

Characterisation of Whey Proteins–Pectin Interaction in Relation to Emulsifying Properties of Whey Proteins

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Abstract: The aim of this work was to characterise influence of whey proteins–pectin interaction on emulsification properties of whey. As the first, structural characteristics of pectin-protein complexes were evaluated for pure β -lactoglobulin by both dynamic light scattering method for measuring of the particle size distributions and Doppler laser electrophoresis for measuring the ζ -potential (surface electrical potential) of particles. In mixed pectin- β -lactoglobulin systems, it was observed that the addition of pectin prevent from the protein-protein interaction, which caused production of huge protein aggregates (2000–2500 nm) at pH values near β -lactoglobulin isoelectric point and at temperatures near its denaturation temperature. However, these protei–pectin complexes had large hydrodynamic diameters (monomodal size distribution at 350 and 1000 nm for high esterified and low esterified amidated pectin, resp.), which can slow down their diffusion to the oil-water interface in emulsions. The ζ -potential values indicated improvement of colloid stability by addition of pectin. The evaluation of the influence of the protein–pectin interaction on emulsification properties was performed by the determination of a surface weighted mean (D [3,2]) of oil droplets in o/w emulsions measured by the laser diffraction, further by microscope observations, the determination of emulsion free oil content and observations of creaming. The emulsifying properties were influenced by the pectin addition, more negatively by the high esterified than by the low esterified amidated pectin addition.

Keywords: whey proteins; pectins; dynamic light scattering; ζ -potential; emulsion; particle size distribution; free oil

INTRODUCTION

The functional properties of whey proteins (globular proteins isolated from whey, a by-product of cheese manufactured from cow's milk), which include water binding, emulsification, foaming and gelation, are related to their structural and other physicochemical characteristics. These characteristics can be influenced by many factors, among others, by the interaction of whey proteins with pectin (CAYOT & LORIENT 1997; DE WIT 1998). The major whey protein is a β -lactoglobulin, which is a compact globular protein with molecular mass 18.3 kDa (SAWYER 2003). Pectin was chosen for this work because it is an important polysaccharide used in a number of foods as a gelling agent, thickener, texturiser, emulsifier and stabiliser.

Commercial pectins used as food additives are linear hetero-polysaccharides which contain at least 65% by weight of galacturonic acid-based units, which may be present as free acid, methyl ester or, in amidated pectins, acid amide (MAY 2000). These various structural forms of pectin can be added to both influence and control functional properties of whey. The two types of interaction are responsible for complex formation and the immiscibility of biopolymers: attraction and repulsion between unlike macromolecules (TOLSTOGUZOV 1997). The interaction between pectin and β -lactoglobulin are mainly caused by hydrogen bonding between carboxyl groups of pectin and peptide linkage of protein. The compatibility of β -lactoglobulin with pectin in aqueous solution is greatly influenced by pH, ionic strength and

the structural features of the pectin (WANG *et al.* 2000; GIRARD *et al.* 2002; BÉDIE *et al.* 2008). Quite often both pectin and whey proteins are present together in dairy and food products, such as yogurt and milk drinks. The control or manipulation of its macromolecular interactions is a key factor in the development of novel food processes and products as well as in the formulation of fabricated food products (TOLSTOGUZOV 1997).

In this work, the aggregation of whey proteins with pectins and the influence of this aggregation on emulsifying properties were evaluated.

MATERIALS AND METHODS

Materials. These pectins were chosen for this work: native pectin from orange peel (HM) (GRINDSTED[®] Pectin XSS ranges, Danisco, DK) as an example of high esterified pectin (degree of esterification 60–70%) and three modified pectins. The commercial low esterified and amidated pectin with low calcium-reactivity (LMA) (GENU pectin LM-101 AS, degree of esterification 35%, degree of amidation 15%) and with medium calcium reactivity (GENU pectin LM-102 AS, degree of esterification 32%, degree of amidation 18%) were purchased from Copenhagen Pectin A/S (Lille Skensved, DK). Potassium pectate, with $\leq 0.5\%$ of esterification, was purchased from Sigma-Aldrich (product number 51186). The β -lactoglobulin (from bovine milk) was purchased from Sigma-Aldrich, too (product number L3908). Whey powder (from rennet whey) was purchased from Promil, a.s. (Nový Bydžov, CZ). The mixed oil CERESOL (rape-seeds and sunflower) produced by Setuza, a.s. (Ústí nad Labem, CZ) was used as a dispersed phase of o/w emulsions.

