Physicochemical properties of native and extruded maize flours in the presence of animal proteins.

Ángela Bravo-Núñez*, Manuel Gómez

Food Technology Area. College of Agricultural Engineering. University of Valladolid, 34071 Palencia, Spain.

*Corresponding author e-mail: angela.bravo.nunez@gmail.com

Abstract

Interest for protein-enriched starchy products has increased, as protein intake moderately higher than current recommendations may provide health benefits for ageing populations. Influence of the substitution of native or extruded flours by 25% or 50% of proteins (collagen, milk, whey or egg white proteins) was studied. Collagen was the only protein that increased water binding capacity ($p<0.05$) up to 233,72% for mixtures with native and up to 40.85% for mixtures with extruded flours. Regarding gelling properties, regardless used flour, mixtures with 50% of collagen increased water absorption index and swelling power ($p<0.05$) by ~100%, while mixtures with 50% of egg white protein decreased water absorption index by ~40% ($p<0.05$). Pasting viscosity decreased in flour-protein mixtures, except the ones with egg white protein, that increased. Endotherm peaks shifted to higher temperatures when proteins were present. Regarding viscoelastic behaviour, when using native flour and higher protein content, gels presented stronger gel behaviour.

Keywords: Maize flour; pregelatinisation; proteins; dairy; collagen; egg white.

1. Introduction

Functional food products have been defined as foods that provide an added health benefit over and above the food product's traditional nutritional value [1]. In this manner, interest for protein-enriched starchy products has increased by both the food industry and consumers during recent years. A protein intake moderately higher than current recommendations may provide health benefits for ageing populations [2]. There is also convincing evidence stating that diets with higher protein content increase thermogenesis and satiety compared to diets with lower protein content [3], and therefore the substitution of starch, main carbohydrate in cereals, by proteins in weight-loss diets could be interesting in weight-loss diets. Animal proteins, such as dairy, egg and meat proteins, seem to be more digestible than vegetal proteins [4]. In addition to digestibility, amino acids from animal proteins are not as likely to be converted to urea as amino acids from vegetal proteins [5]. Although vegetal proteins have also their benefits, it seems clear that proteins coming from animal sources are more efficiently utilized within the body.

Besides the nutritional aspects, there has been continued research interest in polysaccharide– protein systems because they can function as gelling agents, thickeners, emulsifiers, texture modifiers or stabilizers in foods [6]. The underpinning reason for this research resides in the influence that proteins have in the behaviour of flours. When elaborating starchy products, hydration, pasting, thermal, rheological and gelling properties of flours are important parameters that will influence the behaviour of flours when applying to products. Sometimes these properties are not optimal in native starch, and thus need to be modified by various techniques to better suit the relevant end product [7]. Extrusion is one of these such techniques, as this process not only modifies native starch to achieve changes in the technological behaviour, but also gives clean label products, which is something that many consumers are notably concerned about presently.

Many researchers have reported on the interactions between native flours/starches with animal proteins. Regarding dairy proteins, several authors focused on interactions of different starches with casein proteins [8-12], while others focused on starch interactions with whey proteins [13-17]. In each research study, the percentage of protein used was different, ranging from 0.5% to 75%, making it difficult to draw comparisons between the results from each. To a lesser extent, other animal proteins like egg or collagen have also been studied. Regarding egg protein, its effect on the interfacial properties and foaming capacity of rice starches [18], or the effect of this

protein on the solution and emulsion properties of barley starches [19] were studied, including in both studies chemically modified starches. For collagen, its effect on the mechanical properties of corn starch films have been investigated [20].

Regarding interactions between pregelatinised flour/ starch and animal proteins, only one article was found, and it focused on the interaction with whey protein [21], although the pregelatinised flours where not obtained by extrusion process.

Despite the multiple available reports of the effect of animal proteins on the behaviour of flours /starches, limited information is available for comparing the effect of different animal proteins on the same flours/starches, since experimental conditions and protein percentages are not always the same. Therefore, comparing several proteins simultaneously will guarantee these parameters to be the same, which will help to make reliable comparisons regarding the interactions of different proteins with flours.

The aim of the present work was to analyse the influence of different animal proteins (egg white, whey, milk and collagen proteins) on the properties of two maize flours (native and extruded). Interactions between native and extruded maize flours and different animal proteins (collagen, milk, whey and egg white) were studied by substitution of 25 and 50% of the flour. Flour-protein mixtures were prepared by substitution of 25 and 50% of the flour by commercial proteins powders. The mixtures were dry-blended before analysis. Hydration, pasting, thermal and gelling properties of all the mixtures and controls were studied.

2. Materials and methods

Native (NMF) (moisture and protein content of 11.05 g/100g and 7.6g/100g, respectively) and extruded (EMF) maize flours were provided by Molendum Ingredients (Zamora, Spain). The extrusion treatment was performed with an industrial Bühler Basf single screw extruder (Bühler S.A., Uzil, Switzerland). The length to diameter (L/D) ratio for the extruder was 20:1. The extrusion was carried out with a further water addition of 12% at a maximum barrel temperature of 60 ºC, a feed rate of 500 kg/h and a screw speed of 453 rpm. The extruded product was dried by convection air until 10.09 % moisture and then ground with a compression roller to a particle size bellow 200 µm. Extrusion parameters where chosen by the company based on previous research in order to obtain complete gelatinization of the starch with minimum dextrinization. Animal proteins of milk (mixture of native micellar casein and whey proteins) (≥ 85 % protein)

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(Solmilko MPI, Glanbia, Ballyragget, Ireland), whey (≥ 88 % protein) (Provolon 295, Glanbia, Ballyragget, Ireland), collagen (≥ 94 % protein) (Scanpro T95, BHJ Protein Foods, Gråsten, Denmark) and egg white (≥ 80 % protein) (Egg white powder Specialist, Occhiobello, Italy), with solubility's of 0.33, 0.54, 0.03 and 1.00 mg soluble protein/ mg total protein respectively, measured with the Quick Start™ Bradford Protein Assay (Bio Rad, Hercules, California, United States), were used for the flour-protein mixtures.

2.1. Hydration and gelling properties

Hydration properties were analysed following the methodology of Martínez et al. [22] with modifications. Swelling volume (SV), or the volume occupied by a known weight of sample after hydration with water over time, was evaluated by adding 100 mL of distilled water to 5 g (± 0.1 g) of sample and allowing it to hydrate for 20 h at 25 ℃, values were expressed as volume (mL) per gram of solid. Water holding capacity (WHC), defined as the amount of water retained by the sample without being subjected to any stress, was determined on the same suspension used to evaluate SV; the hydrated solid was weighed after removing the excess of water and values were expressed as grams of water per gram of solid. Water binding capacity (WBC), or the amount of water retained by the sample after it has been centrifuged, was determined by adding 25 g of distilled water to 1.25 g (± 0.1 g) of sample, mixing it vigorously and centrifuging at 580 x g for 10 min. The resulting hydrated solid was weighed after removing the excess of water, and results were expressed as grams of retained water per gram of dry sample. Hydration properties were analysed in duplicate form different mixtures batches.

