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Effect of Calcium Chloride on Rheological Properties and Structure of Inulin - Whey Protein Gels

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***Abstract*—**The rheological properties, structure and potential synergistic interactions of whey proteins (1-6%) and inulin (20%) in mixed gels in the presence of $CaCl_2$ was the aim of this study. Whey proteins have a strong influence on inulin gel formation. At low concentrations (2%) whey proteins did not impair in inulin gel formation. At higher concentration (4%) whey proteins impaired inulin gelation and inulin impaired the formation of a Ca^{2+}-induced whey protein network. The presence of whey proteins at a level allowing for protein gel network formation (6%) significantly increased the rheological parameters values of the gels. SEM micrographs showed that whey protein structure was coated by inulin moieties which could make the mixed gels firmer. The protein surface hydrophobicity measurements did not exclude synergistic interactions between inulin and whey proteins, however. The use of an electrophoretic technique did not show any stable inulin-whey protein complexes.

***Keywords*—** gels, hydrophobicity, inulin, SEM, whey proteins.

I. Introduction

INULIN is a reserve carbohydrate present in the roots and tubers of many *Compositae* and other plant families [1]. From a chemical point of view inulin is a natural fructose polymer with β (2 - 1) glycosidic bonds [2]. When concentrations exceed 15%, inulin has the ability to form particle gels, showing a fat-like texture [3]. It can be used as a fat substitute in many foods like cakes [4], pasta [5] or dairy products [6].

Whey proteins can be used as water binding, emulsifying fat and gel forming components [7]. They have also high nutritional value [8]. Modifying heating conditions, pH, ionic strength, types and concentration of salt, reducing agents, can alter the whey protein gel properties [9]. In neutral pH whey proteins can form gels in two ways. In heat-induced gelation whey proteins are heated in the presence of ions. In cold- or ion-induced gelation salt is added to preheated whey proteins solution to cause gelation [10]. The ion-induced gelation technique was chosen in this study.

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Recently rheological properties of inulin – whey protein mixed systems have been studied. Glibowski and Bochynska [11] examined the rheological properties of liquid or semi liquid mixtures containing whey protein (1-7%) and inulin (1-15%) in the presence of NaCl. The increase of inulin concentration positively affected the viscosity increase of the whey protein – inulin mixtures and the main component responsible for increasing viscosity of the whey protein-inulin mixtures were whey proteins.

At high inulin concentrations (20, 35%) inulin gel formation was strongly affected by whey proteins. The presence of whey proteins and NaCl at a level allowing for protein gel network formation (7% and 180mM respectively) significantly increased the G' and G" values of the gels. Whey proteins at low concentrations (1-4%) were not able to form gel and also impaired or did not allow for formation of firm gel by inulin [12]. Because the mechanism of Ca^{2+}-induced gelation of whey proteins differ from Na^{+}-induced one [10], [13], [14] and to the best of our knowledge there are no studies about interactions of inulin and whey proteins in the presence of calcium chloride the aim of this study was to examine the effect of $CaCl_2$ on rheological properties and structure of inulin - whey protein gels.

II. Materials And Methods

A. Materials

Whey protein isolate (91.23% protein) was obtained from DAVISCO Food Ingredients International (Le Sueur, MN, USA). Inulin Frutafit® TEX! was kindly delivered by Sensus Operations C.V. (Roosendaal, The Netherlands). Inulin was extracted from chicory root and its average degree of polymerisation is ≥ 23 (producers data). All other chemicals used were of analytical grade supplied by POCH Katowice SA (Poland).