Characterisation of β -lactoglobulin-pectin interactions. A stock solution (1 mg/ml) of protein or

polysaccharides were prepared by adding of powder to deionised water (18.2 M Ω .cm at 25°C, Millipore Inc., USA) and stirring gently for 1 h at room temperature. Solutions were then stored overnight at 5°C to allow complete hydration and filtered by PVDF syringe filters with porosity 0.45 μ m to remove dust or other huge particles. The mixtures were prepared by combining stock solutions in 1:1 volume ratio (total polymer concentration 1 mg/ml). The pH of prepared solutions was adjusted to 7.0, 6.0, 5.0, 4.0, and 3.0 ± 0.1 using 0.025M HCl or NaOH. Each sample was measured five times in two individual repetitions. Results were expressed as mean with standard deviation.

The particle size was determined by dynamic light scattering (DLS) method using Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). The diameter that is measured in DLS is a value that includes also the double layer of particle and is referred to as hydrodynamic diameter. The software calculates mean of hydrodynamic diameters of all particles presented in sample according intensity of scattered light and it is called as Z-Average. The influence of the temperature on Z-Average was measured at pH 7 in the temperature trend from 40°C to 90°C with step 5°C and 10 min of equilibration time in each step.

The ζ -potential or particle surface electrical potential was measured by Doppler laser electrophoresis using Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK).

Emulsifying properties. The oil-in-water emulsions with oil content 10% w/w were prepared. The whey powder was reconstituted to 7% solution in demineralised water (protein content 0.65% w/w). Pectin was added in 1:1 w/w ratio related on protein content of reconstituted whey. The oil and aqueous phase (reconstituted whey powder with or without addition of pectin) were mixed for 10 min at 40°C using a rotor-stator mixer (Si-

Table 1. Influence of pectin addition on properties of model whey emulsions

Aqueous phase	D [3,2] (μ m)	Free oil (% w/w)	Creaming layer (% vol.)
Whey	4.91 \pm 0.03	1.21 \pm 0.04	17 \pm 1
Whey + LMA	5.27 \pm 0.10	0.27 \pm 0.03	N
Whey + HM	6.01 \pm 0.15	2.36 \pm 0.64	22 \pm 1

D [3,2] – surface weighted mean; free oil – expressed as weigh of unemulgated oil, which was extracted and related to mass of emulsion; LMA – low esterified amidated pectin; HM – high esterified pectin; N – any creaming was not observed; \pm the interval of duplicate measurement

lent Crusher M, Heidolph Instruments GmbH & Co.KG, Schwabach, D) at 24 000 rpm and then were cooled by water bath.

The size distribution of oil droplets was determined by the laser diffraction using the Mastersizer 2000 with the sample unit Hydro 2000G (Malvern Instruments Ltd., Malvern, UK) according MICHALSKI *et al.* (2002). Surface weighted mean ($D [3,2]$) was calculated from the size distribution by the software Mastersizer 2000 Vers. 5.13. Simultaneously, microscopic images of fat globules were done by light microscope Leica 55 (Leica Microsystems Ltd., Wetzlar, D) with a digital camera Leica DFC 320 and processed by PC software Lucia G on VGA (Vers. 4.60).

Free oil was determined gravimetrically using the extraction to organic solvents, where equal volume of diethyl ether and then equal volume of petroleum ether were mixed with the sample. After two hours, unpolar phase was taken away and solvents were evaporated by the vacuum distillation. Finally, the oil was dried to constant weight at 102°C.

RESULTS AND DISCUSSION

As the first, we have characterised the behaviour of mixed β -lactoglobulin–pectin systems, which have a difference in a degree of esterification and amidation, by measuring of the particle size and ζ -potential at different medium conditions. The β -lactoglobulin was chosen because of mainly proportional representation in whey proteins. Polygalacturonic acid, as an example of unesterified pectin, and low esterified and amidated pectins

were compared for a complex production with β -lactoglobulin in this work, however these pectins were further excluded for preparation of emulsions, because of high gelling activity at the present of calcium ions. Finally, two model systems were chosen: commercial low esterified and amidated pectin with low calcium reactivity (LMA) and pectin from orange peel as an example of high esterified pectin (HM). The pH of medium was changed from 7 to 3 as a common range of pH in foods.