Regarding gelling properties, water absorption index (WAI), swelling power (SP) and water solubility index (WSI) were determined following the method of Toyokawa et al. [23], with the modifications of Rosell et al. [24]. Samples (50 ± 1 mg) (Wi) were dispersed in 1 mL of distilled water in a 1,5 mL Eppendorf tube with a rod, and heated at 90 °C for 10 min in a water bath. Samples were then cooled down in a water bath with ice for 10 min. Subsequently, samples were centrifuged at 3000 × g at 4 °C for 10 min. Supernatants were decanted in evaporating plates and the remaining residue in the 1,5 mL Eppendorf was weighed (Wr). Supernatants were allowed to dry at 105 °C for 24 h. Dry solids were weighed (Ws). WAI, SP and WSI were calculated as follows:

$$WAI\left(\frac{g}{g}\right) = \frac{Wr}{Wi}$$

$$WSI\left(\frac{g}{100\,g}\right) = \frac{Ws}{Wi} * 100$$

$$SP\left(\frac{g}{g}\right) = \frac{Wr}{Wi - Ws}$$

Gelling properties were analysed in duplicate form different mixtures batches.

2. 2 Pasting properties

Pasting properties of the samples were analysed using a Rapid Visco Analyser (Model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). 3.5 g (±0.1 g) of sample (dry basis) was dispersed in 25 g (±0.1 g) of distilled water. The obtained slurries were then subjected to heating and cooling cycles programmed to follow the general AACC pasting method [25].

All measurements were performed in duplicate.

2. 3 Differential scanning calorimetry (DSC)

Thermal properties of samples were analysed using a Q-20 differential scanning calorimeter (TA instruments, Crawley, UK) equipped with a refrigerated cooling system RCS40 (TA instruments, Crawley, UK). An empty pan and dry nitrogen at a flow rate of 50 mL/min as the purge gas were used as a reference. A weight of 3.0 mg sample (dry basis) together with distilled water (1:2 w/w) were placed in an aluminium pan (T140613) covered with a hermetic lid (T140804). To avoid moisture loss, pans were sealed hermetically and stored for 24 h at 4 ℃ before experimentation to allow for complete hydration of samples. All samples were maintained isothermally at 20 ℃ for 2 min before being heated from 20 to 98 ℃ at 10 ℃/min. Onset temperature (To), peak temperature (Tp), conclusion temperature (Tc) and enthalpy (J/g) of starch gelatinization were determined. All experiments were performed in duplicate from different mixtures batches.

2. 4 Rheological measurements

Samples for dynamic viscoelastic properties were prepared using RVA AACC pasting method [25]. However, the final temperature of the RVA test was set at 30 ℃ instead of 50 ℃. The time between the end of the RVA analysis and the placement of the samples in the rheometer was of 1 min.

Rheological measurements were performed using a rheometer (Haake RheoStress 1, Thermo Fischer Scientific, Scheverte, Germany) equipped with a titanium parallel serrated plate geometry sensor PP60 Ti (60 mm diameter) and 1 mm gap. The sample was placed into the rheometer and stabilized at 30 ℃ with a Phoenix II P1-C25P water bath (Thermo Fisher

Scientific, Schwerte, Germany). To avoid sample drying, space between the plates was covered with Panreac vaseline oil (Panreac Química S.A., Castellar del Vallés, Spain). The pastes rested for 500 s before the test was started. Two dynamic viscoelastic measurements were made: (1) deformation sweeps at a constant frequency (1 Hz) to determine the maximum deformation attainable by a sample in the linear viscoelastic range, and (2) frequency sweeps over a range of 0.01–100 Hz at a constant deformation within the linear viscoelastic range. For each measurement a fresh sample was used in order to avoid different waiting times that could influence the behaviour of the samples. The storage modulus (G'), loss modulus (G'') and loss factor (tan $\delta = G''/G'$) as a function of frequency (ω) were obtained. All measurements were made in duplicate from different batches.

2.5 Statistical analysis

To study the differences between the characteristics of the mixtures according to the percentage and type of both flour and protein analysis of variance (ANOVA) was used. Fisher's least significant difference (LSD) test was used to describe means with 95 % confidence. The analysis was performed using Statgraphics Centurion XVII software (Statpoint Technologies, Warrenton, USA).

3. Results and Discussion

3.1. Hydration properties

Hydration properties (WHC, WBC and SV) are shown in Table 1. When using native flour, only mixtures with collagen enhanced all hydration properties significantly ($p<0.05$), 62.55/73.13 %, 84.30/233.72% and 73.33/100% higher (depending if protein percentage was 25 % or 50 %) for WHC, WBC and SV respectively. Mixtures with milk proteins enhanced significantly ($p<0.05$) values of WBC (50 % for the highest protein percentage) and SV (53.33 and 66.67 % for 25 and 50 % of protein). Significant differences between percentages for these two proteins were only observed for WBC (81.07 % and 37.23 % higher for mixtures collagen and milk proteins respectively when protein content was 50 %). For mixtures with egg white or whey proteins, a decrease in WHC and WBC was observed when increasing protein content, although significant differences ($p<0.05$) were only observed for the highest protein percentage. WHC and WBC were reduced 41.85 and 70.50 % by the presence of egg protein, while the presence of whey protein resulted in reductions of 51.54 and 75.81 %. No significant differences with respect to

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the control were found for SV. These differences can be due to the hydration properties of the proteins alone, since collagen and milk are the ones that presented the higher hydration properties (WHC 177.97 and 172.25 %higher than normal flour), while egg white presented a relatively low values (WHC 45.37 % lower) and whey protein was not able to retain water.

Protein solubility (shown in materials and methods) seems to be related with hydration properties, since these values follow an inversely proportional relationship with protein solubility. In agreement with this, Shin, et al. [26] observed that other more soluble proteins reduced values of WBC of rice flours.

The fact that collagen protein presented the highest hydration properties, alone and when in mixtures, can be explained on the basis of how collagen interacts with water. A collagen fibril can be described as crystalline due to its regular arrangement of identical subunits in the axial direction and semi-crystalline in the lateral direction corresponding to disorder in the packing arrangement of the collagen fibrils [27]. When interacting with water, the water molecules organize in a semi-clathrate-like structure that surrounds and interconnects triple helices in the crystal lattice, where hydroxyprolyl groups play a crucial role in the assembly [28]. This tight interaction of water with collagen may be responsible for the increase in the hydration properties of this protein. The hydration properties were also augmented when using milk protein (composed of native micellar casein and whey proteins), which may be due to the limited solubility of casein proteins, since a drop in solubility of starch-sodium caseinate systems when compared to a native starch was reported by Doublier et al. [9].

