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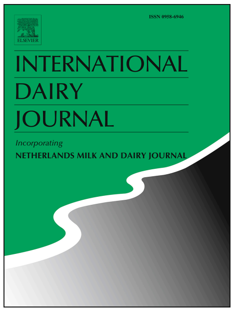

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PII: S0958-6946(20)30250-8

DOI: https://doi.org/10.1016/j.idairyj.2020.104880

Reference: INDA 104880

To appear in: *International Dairy Journal*

Received Date: 5 April 2020

Revised Date: 11 September 2020

Accepted Date: 14 September 2020

Please cite this article as: Pluta-Kubica, A., Černíková, M., Dimitreli, G., Nebesářová, J., Exarhopoulos, S., Thomareis, A.S., Salek, R.N., Buňka, F., Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions, *International Dairy Journal*, https://doi.org/10.1016/j.idairyj.2020.104880.

Influence of the melt holding time on fat droplet size and the viscoelastic properties of model spreadable processed cheeses with different compositions

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ABSTRACT

Spreadable processed cheese (SPC) samples, with 30 and 40% (w/w) dry matter (DM) and 30, 40 and 50% (w/w) fat in dry matter (FDM), were produced with nine individual melt holding times (between 0 and 10 min) and stored for 30 days. Milk fat droplet size and viscoelastic properties were determined. In general, longer holding times resulted in decreased diameter of the milk fat droplets in all tested SPC samples. Furthermore, the size of the milk fat droplets decreased with increasing DM content and decreasing FDM content. Furthermore, for most of the produced SPCs, with the progress of the storage time, the G* values decreased over the first 2 or 3 min (of the applied holding time). In addition, prolonging the holding time and storage period resulted in an increase of the samples G* values. Increased DM content and decreased FDM content in SPC samples resulted in increased G* values.

1. Introduction

According to Codex Alimentarius Commission (2000), processed cheese (PC) is manufactured from one or more varieties of natural cheese. Moreover, other optional dairy ingredients (e.g., anhydrous butterfat, butter, cream, milk powder, whey, buttermilk, caseinates, coprecipitates) or non-dairy ingredients (preservatives, stabilisers, flavouring agents) can be added into the processed cheese blend to improve functional properties or modify composition (Černíková, Nebesářová, Salek, Řiháčková, & Buňka, 2017a; Codex Alimentarius Commission, 2000). Thereafter, the applied raw materials are shredded, blended, melted and emulsified at elevated temperatures in the presence of appropriate emulsifying salts (ES; e.g., sodium, potassium and/or ammonium salts of the citric, lactic, mono-, di- and/or polyphosphoric acids) (Codex Alimentarius Commission, 2000; El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2010). In addition, the relationship between a minimum level of dry matter (DM) and a minimum level of fat in DM (FDM) in PC is also specified by the Codex Alimentarius Commission (2000). However, Codex standards are not legislation. Therefore, on the markets of the European Union (EU), there exist products with DM content lower than the amount required by the standard, whereas they still appear to be named as "processed cheese" (Černíková et al., 2017a). In particular, the above-mentioned products must comply with the internal legal regulations of the individual member EU countries. In general, according to Hickey (2011), legislation on PC and related products varies a lot around the world.

One of the most important stages of PC manufacture is the continuous heating and stirring of the ingredients for a period of time allowing the formation of a homogenous and smooth mass (Fu & Nakamura, 2020). In addition, during blending and melting, ES partially solubilise caseins due to the ion-exchange (calcium to sodium or potassium) phenomenon (Fu et al., 2018b). In particular, the fat present is emulsified and the proteins are hydrated. Both the solubilisation and the hydration of casein, resulting in a temporary loosening of the protein network and a decrease in the viscosity of the melt. However, because of the swelling of the protein units, the protein-protein interactions intensify as the degree of peptisation increases.

The solubilised protein molecules may also associate with lipids. The proteins present in the formed gel network could form hydrogen and disulphide bonds, as well as electrostatic and hydrophobic interactions may occur. Furthermore, denatured β-lactoglobulin can interact with other proteins in the network such as κ-casein and other whey proteins by forming disulphide bridges. These interactions can cause an increase in the firmness of PC and decrease its meltability (Bowland & Foegeding, 2001; Nogueira de Oliveira, Ustunol, & Tamime, 2011). Calcium bridges and calcium-phosphate complexes may also be involved during the processing (Buňka et al., 2014). The re-association of the proteins results in an increase in viscosity.

The creation of the final network of the PC matrix is called creaming. The latter phenomenon is realised during heating, cooling and storage (Dimitreli, Thomareis, & Smith, 2005; Kawasaki, 2008; Lee, Buwalda, Euston, Foegeding, & McKenna, 2003; Mozuraityte, Berget, Mahdalova, Grønsberg, Øye, & Greiff, 2019).

Furthermore, consistency is one of the most important properties of PC and many factors can influence this. The latter factors can be categorised into three main groups: (i) composition of the raw materials applied (type and degree of maturity of natural cheeses, their chemical composition, type and quantity of ES, additional ingredients, etc.), (ii) processing parameters during manufacturing (temperature during melting, speed of agitation, holding time under melting temperature, rate of cooling), and (iii) storage conditions (temperature, time and permeability of the packaging material used) (Černíková, Pachlová, Holas, Moudrá, Slintáková, & Buňka, 2018b; Fu & Nakamura, 2018; Fu et al., 2018a).

The effect of processing parameters, such as holding time of the melt, on the consistency of PC spreads has been studied extensively. Swenson, Wendorff, and Lindsay (2000) investigated fat-free PC (with 40%, w/w, DM content) and stated that, the longer the holding time, the lower the firmness of the product. However, Bowland and Foegeding (2001) examined the effect of processing time (10, 20 and 30 min) on the viscoelastic properties of model PC (49.5–52.5%, w/w, DM; 51.4–54.5%, w/w, FDM) over a decreasing temperature regime from 25 °C to 80 °C (to determine sample solidification). The authors concluded that there was no relationship when the small strain analyses (G´, G´´, G* and δ) were performed at temperatures lower than 80 °C. Moreover, Lee et al. (2003) found that the apparent viscosity of spreadable processed cheese (SPC) melt containing 50% (w/w) DM and 50% (w/w) FDM rose until 25 mins of processing at 80 °C and then decreased. Furthermore, Černíková et al. (2017) and Černíková, Salek, Kozáčková, and Buňka (2018c) investigated the effect of holding time of the melt in a selected temperature on the viscoelastic properties of PC with 35% (w/w) DM and 40% (w/w) and 50% (w/w) FDM content. These authors concluded that the firmness of PC decreased up to the 3rd minute of holding time but then increased significantly (the maximum holding time applied was 20 min). Přikryl et al. (2018) also examined the consistency of PC spreads (37%, w/w, DM and 50%, w/w, FDM) after holding times of 1, 5 and 10 min, they stated that, the longer the melt is maintained at the melting temperature, the more rigid the product becomes.