B. Preparation of samples for rheological measurements

WPI solutions were prepared by hydrating whey proteins in distilled water, mixed with a magnetic stirrer for 1 h at 21˚C followed by heating in flasks at 80˚C for 30 min. to unfold the whey proteins [15]. Immediately after heating the whey protein solution was transferred into a beaker to add inulin powder. During the dissolving procedure a hot plate magnetic stirrer was used to avoid decreasing the temperature of the mixture. To avoid clump formation, inulin was sprinkled into the beaker through a sieve. The dissolving procedure took

approximately 2 min. To initialize whey proteins gelation, 0.25 M calcium chloride solution was added to obtain in the final whey protein-inulin solutions 7.5 and 15 mM of $CaCl_2$. Finally, the solutions were poured into a plastic cylindrical containers 35 mm in inner diameter. The height of the gel in the container was 50 mm. The lids were affixed to prevent evaporation and the containers were kept for 21 hours at 5˚C in a thermostatic cabinet. The final concentration of whey proteins and/or inulin were 2, 4, 6% (w/w) and 20% (w/w) respectively.

C. Preparation of samples for hydrophobicity analysis

One part of the samples was prepared according to *B* section. The second part of the samples was prepared by hydrating inulin powder in water at 95˚C followed by cooling, then farther dissolving the whey proteins and heating the mixture in flasks at 80˚C for 30 min. Subsequently, the heated solutions were cooled down in tap water and water and/or calcium chloride solution was added to obtain the final solutions containing 6% (w/w) whey proteins, 20% (w/w) inulin and 15 mM of $CaCl_2$.

D. Rheological measurements

The apparent viscosity and dynamic oscillatory rheological measurements were conducted using a Haake RS 300 rheometer (Haake GmbH, Karlsruhe, Germany) equipped with parallel plate geometry (both 35 mm diameter and serrated). The samples were analysed after their storage for one hour at room temperature. Most of the samples had spreadable consistency. Elastic samples were sliced into 1 mm slices. When the sample was placed on the plate, the lift moved and the upper plate took the measuring position (1 mm gap). All rheological experiments were conducted at 20˚C. Temperature control was maintained by a Haake DC30 circulator water bath (Haake GmbH). The apparent viscosity was measured at 20 s^{-1} shear rate for 120 s. For analytical purposes the average value was calculated from the 90^{th}, 105^{th} and 120^{th} second of measurement. In the case of elastic samples the results of the apparent viscosity measurements were only approximation because of the sample fracture and were marked in the figure with an asterisk. Small strain measurements were conducted at a frequency of 1.0 Hz and a strain of 0.001. The used strain corresponded to the maximum found within the linear viscoelastic region of the studied material.

E. Surface hydrophobicity

Protein surface hydrophobicity was measured using 8-anilino-1-naphthalenesulfonic acid (ANS) according to the method previously described by Glibowski *et al*. [14]. Briefly, aliquots (20 μL each) of 8 mM ANS stock solution were added to 4 mL of diluted samples ranging in protein concentration between 0.005 – 0.025% (w/w). The relative fluorescence intensity (RFI) of each solution was measured, starting from the lowest to the highest concentration, using a Shimadzu F-5000 spectrofluorometer (Shimadzu Corporation, Kyoto, Japan), with excitation and emission slits set at 5 nm, and an excitation wavelength of 390 nm and emission wavelength of 470 nm. Surface hydrophobicity was expressed as the initial slope of the plot of RFI values vs. % protein concentration, computed by least squares linear regression analysis (Microsoft Excel 5.0, Microsoft, Redmond, WA, USA).

F. Scanning electron microscopy (SEM)

To examine the microstructure the samples were prepared according to *B* section. The samples were plunged into a liquid nitrogen slush at – 205°C. The frozen samples were transferred under vacuum into an attached preparation chamber where they were freeze etched. Afterwards the samples were sputter coated with 5 nm of gold. Sample observations were performed in a LEO 1430 VP (Cambridge, UK) operating at 15kV voltage.

G. Statistical analysis

Hydrophobicity, apparent viscosity and dynamic oscillatory rheological measurements were completed in three independent trials. Each apparent viscosity analysis was performed in triplicate. Dynamic oscillatory rheological data correspond to the average of the measurements. Hydrophobicity and apparent viscosity data were analysed by the Statistical Analysis System (SAS Enterprise Guide 3.0.3.414) using the ANOVA procedure for analysis of variance and Student-Newman-Keuls t-test for ranking the means.