When using egg white or whey protein, the reduction of the hydration properties may be due to the amino acid composition of the protein. Amino acid residues with charged groups bind about 6 mol water/mol residue, the uncharged polar residues bind about 2 mol/mol residue, and the nonpolar groups bind about 1 mol/mol residue [29]. The hydration capacity of a protein therefore is related, in part, to its amino acid composition, as the greater the number of charged residues, the greater the hydration capacity. Kuntz & Kauzmann, [30] confirmed this theory, since they reported that both ovoalbumin, the main protein of egg white proteins, and β-lactoglobulin, the main protein of whey proteins, had hydration capacities of 0.30 and 0.45 g water/ g protein, respectively. Their values were much lower than our results for maize flour, and together with the significantly lower hydration values of our egg and whey protein respect

the native maize flour, could justify the observed decrease in our mixtures containing these two proteins.

When using extruded flour, only collagen protein increased significantly ($p<0.05$) WHC, WBC and SV by 10.27/16.03, 14.47/40.85 and 27.71/45.78 % respectively, depending if protein percentage was 25 or 50 %. Significant differences between percentages were only found for WBC and SV.. Milk protein reduced significantly ($p<0.05$) WHC and SV by 24.81/31.04 and 16.87/18.08 % (depending if protein percentage was 25 % or 50 %). WBC was only significantly ($p<0.05$) reduced by milk protein when protein level was 50 %, by 8.51 %. Egg protein reduced WHC 37.79 and 67.18 % when protein level was 25 or 50 %. For mixtures with whey protein, these reductions were 46.18 and 57.25 %. For WBC and SV, mixtures with egg or whey proteins behave similarly. When protein content was 25 % WBC was reduced 25.35 %, and ~57 % when protein content was 50 %. SV was reduced between 35 and 50 %.

The differences observed between mixtures with native flour and extruded flour may be due to the high hydration properties values that extruded flour presents due to its higher levels of gelatinized starch [31]. Since extruded flour presented higher hydration values than native flours, it seems logical to think that when substituting this flour with protein, even though protein will also contribute to the hydration properties, they would be reduced overall. The fact that collagen was the sole protein that increased hydration properties when mixing with both native and extruded flour confirm the theory that proteins also do contribute to hydration properties.

Gelling properties (WAI, WSI and SP) are shown in Table 2. For WAI and SP no significant differences ($p>0.05$) were observed between native or extruded flours. Differences with hydration properties are attributable to the fact that, for assessing gelling properties, mixtures underwent a heat-moisture treatment that affected the functionality of native flour, since inner starch underwent a gelatinizing process. As starch is already gelatinized in extruded flours, in this case it is normal that no significant differences were found. In addition, no significant differences were found between the analogue mixtures with proteins, although WAI values were much higher than WHC values. This is because with the heat treatment proteins denaturize, modifying their capacity to retain water. For WAI and SP, only collagen mixtures with 50% protein presented higher values of both WAI (103.09-111.90 % higher) and SP (95.69-99.71 % higher) in comparison with both native and extruded flours. On the contrary, egg white protein

mixture with 50% of protein presented a lower WAI value (43.42 % lower) with respect the extruded flour. These differences may be related with the capacity of the proteins to retain water after the thermal treatment, being higher for collagen and lower for egg white protein. The mixtures with the other proteins did not present significant differences with the controls, regardless of the protein percentage in the mixtures.

Regarding WSI, which measures the solubility of the mixtures, values extruded flour were 298.11 % higher than values for native flour, although no significant differences were found, probably due to the high WSI of whey and egg proteins alone. Nevertheless, this difference between flours affected the WSI values of protein-flour mixtures, were it was observed that mixtures with extruded flour presented higher WSI values than the analogue mixtures with native flour. These results are in agreement with Hagenimana et al. [32]. These authors explained these differences by the dextrinization of starch during the extrusion process. Egg white proteinenhanced WSI values for both native and extruded flours, 927.67 and 1950.94% with native flour (depending in protein percentage), and 363.67 and 635.07% when mixtures were made with extruded flour. Milk and whey protein enhanced WSI for mixtures with 50 % of protein. Respect native flour, this increase was 657,86 and 1276.10 % for milk and whey proteins respectively; while for mixtures with extruded flour was 233.33 and 441.70 %. On the contrary, collagen did not modify WSI values of the flours, with the exception of the mixture of collagen with extruded flour at 50% of substitution, in which case WSI was reduced to 0.

It can be said that behaviour of collagen protein-flours mixtures provided strong evidence for the high affinity that collagen proteins have with water and their ability to become integrated within the gel matrix, while behaviour of egg white protein-flours mixtures showed the opposite. It was also observed that the results obtained for the protein-flour mixtures are in agreement with the given solubility of proteins.

3.2. Pasting properties

Pasting properties of the mixtures and flours are shown in Figure 1. Native flour presented the typical behaviour of this type of flour with a sudden increase in the viscosity of the paste at temperatures above 70ºC corresponding to starch gelatinisation. Meanwhile, extruded flour showed a higher initial viscosity with no heating, due to its ability of gelatinizing in cold water due to the high levels of gelatinised starch [31], followed by a decrease in viscosity as temperature increased, and a final slight increase in viscosity during the cooling stage. Overall

viscosity of extruded flour was much lower than that of native flour, which is related to the extrusion process that this kind of flours undergo, as it might make the amylose chains lose the ability to retrograde during cooling due to their fragmentation during extrusion [31].

When using native flour, the incorporation of proteins increased the pasting temperature of the mixtures, with a more noticeable effect at higher protein levels. This increase is probably related to the water retention capacity of the samples and to competition phenomenon for available water, as reported by Eliasson [33], who studied the behaviour of wheat starch-gluten protein mixtures. Noisuwan et al. [34] reported that proteins adsorb onto starch granules, and this adsorption would restrict the diffusion of water into the starch granule during pasting, which could affect both pasting temperature and viscosity. For mixtures with native flour, the incorporation of dairy proteins (whey and milk proteins) and collagen decreased viscosity of the RVA curve after gelatinization, while egg white protein had the inverse effect. In all the cases the effect was higher with higher protein content of the mixture. It is known that egg white protein coagulates with a rise in temperature [35], which increases its viscosity [36, 37]; this is a probable reason for the higher viscosity of mixtures with this type of protein than that of the control. For the other proteins, the decrease in overall viscosity is likely due to the reduction in total starch, which is mainly responsible for the increase in viscosity during the gelatinization process. The observed viscosity reduction with mixtures of whey protein is in agreement with the observations of Noisuwan et al. [14] with rice starch and Onwulata et al. [15] with several starches. Regarding casein, a principal component of milk protein, the majority of investigations have observed an increase in the viscosity of the RVA curve with its incorporation [9, 11, 12]. Nevertheless, in these investigations protein was added and not used as a substitution for starch/flour, and therefore the dilution effect of starch was not an existing factor. In fact, Goel et al. [10] observed that while the addition of casein increased viscosity, the substitution of starch by protein had a reduction effect, which is in concordance with our results for milk protein.

