; A, B) or continuous-flow sterilization treatment at 124°C for 5 min (C, D) or 140°C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20°C (A, C, E) or 40°C (B, D, F) on the particle size of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to particle size before heat treatments.

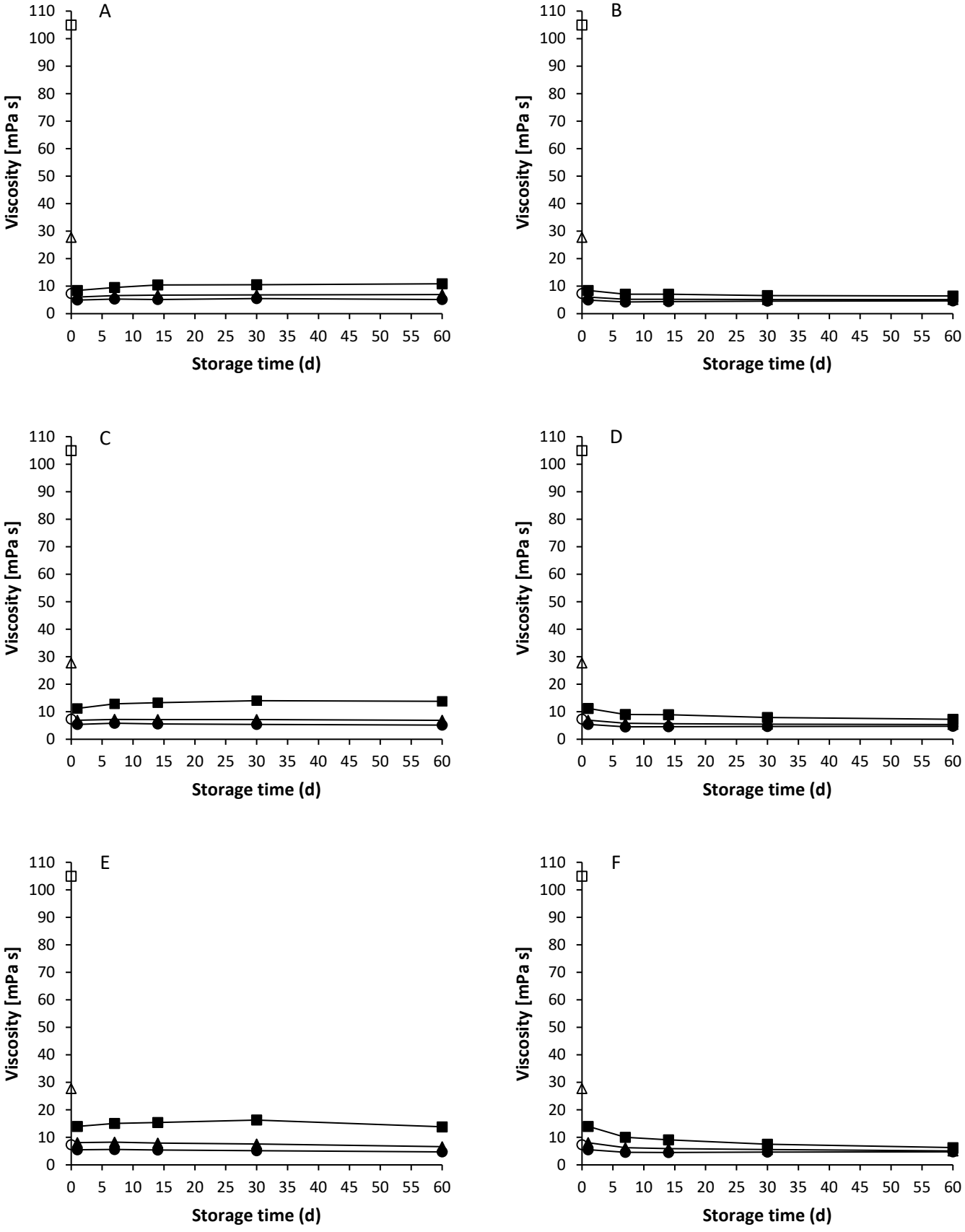

Fig. 8. Influence of in-container sterilization (121°C for 8 min; A, B) or continuous-flow sterilization treatment at 124°C for 5 min (C, D) or 140°C for 5.8 s (E, F) and subsequent storage for up to 60 d at 20°C (A, C, E) or 40°C (B, D, F) on the level viscosity (at a shear rate of 100 s^{-1}) of 9% MCI solutions containing 0 (●), 12 (▲) and 24 (■) mEq L^{-1} of added sodium hexametaphosphate (SHMP). Open symbols correspond to viscosity before heat treatments.