III. Results And Discussion

Fig. 1 shows mechanical spectra of 20% inulin and/or 6% whey protein gels with 15 mM $CaCl_2$. Storage modulus (G') is related to elastic properties and loss modulus (G") is associated with viscous properties of the analyzed material [16]. All analysed samples were gels because they revealed higher values of the elastic modulus than the storage modulus. The highest moduli values were recorded for inulin-whey protein gels with $CaCl_2$. G' and G" values for the other samples were significantly lower. The presence of whey protein without calcium chloride caused the gel to exhibit lower elastic properties than the inulin gel with $CaCl_2$. These results may suggest a weakening effect of the whey protein on inulin gel formation. This tendency is in accordance with the results of the previous work which described rheological properties of inulin whey protein mixed gels with NaCl [12].

To explain the effect of whey proteins on rheological properties of inulin gels, apparent viscosity measurements were carried out (Fig. 2). 2% whey proteins addition did not have significant ($p \leq 0.05$) influence on apparent viscosity. 4% addition caused relevant drop in apparent viscosity value whereas samples with 6% whey proteins addition revealed significant increase of apparent viscosity even though the placed data are only an approximation, samples with 6% whey proteins addition were elastic gels, which was connected with rupturing the samples during analysis. In the previous study Glibowski [12] noticed a similar tendency. In the NaCl presence, increase in whey protein concentration up to 3% caused significant decrease in the rheological parameters values but farther increase in whey protein addition caused increase in apparent viscosity, G' and G" values. Although Na^+-induced gelation differs from the Ca^{2+}-induced one [10],

apparently, in inulin-whey protein mixed gels the type of salt does not change the tendency. Much lower concentrations of divalent ions are needed to cause aggregation than monovalent ions [13], [15] because they are much more effective at screening electrostatic interactions and because of their ability to form salt bridges [10].

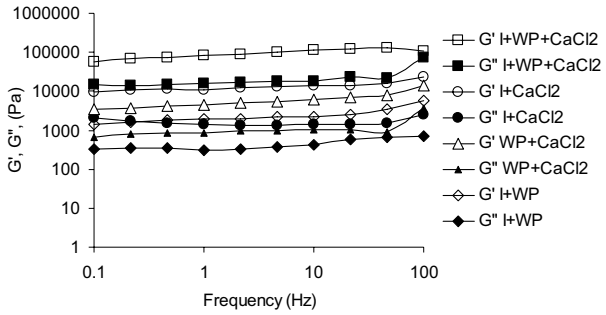

Fig. 1. Mechanical spectra of 20% (w/w) inulin (I) and/or 6% (w/w) whey protein (WP) gels with 15 mM $CaCl_2$

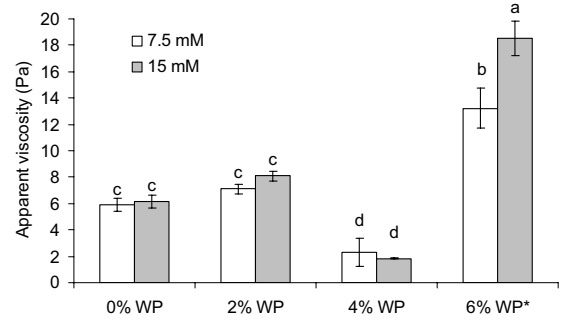

Fig. 2. Apparent viscosity (± S.D.) of the 20% (w/w) inulin gels with addition of 2, 4, 6% (w/w) whey proteins (W) at 7.5 and 15 mM $CaCl_2$.
[a-d]Means with different superscripts are significantly different, $p \leq 0.05$.
* Elastic samples

In inulin-whey protein mixed gels whey proteins at low concentration do not influence significantly on rheological properties of the mixed gel. Such low concentration (2%) probably does not impair in inulin gel formation. Increasing whey protein concentration is more and more important and finally it does not allow for gel formation by inulin at all. 4% whey protein addition is big enough to impair inulin gelation and although at this concentration of whey proteins an calcium chloride it is possible to form Ca^{2+}-induced gel [15] inulin apparently impairs formation of rigid structure. 6% addition of whey proteins causes significant increase in apparent viscosity. This concentration is not only sufficient to form stable three dimensional structure but such formed gel reveals significantly higher rheological parameters values (Fig. 2).