When using extruded flour similar trends were observed, although overall viscosity was significantly lower in all the cases. It is important to note the fact that mixtures made with egg white protein showed a clear rise in viscosity respect the extruded flour during heating, which again can be attributed to protein coagulation [35-37]. However, the viscosity increase observed with extruded flour is much lower than that of native ones. This difference may be related to

interaction phenomena between native and egg white protein, or to water competition between the two polymers in the case of extruded flours, with high WHC and WBC.

3.3. Thermal properties

Calorimetric results are shown in Table 3. Mixtures with extruded flours are not shown since these flour did not exhibit a gelatinization peak, which indicates that complete gelatinization of starch was achieved during the previous extrusion process [38]. For native flour mixtures, an enthalpy decrease was observed with the incorporation of proteins. This decrease was more pronounced for mixtures containing collagen, decreasing 23.09 % when protein content was 25 % and 62.01 % when protein content was 50 %. For the rest of the proteins a significant reduction was only observed when protein content was 50%, being 14.34, 50.47 and 20.67 % for egg white, milk and whey proteins respectively. These differences between gelatinization enthalpies (energy needed to dissociate amylopectin double helixes) may be due to the interaction between proteins and amorphous regions of starch granules [39] and water competition between polymers [33]. In fact, differences between mixtures containing collagen and the mixtures with the other proteins may be triggered by the higher hydration properties of this protein, which reduces the available water to a greater extent. Furthermore, decreasing available water for starch shifts endotherm peaks to higher temperatures [40]. Focusing on the endotherm peaks, it was observed that the endotherm shifted to higher temperatures as protein percentage increased, confirming the observations of the pasting properties. This effect is in accordance with observations from other studies of the interactions between starches and soy proteins [41, 42]. In our case this effect was more pronounced for egg white proteins. As reported before, this shift is associated with a competition between protein and starch polymers for water during the gelatinization stage [33]. In our case, differences between proteins cannot only be related with competition phenomena for water, since mixtures with egg white proteins were the ones that presented lower hydration values but experienced the greatest shift in the endotherm peak to higher temperatures (at least 2 ºC more than when the other proteins where present for all To, Tp and Tc). The shift with egg white protein could be driven by the fact that at this temperature range both phenomena of starch gelatinization and egg white protein coagulation overlap [35]. It is interesting to note the temperature range where collagen protein gives an enthalpy peak, as no peak was observed in this range for the flour-collagen protein mixture. Explanation to this may be due to protein-flour interaction, but remains unclear.

3.4. Viscoelastic behaviour

Mechanical spectra of the flour-protein mixtures obtained from the slurries after an RVA cycle are plotted in Figures 2 and 3. For native flour it was observed that G′ values were strongly dependent on the frequency at higher protein contents, while G″ were somewhat more independent but followed the same tendency. Some authors [43, 44] reported that when G′ is independent of the frequency and G″ is somewhat dependent, a weak-gel behaviour results. For extruded flour, the pastes presented weaker behaviour than the ones with native flour. Loss factor increased in all the mixtures as frequency increased, and this increase was more pronounced when using extruded flour. Therefore, both presence of proteins and extruded flour had a negative effect in gels elasticity.

When using native flour, the incorporation of collagen and milk proteins did not modify G" values but did decrease G' values, with this effect being more evident at higher protein levels. Egg white and whey protein did not modify G" and only slightly reduced G' values for protein levels of 25 %. Meanwhile, for levels of 50 % of protein both values increased with respect to the control, being more noticeable for mixtures with whey protein. Yang et al. [17] also reported that whey protein weakened the gel structure of the starch–protein mixtures at low protein content (0.3:1, 0.2:1 and 0.15:1 protein-starch ratios). These authors also observed that this decrease was more pronounced for lower protein levels, which could explain our results at high protein content. For its part, the increase of both G′ and G″ at high egg white protein levels is probably related to heat aggregation of proteins [36].

 When using extruded flour, mixtures presented lower G′ values and similar G″ compared to the control as protein content increased, with the exception of the mixture with egg white protein at 50% protein level, that showed similar G´ and higher G″, again probably due to heat aggregation of proteins [36]. Although rheology was measured from the obtained pastes after an RVA cycle, no correlations were found between final viscosity values of RVA and rheological values. RVA curves showed that the presence of egg white protein increased final viscosity regardless type of flour or protein percentage, while the presence of whey protein decreased final viscosity for both protein levels. Internal gel structure depends on the interactions between proteins and gelatinized starch, which can be developed during gelatinization or subsequently.

4. Conclusion

The type and quantity of protein noticeably affects protein-flour interactions. Collagen protein was the only protein that enhanced hydration properties of both native and extruded flour (WHC was increased by 73.13 % for mixtures with 50 % of protein when flour was unmodified, and by 16.03 % for this same percentage when flour was extruded). Milk protein increased WBC and SV when mixtures where made with native flour, while a decrease was observed when extruded flour was used. Egg and whey proteins reduced hydration properties when mixing with both native or extruded flours. Regarding gelling properties, for WAI and SP no significant differences were found between flours nor between analogue mixtures made with one flour or another. Only collagen mixtures with 50% protein presented higher values of both WAI (~105 % higher) and SP (~97 % higher) than either of the flours. On the contrary, egg white protein mixture with 50% of protein presented a lower WAI value (43.42 % lower) with respect the extruded flour. Regarding WSI, higher values were found for mixtures made with extruded flour than for mixtures made with native flour, most probably because dextrinization of the extruded flour. Pasting properties decreased by the presence of collagen, whey and milk proteins, while egg white protein increased them and also changed the curve-shape for the extruded flour. Endotherm peaks of all mixtures shifted to higher temperatures because of water competition phenomena between polymers and denaturation of proteins, being this shift more evident when mixtures were made with egg protein than when made with the other proteins (2ºC or more). In general terms, it can be stated that behaviour of protein-flour mixtures will affect physical properties of the doughs and batters and final quality of the protein-enriched product. This research can be useful for further development of bakery products with high protein content.