Nevertheless, the above-mentioned results are contradictory and the effect of holding time on the consistency of PC spreads with different DM and FDM contents remains unclear. Especially, the effect of holding times below 10 min (in close gaps within the holding time range) on SPC samples (with different DM and FDM contents; produced under identical processing protocol) viscoelastic properties described by the complex modulus and phase shift up to now is missing from the existing scientific literature. In general, it is accepted that the short duration of the holding time is economically advantageous. In the present study, model SPCs, manufactured with identical raw materials and under constant processing parameters (temperature, agitation speed) as well as using the same laboratory equipment, were examined. The aim of the research was to determine the effect of the holding time (0, 1, 2, 3, 4, 5, 6, 8 and 10 min) of the SPC melt (at 90 °C) on the size of milk fat droplets and selected viscoelastic properties (complex modulus and phase shift) of model SPC samples with different DM (30 and 40%, w/w) and FDM (30, 40 and 50%, w/w) contents during storage.

2. Materials and methods

2.1. Manufacture of the samples

SPC samples [6 different PC formulations (2 DM × 3 FDM = 6)] × 9 (holding times) = 54 samples in total] were manufactured according to the protocol previously described by Černíková et al. (2017b). The formulation of the PC samples is presented in Table 1. The total weight of the produced SPC samples ranged within the interval of 1105.6 to 1166.4 g per batch. The composition of the ES used was as in the research of Černíková et al. (2017b). However, their total amount was calculated as a constant ratio of ES to protein (0.15). The relative amount of ES applied is given in Table 1. Total masses of ingredients prepared for the manufacture of the SPC samples were calculated to be similar so as to provide comparable heat transfer.

The model SPCs were manufactured under laboratory conditions using a Stephan UMC-5 (Stephan Machinery GmbH, Halmen, Germany) equipped with indirect heating. The target temperature was 90 °C (reached after approximately 12 min of processing) and the mixture was heated under partial vacuum with an agitation speed of 1500 rpm. The applied holding times at 90 °C were: 0, 1, 2, 3, 4, 5, 6, 8 and 10 min (a separate batch of PC for each holding time was prepared). Furthermore, the hot melt (immediately after production) was packed in polypropylene containers (cuboid shape; length: 95 mm, width: 75 mm, height: 30 mm). The weight of the sample in one container was approximately 85 ± 5 g. Containers were sealed with aluminium lids, left to cool down at ambient temperature (target temperature 25 ± 1 °C; approximately 5 h) and then the samples were transferred into a refrigerator (6 ± 2 °C) where they were stored over the whole experiment. The samples were analysed 24 h after the manufacturing and at the 14th and 30th day of storage.

2.2. Basic chemical composition analysis of the samples

The DM and fat contents were determined according to ISO (2004a) and ISO (2004b), respectively. The FDM content of the PC samples was calculated as fat content divided by DM. The pH was measured using a pH-meter equipped with a glass tip electrode (pH Spear, Eutech Instruments, Oakton, Malaysia) into the samples at three randomly chosen locations. Analyses were performed in triplicate.

2.3. Rheological analysis of the samples

A dynamic oscillatory shear rheometer (Rheostress 1, Haake, Bremen, Germany) equipped with a plate-plate geometry (35 mm diameter, 1 mm gap) was used for the determination of the SPC viscoelastic properties. Furthermore, all tested samples were measured in the control shear stress mode at a frequency ranging from 0.05 to 100.00 Hz (at 20.0 ± 0.1 °C). The amplitude of shear stress (20 Pa) was selected in the linear region of viscoelasticity. Additionally, the exposed edge of the parallel-plates geometry was covered with a thin layer of silicone oil to prevent sample dehydration. In oscillatory shear tests, the overall response of the sample may be characterised by the complex modulus $G^* = [(G')^2 + (G'')^2]^{1/2}$, where G´ is the elastic modulus (kPa) and G´´ is the viscous modulus (kPa). The G* describes the total resistance to deformation of a material (considered as an elastic solid) and is therefore, a measure of its consistency (Dimitreli & Thomareis, 2008). Moreover, phase shift is the phase angle between stress and strain. In particular, if $\delta < 45^o$ or tanδ (G´´/ G´) < 1 or G´ > G´´, the material is more elastic than viscous (solid-like behaviour). On the contrary, if $\delta > 45°$ or $\tan\delta > 1$ or $G'' > G'$, the material is more viscous than elastic (liquid-like behaviour) (Dimitreli & Thomareis, 2008; Sołowiej, Cheung, & Li-Chan, 2014).

2.4. Scanning electron microscopy analysis of milk fat droplet size

The analysis of the size of milk fat droplets was performed using a scanning electron microscope JEOL JSM-7401F (Jeol, Japan) and ImageJ software (Wayne Rasband, Maryland, USA). Before viewing the samples were prepared by chemical fixation, dried using Leica EM CPD300 (Leica Microsystems, Austria) (Černíková et al., 2017a) and gold-plated in Sputter Coater SCD 050 (Bal-tec, Liechtenstein). The microphotograph of each sample was analysed to determine the fat droplet diameter (expressed in μm). Each sample was analysed twice (2 repetitions × 3 batches; $n = 6$), and the results were expressed as median ± standard error. The analysis of the size of milk fat droplets of the SPC samples was performed after 30 days of storage.

2.5. Statistical analysis

The results obtained were evaluated using Kruskal-Wallis and Wilcoxon tests (the significance level was 0.05). The chi-square test was applied for the comparison of the fat droplet size of model SPC. Unistat® 6.5 software (Unistat, London, UK) and Microsoft Excel (Microsoft Corporation, Santa Rosa, CA, USA) were used for the statistical analysis.

3. Results and discussion

3.1. Basic chemical composition of the samples

The chemical composition of the model SPC is presented in Table 2. The values of DM were comparable during the 30-day storage time and ranged from 31.11 to 31.39% (w/w) for 30% DM SPC and from 41.09 to 41.49% (w/w) for 40% DM SPC. The calculated FDM levels were also in agreement with the target values (Table 2; $P > 0.05$). Therefore, these samples can be used to determine the effect of the holding time on the size of milk fat droplets and the viscoelastic properties.