Because rheological properties of inulin gel with 6% whey protein addition differ significantly in comparison to samples containing inulin alone, scanning electron microscopy was used to examine the microstructure of the inulin, Ca^{2+}-induced whey protein and mixed gels (Fig. 3). The structure of the inulin gel is quite compact (Fig. 3 A i B) whereas the structure of whey protein gel is a porous one (Fig. C i D). In the mixed gels it is difficult to distinguish protein and inulin phases at the applied microscopic technique (Fig. E i F) however the formed structure is less porous than in Ca^{2+}-induced whey protein and more compact than in inulin gel. It can be supposed that the formed whey protein structure was coated by inulin moieties which was also observed in the Na^{+}-induced whey protein-inulin mixed gels [12].

Compact structure of Ca^{2+}-induced whey protein-inulin mixed gels puts a forward question regarding potential interactions between inulin and whey proteins. Previous findings suggest interactions between inulin and β-lactoglobulin, the most abundant whey protein, although α-lactalbumin, the second in abundance whey protein, did not interact with inulin [17].

Mixed gels obtaining procedure based on inulin dissolving followed by heating of whey protein solution. For hydrophobicity measurements, besides earlier mentioned procedure, whey proteins were also heated for 30 minutes together with inulin. Extending the time of contact between inulin and whey protein in dissolved state could intensify formation of potential complexes. For rheological measurements inulin was only dissolved in a hot preheated whey protein solution. Long heating of inulin solution has negative impact on rheological properties of final inulin gels [18].

Heating of whey proteins caused an increase in hydrophobicity values (Table I) as a consequence of exposing hydrophobic amino acids residues buried in the interior of globules [10]. Inulin dissolving in the preheating whey protein solution did not change S_o value significantly ($p \leq 0.05$)

TABLE I
CHANGES IN SURFACE HYDROPHOBICITY OF WHEY PROTEINS (WP) AS A FUNCTION OF INULIN AND $CaCl_2$ ADDITION

	So ($\%^{-1}$)	SD
Unheated WP	849.3[c]	50.7
Heated WP	1223.8[a b]	118.7
Heated WP + unheated inulin	1318.1[a]	124.4
Heated WP + heated inulin	1122.1[b]	104.9
*Heated WP + $CaCl_2$	161.1[d]	7.5
*Heated WP + $CaCl_2$ + unheated inulin	203.0[d]	8.5
*Heated WP+$CaCl_2$ + heated inulin	244.3[d]	14.6

[a-d] Means with different superscripts are significantly different, $p \leq 0.05$.
* Elastic samples

however, heating inulin together with whey proteins caused a considerable decease in surface hydrophobicity. This may suggest formation of some complexes between non-polar amino acids residues and non-polar parts of inulin molecules. The presence of calcium chloride caused significant drop in S_o