5. Acknowledgements

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Table 1. Hydration properties of native maize flour (NMF), extruded maize flour (EMF) and mixtures.

Flour	Protein	% of protein	WHC (g water/ g solid)	WBC (ml water/ g solid)	SV (g water/ g solid)
NMF	-	-	2.27cd	1.72c	0.30ab
EFM	-	-	7.86l	4.70i	0.83i
NMF	Egg	25%	1.95bc	1.08b	0.36bc
NMF	Collagen	25%	3.69fg	3.17f	0.52efg
NMF	Milk	25%	2.72e	1.88cd	0.46def
NMF	Whey	25%	1.85b	1.07b	0.25a
NMF	Egg	50%	1.32a	0.51a	0.24a
NMF	Collagen	50%	3.93gh	5.74k	0.60gh
NMF	Milk	50%	2.79e	2.58e	0.50def
NMF	Whey	50%	1.10a	0.42a	0.22a
EMF	Egg	25%	4.89i	3.51g	0.54fg
EMF	Collagen	25%	8.70m	5.38j	1.06j
EMF	Milk	25%	5.91k	4.73i	0.69h
EMF	Whey	25%	4.23h	3.51g	0.47def
EMF	Egg	50%	2.59de	2.03d	0.42cd
EMF	Collagen	50%	9.12m	6.62l	1.21k
EMF	Milk	50%	5.42j	4.30h	0.68h
EMF	Whey	50%	3.36f	1.99cd	0.44cde

Water holding capacity (WHC). Water binding capacity (WBC). Swelling volume (SV). Values followed by different letters indicate significant differences.

Table 2. Gelling properties of native maize flour (NMF), extruded maize flour (EMF) and mixtures.

Flour	Protein	% of protein	WAI (g/g)	WSI (g/ 100 g)	SP (g/ g)
NMF	-	-	10.02cde	1.59ab	10.18ab
EFM	-	-	9.58bcde	6.33bc	10.22ab
NMF	Egg	25%	8.27abcde	16.34de	9.89ab
NMF	Collagen	25%	10.44de	6.93bc	11.22ab
NMF	Milk	25%	9.26bcde	6.82bc	9.94ab
NMF	Whey	25%	9.67bcde	9.80cd	10.72ab
NMF	Egg	50%	6.71abc	32.61g	9.95ab
NMF	Collagen	50%	20.35f	-0.13ab	20.33c
NMF	Milk	50%	8.15abcde	12.05cd	9.28a
NMF	Whey	50%	7.62abcd	21.88ef	9.76ab
EMF	Egg	25%	7.25abcd	29.35g	10.26ab
EMF	Collagen	25%	11.69e	12.68cd	13.38ab
EMF	Milk	25%	7.74abcd	32.50g	11.64ab
EMF	Whey	25%	6.35ab	28.37fg	8.84a
EMF	Egg	50%	5.42a	46.53h	10.13ab
EMF	Collagen	50%	20.30f	-1.49a	20.00c
EMF	Milk	50%	7.35abcd	21.10e	9.32a
EMF	Whey	50%	10.00cde	34.29g	15.86bc

Water absorption index (WAI). Water solubility index (WSI). Swelling power (SP). Values followed by different letters indicate significant differences.

Table 3. Thermal properties of native maize flour (NMF) and mixtures with proteins.

Flour	Protein	% of protein	Entalpy (g/J)	To(ºC)	Tp(ºC)	Tc(ºC)
NMF	-	-	5.37d	66.34a	72.59a	83.47a
NMF	Egg	25%	5.22d	69.91c	77.27e	88.94cd
NMF	Collagen	25%	4.13c	67.74bc	74.10b	86.37b
NMF	Milk	25%	5.43d	67.85bc	73.78ab	86.69b
NMF	Whey	25%	5.11d	67.66b	75.96cd	85.90b
NMF	Egg	50%	4.60c	72.30e	81.44f	90.25d
NMF	Collagen	50%	2.04a	68.86cd	77.00de	87.55bc
NMF	Milk	50%	2.66b	69.04d	75.58c	82.82b
NMF	Whey	50%	4.26c	71.11e	77.39e	86.99bc

Onset temperature (To). Maximum peak temperature (Tp). Final temperature (Tf). Values followed by different letters indicate significant differences.

Table 4. Textural properties of native maize flour (NMF), extruded maize flour (EMF) and mixtures with proteins.

Flour	Protein	% of protein	Hardness(N)	Slope (Kg/s^2)
NMF	-	-	4.62f	997h
EMF	-	-	4.40f	631f
NMF	Egg	25%	3.09de	790fg
NMF	Collagen	25%	11.52g	2850i
NMF	Milk	25%	1.21abc	259bcde
NMF	Whey	25%	1.32abc	334cde
NMF	Egg	50%	2.05cd	205abcd
NMF	Collagen	50%	42.41i	4903k
NMF	Milk	50%	0.38a	52a
NMF	Whey	50%	4.13ef	413e
EMF	Egg	25%	1.95bc	342de
EMF	Collagen	25%	4.89f	908gh
EMF	Milk	25%	0.97ab	156abc
EMF	Whey	25%	1.09abc	167abcd
EMF	Egg	50%	1.25abc	227abcd
EMF	Collagen	50%	31.19h	3183j
EMF	Milk	50%	0.40	50a
EMF	Whey	50%	0.472a	95ab

Values followed by different letters indicate significant differences.