Regardless of the combination of DM, FDM and storage time, prolonging the holding time did not significantly affect the pH of samples ($P > 0.05$). Hence, the samples with 40% (w/w) DM and 50%, w/w, FDM stored for 1 day showed pH values in the range 5.66 to 5.76 and 5.62 to 5.72 after 0 and 10 min of holding time, respectively. The ES applied can stabilise the pH of the PC due to high buffering capacity (Fox, Guinee, Cogan, & McSweeney, 2017). Moreover, regardless of the combination of DM, FDM and holding time applied, storage for 30 days resulted in a slight but statistically significant ($P < 0.05$) decrease in sample pH. In addition, the pH of the SPC samples ranged from 5.68 to 5.78 after 1 day and from 5.56 to 5.64 after 30 days of storage in the samples with 30% (w/w) DM and 30% (w/w) FDM produced with 0 min of holding time.

These results are in agreement to those previously reported by Černíková, Nebesářová, Salek, Popková, and Buňka (2018a), Černíková et al. (2017b, 2018c) and Salek et al. (2015). A possible explanation could be hydrolysis of polyphosphate salts, which are more susceptible to the nucleophilic attack of water at pH 5.6 than at pH 6.0 (Barth, Tormena, & Viotto, 2017). The pH values depended on the DM and the FDM content ($P < 0.05$). The lowest values were determined for the samples with 40% (w/w) DM and 30% (w/w) FDM (5.50–5.68 and 5.38–5.57 after 1 and 30 days of storage, respectively), with the highest values for those with 30% (w/w) DM and 50% (w/w) FDM (5.83–6.03 and 5.72–5.91 after 1 and 30 days of storage, respectively) ($P < 0.05$). The current observation could be attributed to higher concentration of lactic acid (from the applied natural cheese – Edam) and ES. Furthermore, the addition of ES promotes an increase in electrostatic repulsion, and greater casein dispersion (or peptisation) might occur (Lu, Shirashoji, &

Lucey, 2008). The pH of PC is affected by a few main factors regarding the applied ingredients and ES: the proportions and types of different raw materials, their acidity and buffering capacity as well as the level, type and buffering capacity of the ES (Fox et al., 2017). The model SPCs examined in this study were manufactured using the same ingredients and types of ES, although in different proportions.

3.2. Viscoelastic properties of the samples

The inner structure of the PC samples was evaluated by the complex modulus and loss angle δ. Hence, the loss angle is related to PC melting properties and provides information about its viscoelastic properties. In addition, higher loss angle values indicate higher degree of flowability (Schädle, Eisner & Bader-Mittermaier, 2020).

The results of the complex modulus (G*) and the phase shift (δ) of the model SPCs are shown in Figs. 1 and 2, respectively. These parameters were not determined for the samples with 30% (w/w) DM and 50% (w/w) FDM at 24 h after manufacturing because they presented very liquid-like behaviour. Furthermore, for most of the SPC samples produced, it was demonstrated that, with longer storage times, the G* value significantly decreased in the first 2 or 3 mins of the holding time ($P < 0.05$). Nevertheless, a different pattern was observed in the sample with 30% (w/w) DM and 50% (w/w) FDM contents, the G* of which was constant up to the 5th min of holding time ($P > 0.05$). In all tested samples, prolonging the holding time (up to 10 min) resulted in an increase of the G* values ($P < 0.05$). Moreover, a similar trend was previously reported by Černíková et al. (2017b, 2018c) in PC with 35% (w/w) DM and 40 or 50% (w/w) FDM contents, respectively. However, the current decreasing trend was identified only in the first three mins of processing ($P < 0.05$). Fu et al. (2018a) found that stirring at 1500 rpm at 90 °C could increase the viscosity of PC after approximately 4–6 min. However, those PCs had pH from 5.8 to 5.9 and higher DM levels (54–55%, w/w) than those which were investigated in the present study.

In addition, during the continuous heating and stirring of the ingredients for SPC manufacture, the formation of a homogenous mass occurred. Firstly, ES solubilise para-casein molecules by breaking calcium phosphate bridges. Then, the fat becomes emulsified and the proteins are hydrated (Mozuraityte et al., 2019). Therefore, casein peptisation could occur during the initial holding time (2–3 min) leading to a decrease in the G^* values of the examined SPC samples. Furthermore, a new protein network needs some time to be created. Probably the presence of milk fat droplets with higher values of diameter could extends this time. In addition, the complex modulus in the samples with 30% (w/w) DM and 50% (w/w) FDM started to increase after the 6^{th} min of holding time and these SPCs were characterised by milk fat droplets of the largest diameter (Table 3, Figs. 1 & 3). Hence, due to swelling of protein units, the interactions between proteins increased and association with lipids may have occurred. Thereafter, the re-association of the proteins during the creation of a new protein network resulted in increasing firmness. The continuous increase in G* values of the SPC samples, observed during the holding time up to 10 min, corresponds to the progressive evolution of the creaming action and may be due to the following reasons. Firstly, the size of milk fat droplets decreases when the holding time is prolonged (Table 3 and Figs. 3 & 4). Moreover, the agitation process causes mechanical stress, which accelerates solubilisation and hydration of the present proteins and peptides (Bowland & Foegeding, 2001; Buňka et al., 2014; Černíková et al., 2018c; Lee et al., 2003). Furthermore, interactions between proteins can be enhanced by calcium ions, which may neutralise the charge repulsion between caseins. On the other hand, interactions may occur by cross-linking or bridging between proteins. The strengthening of interactions between proteins can cause a more rigid structure of PC (Sharma, Munro, Dessev, & Wiles, 2016). In parallel with the increase in G* values, the observed decrease in δ values ($P < 0.05$), during the holding time up to 10 min, showed that SPC samples became more elastic.

Regardless of the combination of DM and FDM applied, the values of the complex modulus increased during the 30-day storage period ($P < 0.05$). A more pronounced increase in G* was observed in the samples with 30% (w/w) DM, as the difference between the 1st and the 30th day of storage was often over 100%. The increase in the G* values could be caused by a decrease of the pH which was most likely a result of hydrolysis of the applied ES or dissociation of other compounds present in the PC (Černíková et al., 2018a). Increasing G* can also be triggered by changes in the crystalline form of polymorphic milk fat (Černíková et al., 2018c). A decrease of pH can cause an increase of hardness of PC when disodium orthophosphate is used as ES, probably be due to the decreased electrostatic repulsion (Lu et al., 2008). In contrast, phase shift values decreased over the 30-day storage period ($P < 0.05$), with SPC samples becoming increasingly elastic.