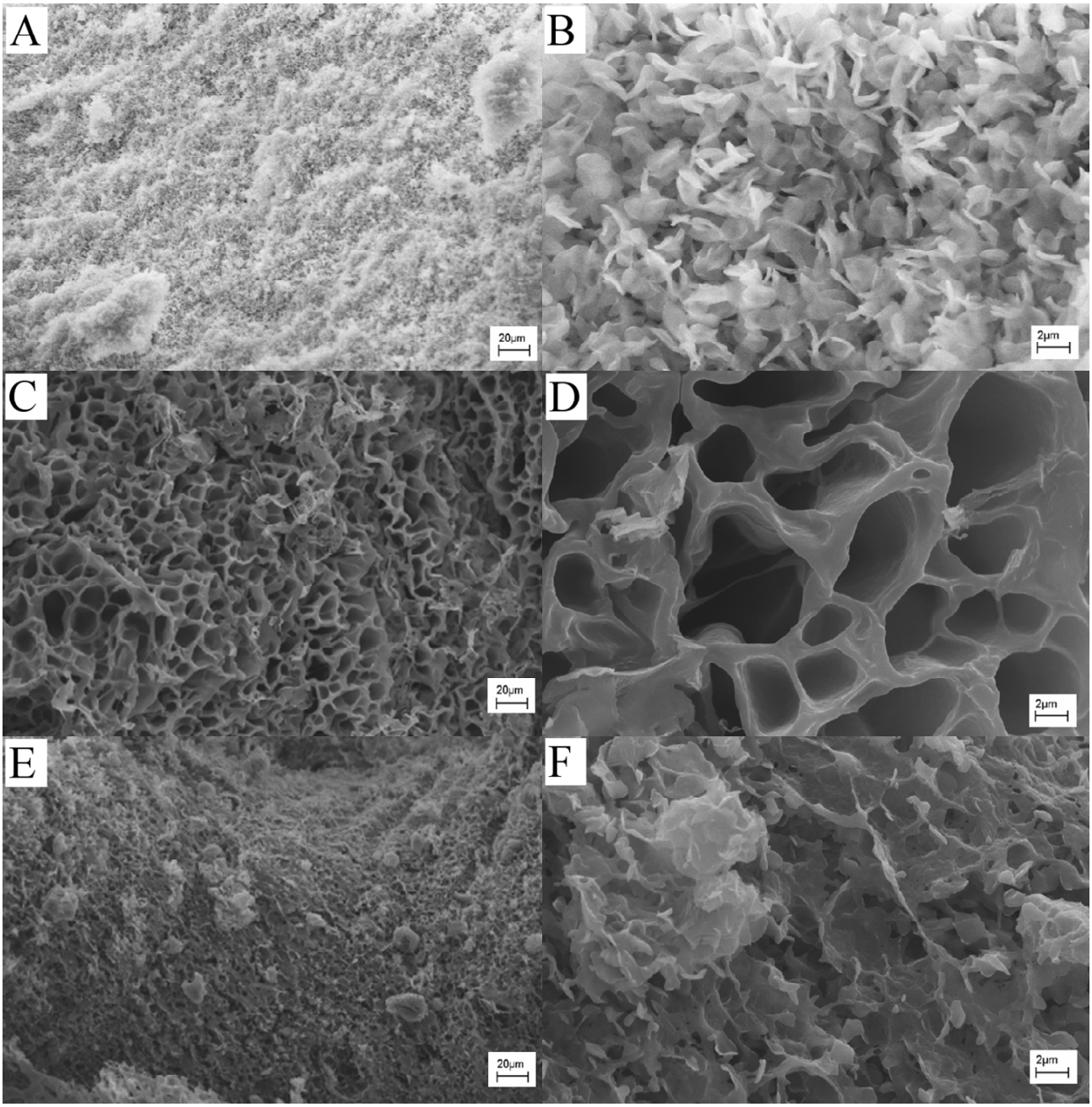

Fig. 3. Scanning electron micrographs of whey protein (WP), inulin and WP-inulin gels. A, B - 20% inulin, C, D - WP with 15 mM $CaCl_2$, E, F - WP with inulin and 15 mM $CaCl_2$. Magnification: A, C, E – 1000x, B, D, F – 10000x.

values as a result of intensive protein aggregation. During this process exposed non-polar residues were covered by aggregating protein molecules. Inulin presence or preparation procedure did not have an impact on hydrophobicity values in the gelled samples.

The obtained results from hydrophobicity measurements and previous studies [17] suggested formation of some inulin-whey protein complexes. Native polyacrylamide gel electrophoretic technique used also in this study (data not shown) did not show any stable inulin-whey proteins complexes which could change the electrophoretic depositions of whey proteins on the electrophoregrams. However, results from electrophoresis does not completely exclude possibilities of the formation of fine inulin-whey protein interactions.

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