Figure 1. RVA curves of flour-protein mixtures of: native flour with 25% (A) and 50% (B) protein content, and extruded flour with 25% (C)and 50% (D)protein content. Flours (black continuous line). Mixtures with milk proteins (black circles). Mixtures with whey proteins (grey triangles). Mixtures with collagen proteins (discontinuous line). Mixtures with egg proteins (spotted line). Temperature profile (Upper continuous grey line). Different scale of y-axis between curves with native flour and extruded flour should be considered.
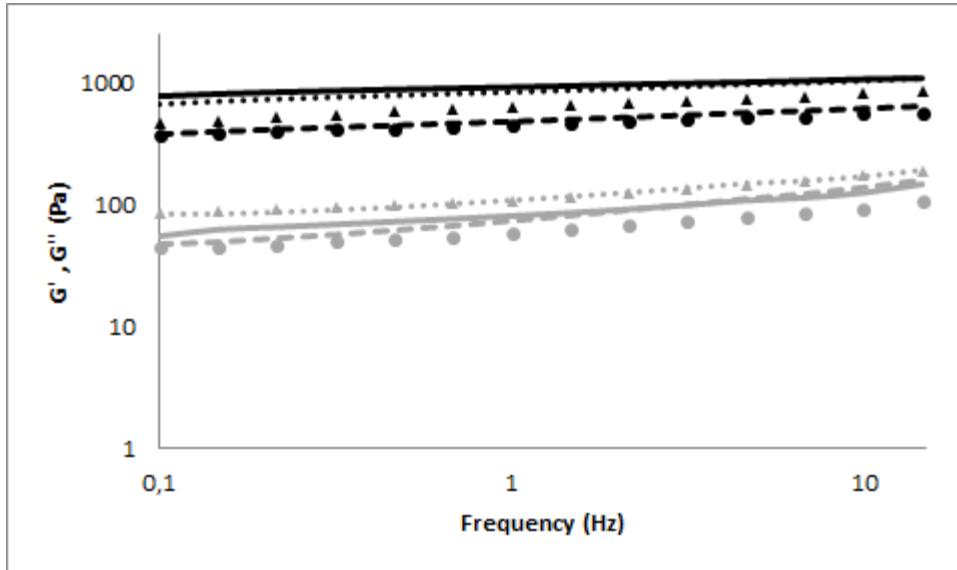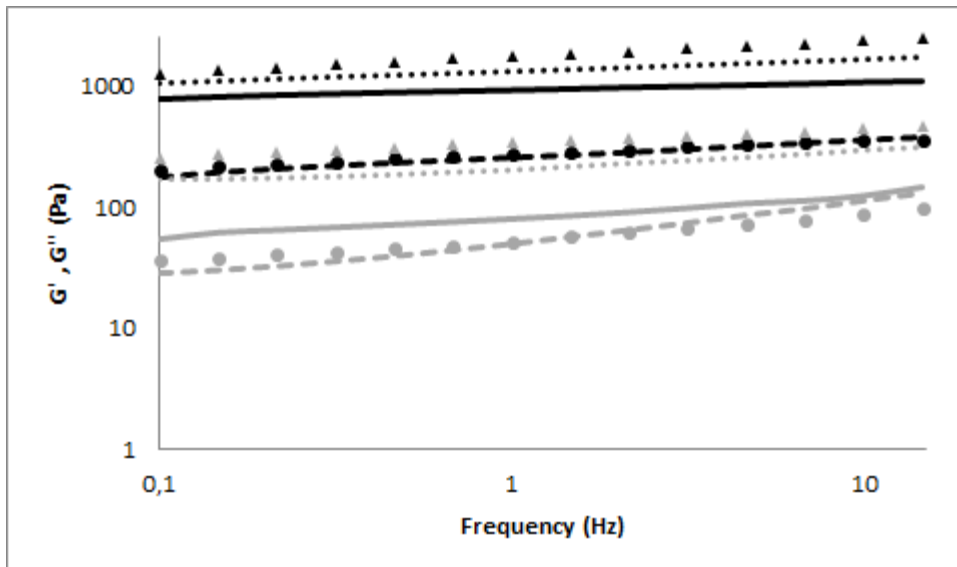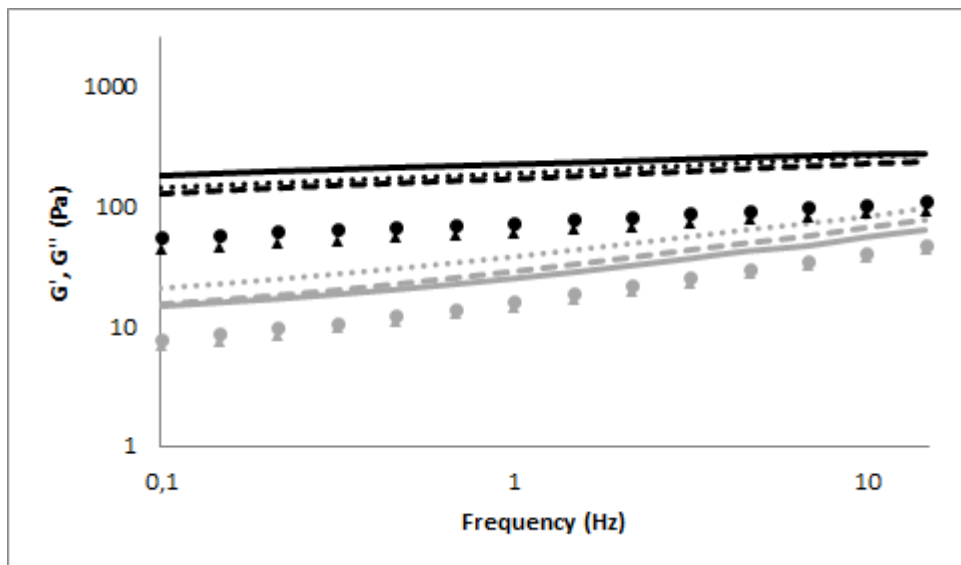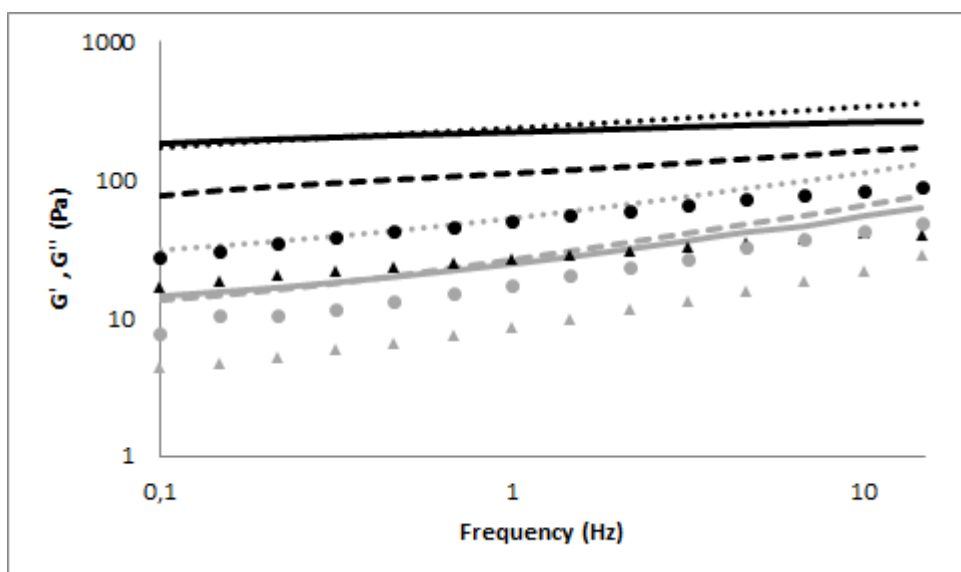