The complex modulus was also dependent on the DM and the FDM contents. The lowest G* was determined in the samples with 30% (w/w) DM and 50% (w/w) FDM ($P < 0.05$). The G* increased as the DM increased and the FDM decreased ($P < 0.05$). In particular, the highest values of G* were reported in the samples with 40% (w/w) DM and 30% (w/w) FDM. Generally, we could assume that, the higher DM and the lower FDM contents, the more rigid the SPC became and a tougher and less spreadable consistency was seen. This could be probably attributed to the increase in NFS and protein contents and, hence, to the strengthening of the protein network of the samples. Similar findings were demonstrated by Černíková et al. (2017a), Dimitreli and Thomareis (2008) and Guinee and O'Callaghan (2013). Moreover, analysis of the phase shift showed that all SPC samples with 40% (w/w) DM and the those with 30% (w/w) DM and 30% (w/w) FDM, independently of the holding time applied and the time of storage, exhibited more elastic than viscous consistency (solid-like behaviour; phase shift less than 45°). Most of the samples with 30% (w/w) DM and 40% (w/w) FDM also had this feature, except one, the sample manufactured with 2 min of holding time. However, its consistency changed into more elastic after 14 days of storage. The samples with 30% (w/w) DM and 50% (w/w) FDM were found to be more viscous than elastic (liquid-like behaviour; $\delta > 45°$), regardless of the holding time applied and the storage period.

It could be concluded that the DM, FDM contents, holding time and length of the storage time affected the rheological and thus, the sensory properties of the PC samples. In particular, the increasing fat content reduced the values of complex modulus, resulting in softer PC products. Moreover, the PCs with low DM content were more viscous than samples with higher level of DM content. In general, some sensory properties (hardness, gumminess, chewiness and meltability) can be affected similarly such as rheological properties with the prolonging of the storage time. Furthermore, increasing holding time resulted in higher values of the G* modulus.

Furthermore, from an economic point of view, shorter holding times could be evaluated as more advantageous for the producers of PC. However, the production cost for PC manufacture can vary significantly and could be affected by multiple factors (raw materials, energy costs, operation costs, location, inflation, taxes, etc.) which could differ between countries. With respect to the applied ingredients (natural cheese – Edam; butter; water and ES) cost implemented in the current study the estimated production cost (€ kg^{-1}; prices are for year 2019) of 1 kg of final product could be as follows: €1.70, 30% (w/w) DM, 30% (w/w) FDM; €1.57, 30% (w/w) DM, 40% (w/w) FDM; €1.43, 30% (w/w) DM, 50% (w/w) FDM; €2.26, 40% (w/w) DM, 30% (w/w) FDM; €2.09, 40% (w/w) DM, 40% (w/w) FDM; €1.91, 40% (w/w) DM, 50% (w/w) FDM. In general, PC cheese formulation can have an impact on final product price. Hence, higher DM content can result in higher PC price. However, in the case of FDM content (comparing PCs with the same DM content) the higher the FDM content, the lower the price of the final product.

3.3. Scanning electron microscopy of the samples and size of milk fat droplets

The development of the size of milk fat droplets of the model SPCs after 30 days of storage in relation to the duration of the holding time is shown in Table 3 and Figs. 3 and 4. In general, most of the samples presented diameter values lower than 1 μm. Furthermore, similar findings were previously reported by Gliguem, Lopez, Michon, Lesieur, and Ollivon (2011). Regardless of the combination of DM and FDM applied, prolonging the holding time up to 10 min resulted in decreased diameter of the milk fat droplets (*P* < 0.05), probably due to the extended rate of shear. Moreover, a significant difference was observed between 0 and 2 or 3 min of the holding time (*P* < 0.05). According to Sutheerawattananonda, Fulcher, Martin and Bastian (1997) prolonging the holding time can result in a reduction in the diameter of the milk fat droplets over the first 5 min. However, in the aforementioned study, trisodium citrate was used as ES, which strongly chelates micellar calcium, forms soluble complexes, and causes the dispersion of the proteins present, leading to sufficient emulsification of the fat present within the PC matrix (Fu et al. 2018b, Sutheerawattananonda et al. 1997). In addition, according to Fu et al. (2018a,b), longer stirring times result in decreasing size of the milk fat droplets.

The size of the milk fat droplets depended on the DM and FDM contents and also on the processing parameters. In particular, the diameter of the milk fat droplets decreased as the DM content increased and the FDM content decreased (*P* < 0.05). Thus, the smallest diameter of milk fat droplets was observed in the samples with 40% (w/w) DM and 30% (w/w) FDM contents (Figs. 3 and 4). The largest fat droplets were determined in processed cheese samples produced with 0 minute (Figs. 3 and 4, panels A, C, E) of holding time and the smallest fat droplets were present in processed cheeses with the holding time 10 min (Figs. 3 and 4, panels B, D, F). This could be attributed to the viscosity of the melt, which, as it increases, impedes the movement of the fat droplets and contributes to their shearing during stirring. In fact, the more the DM increases and the FDM decreases, the more the non-fat solids (NFS) and the protein contents increase.

The increase in firmness of processed cheeses was explained by Sutheerawattananonda et al. (1997) by reducing the size of fat droplets, where a larger number of small fat droplets disrupt the continuity of the protein matrix less intensely compared with the presence of a smaller number of order of magnitude larger fat droplets. Simultaneously with the decrease in the size of fat beads, those authors found that the stiffness of the monitored samples also increases with increasing holding time. However, the above-mentioned authors also stated that the reduction in the size of the fat droplets stops after about 3 mins of holding. Dimitreli and Thomareis (2004) found that increasing the protein content resulted in higher viscosity values of PC melt. Moreover, the samples with 30% (w/w) DM and 30% (w/w) FDM and those with 40% (w/w) DM and 50% (w/w) FDM did not differ in fat droplet size ($P > 0.05$), as they had similar NFS contents (20.50–22.10%, w/w). Černíková et al. (2017a) reported that also for PC with 35% (w/w) and 45% (w/w) DM and 40% (w/w) and 50% (w/w) FDM the diameter of the milk fat droplets increased with the increasing level of FDM. In addition, Lee, Klostermeyer and Anema (2015) also observed that the milk fat droplet diameter decreased as the DM increased.