Primarily, the characterisation of individual polymers was carried in water solution. The β -lactoglobulin had a bimodal size distribution with main fraction at 3 nm (80% intensity peak area) at neutral pH. This fraction represents particles of β -lactoglobulin molecules, which have occurred at this pH as a dimer, and results are consistent with literature: SAWYER (2003) has reported 3.19 nm for dimer hydrodynamic radius of β -lactoglobulin (2.04 nm for monomer). The second fraction at 100 nm represents some unremovable impurities. However, β -lactoglobulin produced very large aggregates (2000–2500 nm) around pH which is near its isoelectric point (Figure 1A). The isoelectric point of β -lactoglobulin has been reported to be around 4.8–5.4 and production of huge aggregates, of which presence is undesirable in many processed foods, is caused by protein–protein interaction (TOLSTOGUZOV 1997; SAWYER 2003). This fact was also consistent with the results of ζ -potential measurement (Figure 1B), where zero value of the particle surface electrical potential was observed in range of pH 4–5. The size of all pectins (monomodal distributions at 500 nm) was not significantly affected by de-

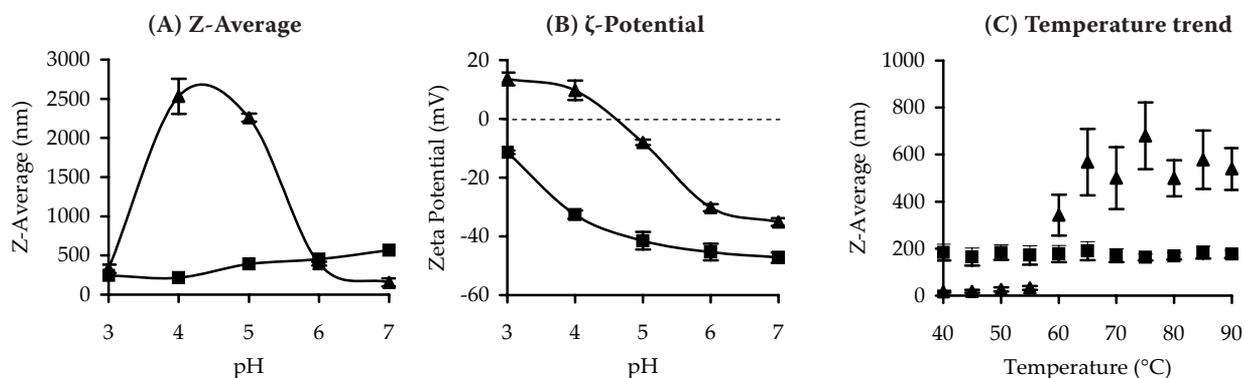


Figure 1. The influence of pH on Z-Average (A) and ζ -potential (B) and influence of the temperature at pH 7 (C) on β -lactoglobulin (▲) and β -lactoglobulin-high esterified pectin mixed solution (■) at 1 mg/ml total polymer concentration. The error bars represent the standard deviation of the mean of ten values

ing of medium pH. Furthermore, the mixed samples were prepared. There were observed macroscopic white settling (mainly fiber shape) aggregates in β -lactoglobulin-low esterified pectins solutions (especially for polygalacturonic acid), of which the particle size were not measured because it was out of the range of instrument. Amount of these visible aggregates increased with decreasing pH. The mixed solutions of β -lactoglobulin-high esterified pectin were clear, without macroscopic aggregates, except of the solution at pH 3. In comparison this with size measurement of pure protein solution, we can conclude, that addition of high esterified pectin successfully prevent to huge aggregation of β -lactoglobulin in pH near its isoelectric point (Figure 1A) as well as at denaturation temperatures (Figure 1C). This conclusion was consistent with the literature (GIRARD *et al.* 2002; BEAULIEU *et al.* 2005; BÉDIE *et al.* 2008). The ζ -potential of this mixture (Figure 1B) is negative in all measured range and absolute values higher than 35 mV indicate good colloid stability of this system for pH range from 4 to 7. However, these complexes had large hydrodynamic diameters (monomodal size distribution at 350 and 1000 nm for high methylated and amidated pectin, respectively), which can slow down their diffusion to the oil-water interface in emulsions.

As the second, the model 10% oil-in-water emulsions were prepared from whey powder, as a commercial mainly used whey substrate, to have an equal protein:pectin ratio in aqueous phase. The oil droplets' size distributions were evaluated by laser diffraction. Distributions pattern was

conformable and it was given by equipment used for emulsifying (Figure 2). It is possible to see the largest oil droplets for emulsions with high esterified pectin.

From microscope images (Figure 3) we can confirm the presence of large fat globules (with size 100 μm). There were no clusters production demonstrated. The agglomeration of fat globules, which is possible to see on these images, were dispersed by dilution with water.

In spite of the fact of large aggregates of pectins with β -lactoglobulin measured by DLS, the surface weighted mean of oil droplets in model emulsions was not influenced dramatically (Table 1), but the difference was significant. Low esterified amidated pectin showed better emulsifying properties than high esterified, because smaller oil droplets were obtained at the same conditions. The significant part of oil was not emulsified in high esterified pectin stabilised emulsions. This free oil is then possible to see on the surface of the emulsion and it is unattractive for customers.