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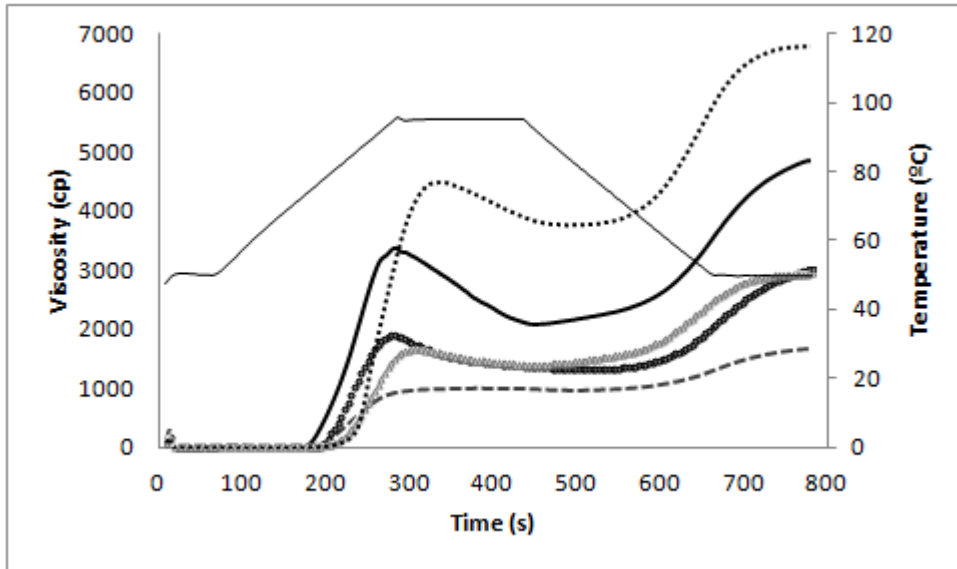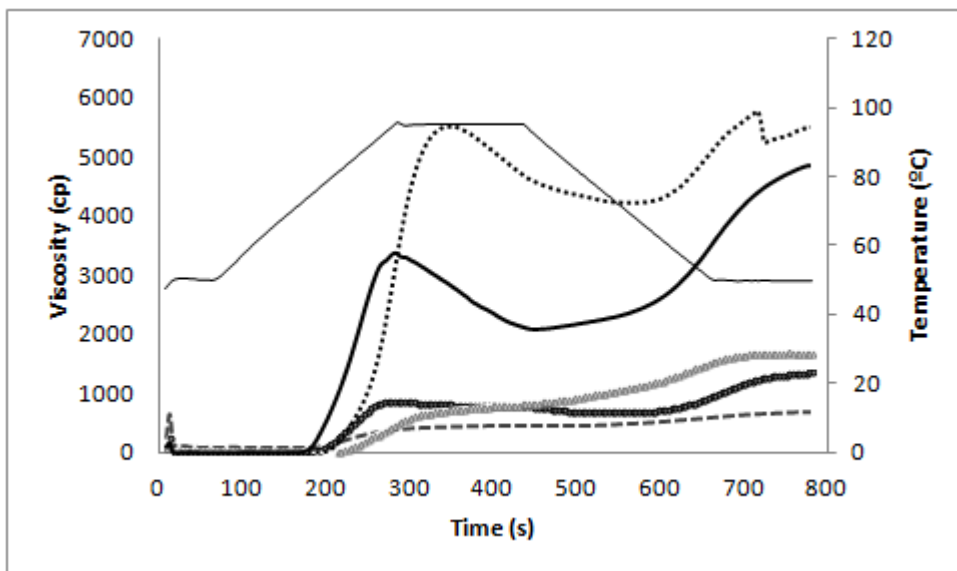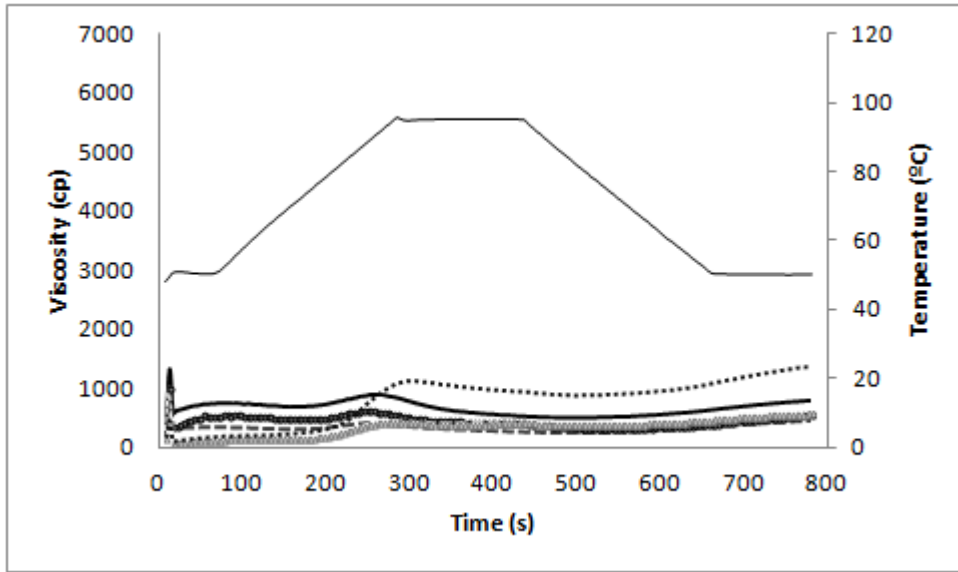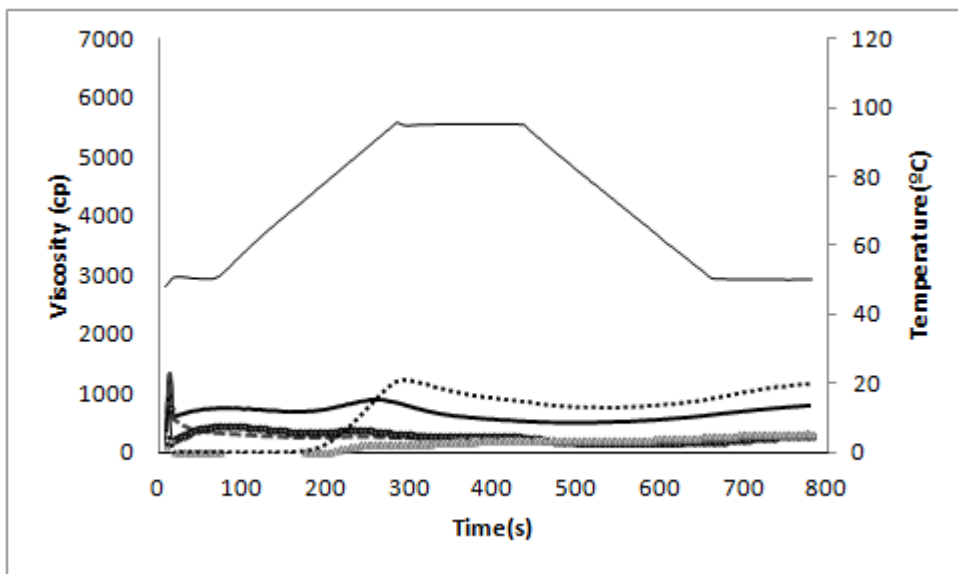
Figure 2. Frequency sweep tests at 30 °C of flour-protein mixtures of: native flour with 25% (A) and 50% (B) protein content, and extruded flour with 25% (C) and 50% (D) protein content. Flours (continuous line). Mixtures with milk proteins (circles). Mixtures with whey proteins (grey triangles). Mixtures with collagen proteins. (discontinuous line). Mixtures with egg proteins (spotted line).