4. Conclusions

The study of six different types of model SPCs prepared and stored for 30 days showed that the viscoelastic properties depend on the holding time, time of storage and DM and FDM contents. For most of the produced SPCs, it was demonstrated that, on the 1st, 14th and 30th day of storage, G* (a measure of consistency) decreased in the first 2 or 3 min of the holding time and gradually increased afterwards. In the most cases of DM and FDM contents, prolonging the holding time from the 3rd min up to the 10th min and storage for 30 days increased the G* in all samples examined. Also, G* increased with increasing DM content at constant FDM and also with decreasing FDM. The same DM content and increasing FDM content caused decreasing value of G*. Nevertheless, inverse relationships were observed in the case of the phase shift evaluation. In addition, most of the SPCs produced exhibited more elastic than viscous consistency (solid-like behaviour).

It could be concluded that DM and FDM contents, holding time and length of the storage time affected the rheological properties of the PC samples. In particular, increasing fat content reduced the values of complex modulus, resulting in more soft PC final products. Moreover, the PCs with low DM content were more viscous than the samples with higher level of DM content. This information may be relevant to industry practice. Moreover, longer holding times of the melt can result in smaller diameter of milk fat droplets in the final product. However, a significant decrease in size was observed after 2 or 3 min. Furthermore, the size of milk fat droplets decreased as the DM content increased and the FDM content decreased. In general, from an economic point of view, shorter holding times could be evaluated as more advantageous for producers of PC. In addition, PC cheese formulation can have an impact on final product price, as higher DM content can result in higher PC price. Comparing PCs with the same DM content, the higher the FDM content, the lower the price of the final product.

Acknowledgements

This research was supported by Own Scholarship Fund of the University of Agriculture funded by the Rector of University of Agriculture in Cracow. We acknowledge the core facility HR SEM JEOL7401F, Institution Laboratory of Electron Microscopy, Biology Centre of CAS, České Budějovice, supported by the MEYS CR (LM2015062 Czech-BioImaging) and ERDF (No. CZ.02.1.01/0.0/0.0/16_013/0001775).

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Figure legends

Fig. 1. The dependence of the complex modulus (G*; kPa) of the model processed cheese (PC) 1 day (24 h; ■), 14 days (○) and 30 days (▼) after manufacture using different holding times (0–10 min) at a melting temperature of 90 °C. Panels A, B and C: samples with 30% (w/w) dry matter content. Panels D, E and F: samples with 40% (w/w) dry matter content. Panels A and D, B and E, and C and D: PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Values are expressed as mean ± standard deviation (n = 8).

Fig. 2. The dependence of the phase shift (δ; °) of the model processed cheese (PC) 1 day (24 h; ■), 14 days (○) and 30 days (▼) after manufacture using different holding times (0-10 minutes) at a melting temperature of 90 °C. Panels A, B and C: samples with 30% (w/w) dry matter content. Panels D, E and F: samples with 40% (w/w) dry matter content. Panels A and D, B and E, and C and D: PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Values are expressed as mean ± standard deviation (n = 8).

Fig. 3. Scanning electron microscopy images of processed cheeses (PCs) with 30% (w/w) dry matter content (scale bar 5 µm; magnification 2500×). Panels A and B, C and D, E and F: show PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Panels A, C, E: PCs produced with holding time 0 min. Panels B, D, F: PCs produced with 10 min holding time. FD*, place after milk fat droplets removed; P, protein; IP, insoluble phosphate.

Fig. 4. Scanning electron microscopy images of processed cheeses (PCs) with 40% (w/w) dry matter content (scale bar 1 µm; magnification 10,000×). Panels A and B, C and D, E and F: show PCs with 30%, 40% and 50% (w/w) fat in dry matter content, respectively. Panels A,

C, E: PCs produced with holding time 0 min. Panels B, D, F: PCs produced with 10 min holding time. FD*, place after milk fat droplets removed; P, protein.

Table 1

Formulation of the processed cheese samples with different dry matter content (DM) and fat in dry matter content (FDM) [a].

Raw materials (%)	Type of processed cheese (%, w/w)					
	30% DM			40% DM		
	30% FDM	40% FDM	50% FDM	30% FDM	40% FDM	50% FDM
Dutch-type cheese	53.5	45.4	37.6	71.2	61.0	50.2
Butter	1.1	6.4	11.4	1.6	8.4	15.2
Emulsifying salt components						
Na_2HPO_4	1.0	0.8	0.6	1.3	1.1	0.9
NaH_2PO_4	0.4	0.4	0.3	0.6	0.5	0.4
$Na_4P_2O_7$	0.5	0.4	0.4	0.6	0.5	0.5
Sodium salt of polyphosphate	0.5	0.4	0.4	0.7	0.6	0.5
Water	43.0	46.2	49.3	24.0	28.2	32.3
Emulsifying salts-to-protein ratio	0.15	0.15	0.15	0.15	0.15	0.15
Relative amount of emulsifying salts	2.4	2.0	1.7	3.2	2.7	2.3

[a] The total weight of the melt (g) ranged from 1105.6 to 1166.4; the percentages of emulsifying salts applied were: Na_2HPO_4, 39%; NaH_2PO_4, 18%; Na_4P_2O, 21%; sodium salt of polyphosphate, 22%.

Table 2

Basic chemical analysis of the processed cheese samples with different dry matter content (DM; % w/w) and fat in dry matter content (FDM; % w/w) during 30-day storage. [a]

Parameters	Type of processed cheese (% w/w)					
	30 % DM			40 % DM		
	30 % FDM	40 % FDM	50 % FDM	30 % FDM	40 % FDM	50 % FDM
Dry matter content (%, w/w) [b]	31.27 ± 0.33 [a]	31.39 ± 0.27 [a]	31.11 ± 0.26 [a]	41.49 ± 0.25 [b]	41.09 ± 0.33 [b]	41.36 ± 0.31 [b]
Fat content (%, w/w) [b]	9.19 ± 0.30 [a]	12.75 ± 0.36 [b]	15.83 ± 0.38 [c]	12.43 ± 0.32 [b]	16.58 ± 0.34 [d]	20.70 ± 0.48 [e]
Fat in dry matter content (%, w/w) [c]	29.40 ± 1.10[a]	40.63 ± 0.71[b]	50.88 ± 0.68[c]	29.95 ± 0.78[a]	40.35 ± 0.97[b]	50.04 ± 0.65[c]