Creaming of model emulsions was obvious in unstabilised emulsion because of low viscosity of continuous phase, but coalescence (preserved samples were evaluated in time) was not confirmed during the storage. The creaming was not observed for emulsions stabilised by low esterified amidated pectin, because of the viscosity increase caused by weak gellation of pectin. Creaming layer of high esterified pectin stabilised emulsions was thicker than in unstabilised emulsions for the same oil content, which indicate lower density of this layer for stabilised emulsions.

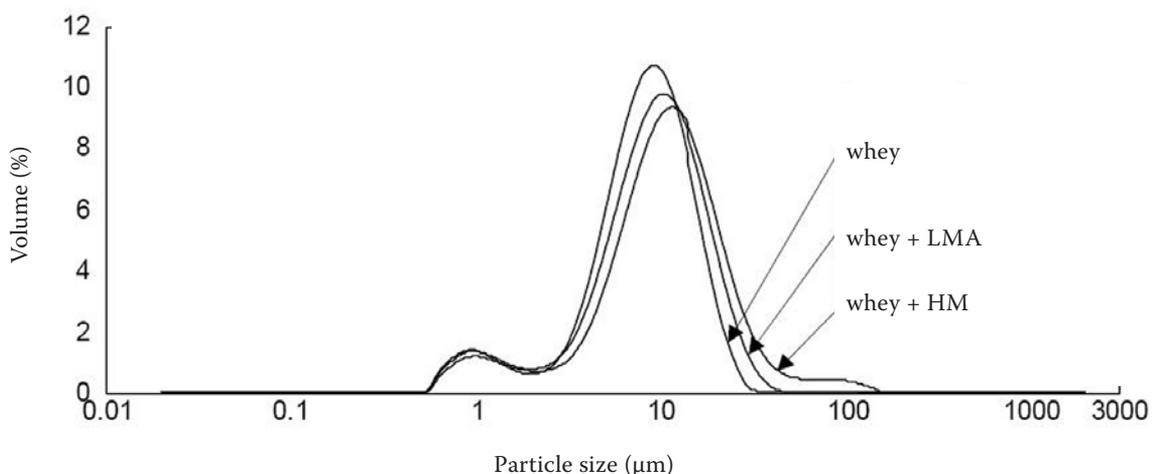


Figure 2. The oil droplets' size distributions of model emulsions with different type of aqueous phase (LMA – low esterified amidated pectin, HM – high esterified pectin)

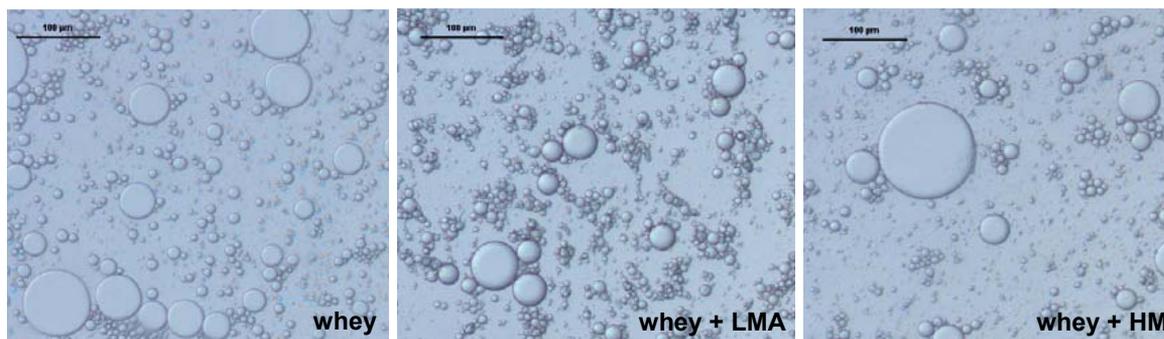


Figure 3. Microscopic images of oil droplets of model emulsions with different type of aqueous phase (LMA – low esterified amidated pectin, HM – high esterified pectin)

CONCLUSIONS

The influence of protein-pectin aggregates production on emulsifying properties of whey proteins was discussed for high esterified and low esterified amidated pectin. The surface weighted mean of oil droplets was higher in emulsions with addition of both pectins. The complexes of whey proteins with low esterified amidated pectin showed better emulsifying properties than complexes with high esterified pectin, which was possible to see both in size of oil droplets and content of free oil, in spite of the fact that low esterified amidated pectin produced larger aggregates than high esterified pectin.

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