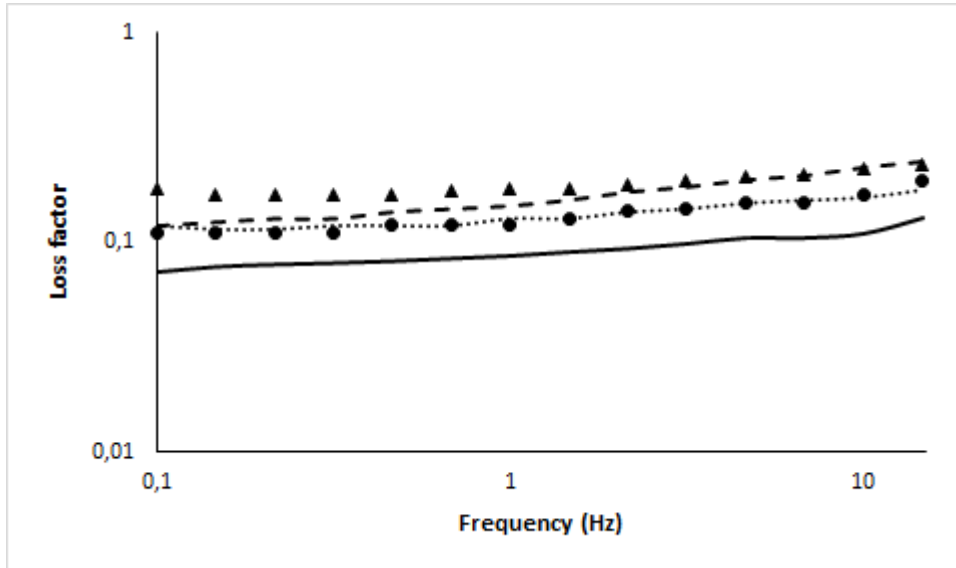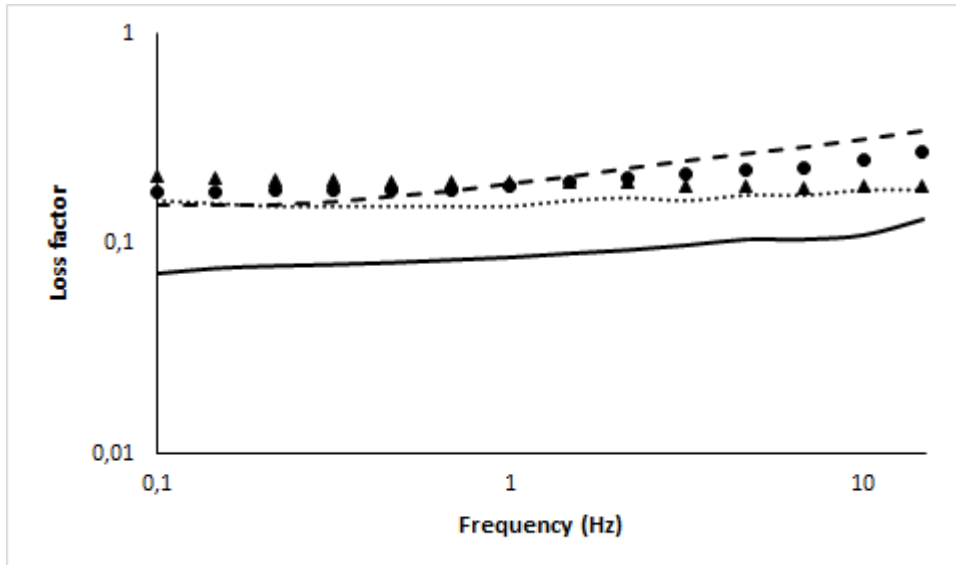
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Figure 3. Loss factor as function of frequency of flour-protein mixtures of native flour with 25%(A) and 50%(B) protein content, and extruded flour with 25% (C)and 50% (D) protein content. Flours (continuous line). Mixtures with milk proteins (circles). Mixtures with whey proteins (grey triangles). Mixtures with collagen proteins. (discontinuous line). Mixtures with egg proteins (spotted line).
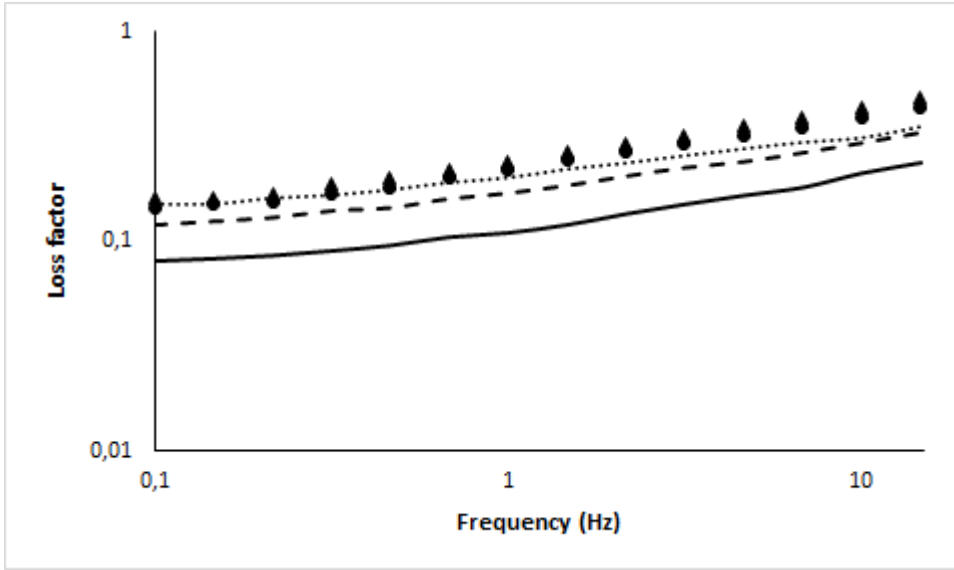
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