[a] For dry matter and fat content expressed as 95% confidence interval for mean of samples manufactured with different holding times and stored 30 days; fat in dry matter content calculated from means of dry matter and fat contents and expressed only as mean values. Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

Table 3

Size of milk fat droplets of model processed cheese samples after 30 days of storage. [a]

Holding time (min)	Size of milk fat droplets (µm)					
	30 % DM			40 % DM		
	30 % FDM	40 % FDM	50 % FDM	30 % FDM	40 % FDM	50 % FDM
0	0.675 ± 0.013 [Fd]	1.027 ± 0.037 [Ee]	1.366 ± 0.064 [Gf]	0.207 ± 0.009 [Da]	0.285 ± 0.007 [Eb]	0.589 ± 0.017 [Fc]
1	0.668 ± 0.025 [Fd]	1.024 ± 0.017 [Ee]	1.355 ± 0.086 [F,Gf]	0.204 ± 0.007 [Da]	0.283 ± 0.009 [Eb]	0.581 ± 0.015 [Fc]
2	0.656 ± 0.029 [Ed]	1.018 ± 0.062 [D,Ee]	1.338 ± 0.042 [Ff]	0.198 ± 0.009 [C,Da]	0.279 ± 0.010 [D,Eb]	0.566 ± 0.014 [Ec]
3	0.613 ± 0.023 [Dd]	1.015 ± 0.037 [De]	1.238 ± 0.079 [Ef]	0.197 ± 0.006 [Ca]	0.272 ± 0.008 [Db]	0.544 ± 0.014 [Dc]
4	0.519 ± 0.024 [Dd]	1.010 ± 0.026 [De]	1.077 ± 0.094 [Df]	0.196 ± 0.006 [Ca]	0.255 ± 0.009 [Cb]	0.504 ± 0.009 [Cc]
5	0.511 ± 0.019 [Cc]	0.991 ± 0.036 [C,Dd]	1.064 ± 0.068 [C,De]	0.195 ± 0.006 [B,Ca]	0.247 ± 0.007 [Cb]	0.500 ± 0.013 [Cc]
6	0.496 ± 0.016 [Cc]	0.951 ± 0.030 [Cd]	1.036 ± 0.048 [B,Ce]	0.193 ± 0.005 [B,Ca]	0.234 ± 0.007 [Bb]	0.492 ± 0.012 [Cc]
8	0.462 ± 0.015 [Bd]	0.916 ± 0.028 [Be]	1.009 ± 0.044 [A,Bf]	0.186 ± 0.006 [A,Ba]	0.230 ± 0.006 [A,Bb]	0.439 ± 0.005 [Bc]
10	0.449 ± 0.021 [Ad]	0.844 ± 0.026 [Ae]	0.993 ± 0.052 [Af]	0.182 ± 0.004 [Aa]	0.224 ± 0.007 [Ab]	0.403 ± 0.020 [Ac]

[a] Abbreviations are DM, dry matter content (%, w/w); FDM, fat in dry matter content (%, w/w). Values are expressed as mean ± standard error (n = 6); means within a column (the difference between the different holding times) and within a row (the difference between the dry matter content and the fat in dry matter content) followed by different superscript uppercase and lowercase letters differ significantly ($P < 0.05$).

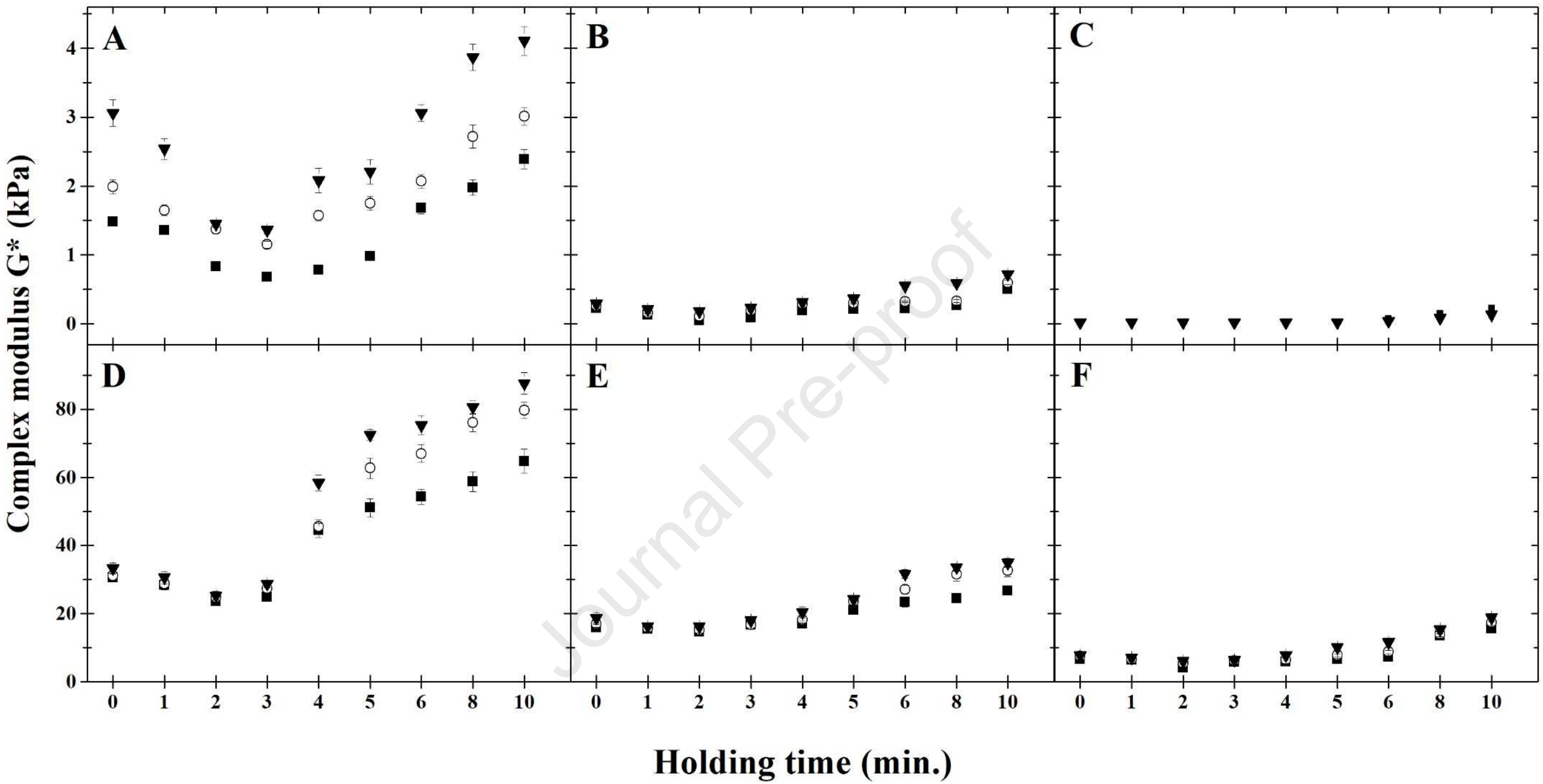
A
B
C
D
E
F
Complex modulus G* (kPa)
Holding time (min.)
0
1
2
3
4
5
6
8
10
20
40
60
80

Figure 1

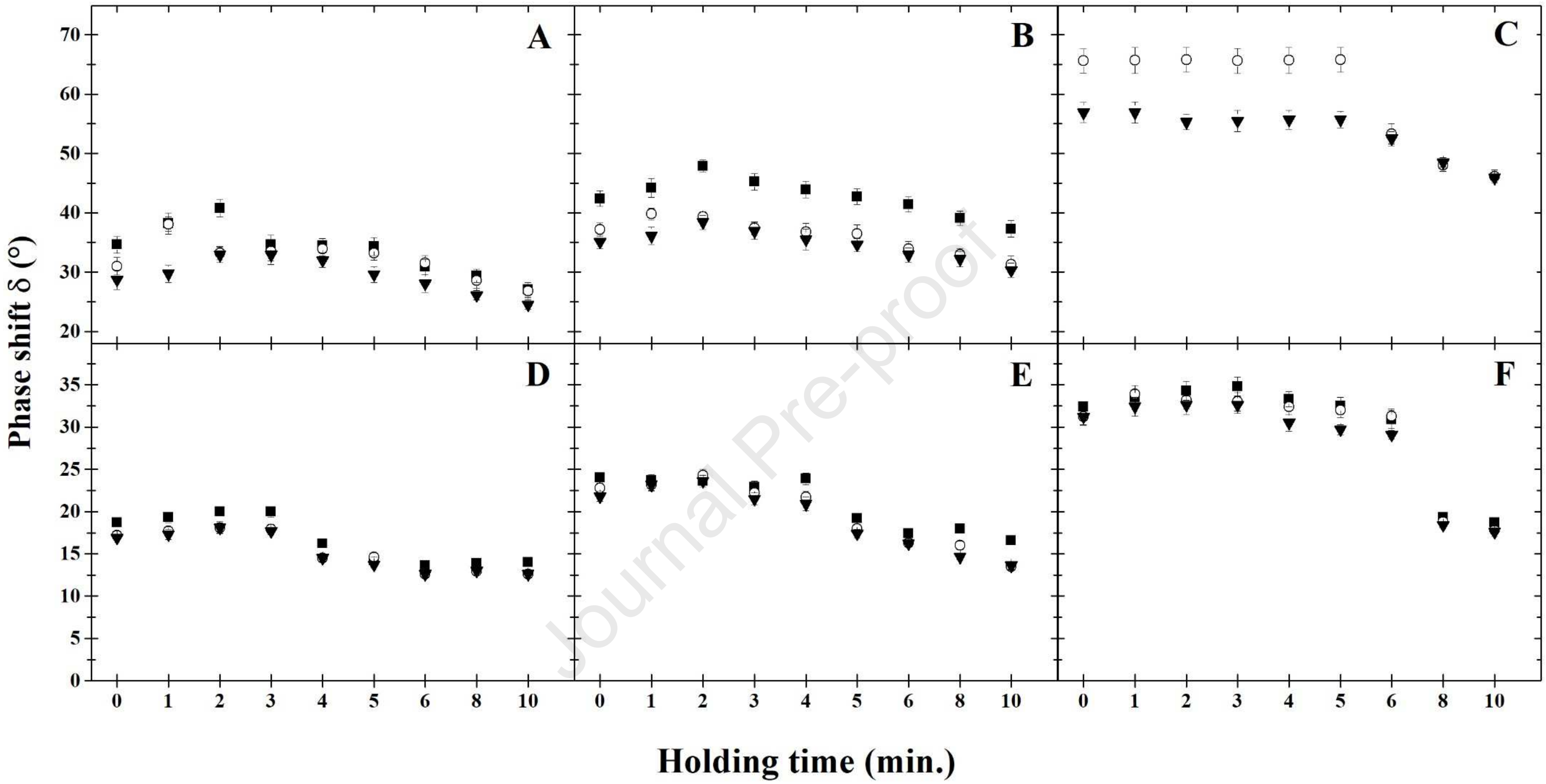
A
B
C
D
E
F
Phase shift δ (°)
Holding time (min.)
70
60
50
40
30
20
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
8
10

Figure 2

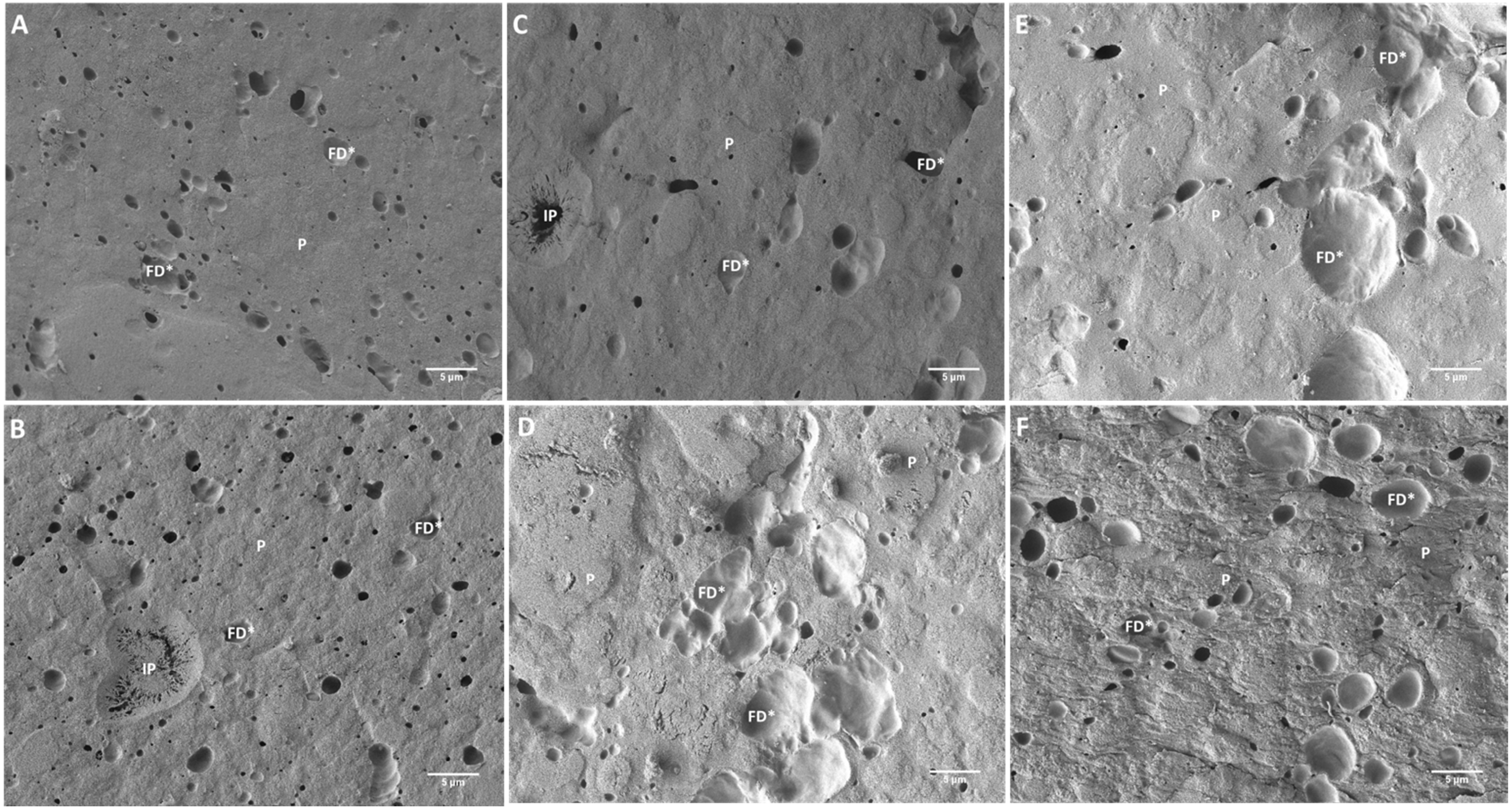
A
FD*
FD*
P
5 µm
C
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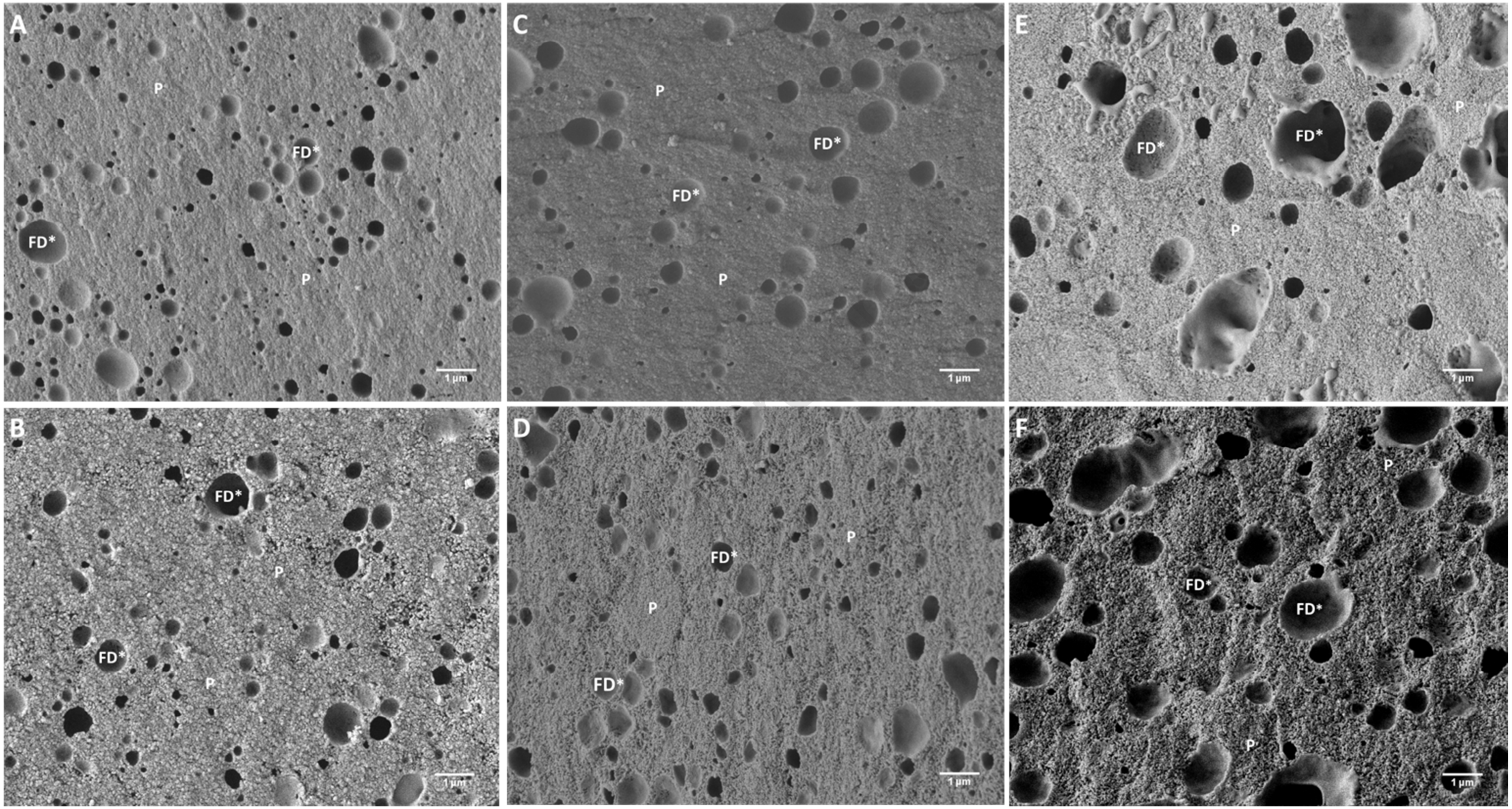
Figure 3


A
P
FD*
FD*
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1 µm
C
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FD*
FD*
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1 µm
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B
FD*
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D
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FD*
P
1 µm

Figure 4