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Emulsifying properties of tribomechanically treated whey proteins

Marija Badanjak Sabolović, Suzana Rimac Brnčić, Vesna Lelas*

University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

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Summary

Whey proteins are used in a wide range of food products because of their high nutritional value and the ability to contribute to the unique functional properties of the final products. The functional properties of whey proteins are affected not only by the whey origin, season dependent variations of protein and non-protein components amount, but also by the conditions of processes involved in their isolation, purification and modification (temperature, pH, pressure, chemicals). In this research, tribomechanical micronization (TM) was used as a technique that could be useful in modification of some functional properties of whey proteins. Therefore, two different commercial powdered whey protein isolates (WPI) were used for analysis. Surface hydrophobicity and emulsifying properties (emulsifying activity and emulsion stability) were determined before and after TM treatment. The results obtained showed increases in surface hydrophobicity of WPI after TM treatment indicating that TM could induce changes of protein conformation and increase exposure of the previously buried hydrophobic regions. Emulsions prepared with tribomechanically treated WPI showed higher emulsion activity and better emulsion stability. The results obtained suggest that TM can be useful and fast process technique for improvement of functional properties of WPI.

Key words: whey protein, emulsifying properties, tribomechanical micronization

Introduction

Whey proteins have valuable nutritional characteristics and versatile desirable functional properties in food products. They are widely used in the food industry to improve solubility, facilitate whipping and improve gelation, water binding, foaming and emulsifying properties of many formulated food products (Aguilera, 1995). The functionalities of WPI depends on intrinsic factors such as amino acid sequence, amino acid composition, secondary and tertiary structures, molecular size, shape, flexibility, net charge, hydrophobic/hydrophilic character of protein surface and extrinsic factors such as temperature, pH and ionic strength. The knowledge of relationship between intrinsic and extrinsic factors is required to control functional behaviour in different applications and modify protein and process conditions to optimize desirable functions. Modification of whey proteins usually refers to physical (Herceg at al., 2004a; Herceg at al., 2004b; Herceg at al., 2005; Krešić at al., 2008; Sui at al., 2011; Xu at al., 2011; Chandrapala at al., 2011; Brnčić at al, 2011; Dissayanake at al., 2013), chemical (Li at al., 2005; Corzo-Martinez at al., 2010; Morand at al., 2012; Liu at al., 2012) or enzymatic treatments (Gerrard at al., 2012; Damodaran i Agyare, 2013) changing its conformation, structure and consequently its physicochemical and functional properties. The objective of this study was to compare influence of tribomechanical micronization (TM treatment) on ability of two commercially available whey protein isolates to form and maintain stable emulsions.

*Corresponding author/Dopisni autor: Phone/Tel: +385 1 4605 016; E-mail: srimac@pbf.hr

Materials and methods

In this research the investigations were carried out with two powdered whey protein isolates (WPI), BiPro produced by Davisco Foods International, Le Suer, MN, USA (IP-1) and RT-90 produced by Main Street Ingredients, La Crosse, WI, USA (IP-2). Chemical composition has been declared by manufacturer. Whey protein isolates BiPro contained 92 % protein, 1 % lactose, 0,3 % fat, 5 % water, 2 % ash while whey protein isolates RT-90 contained 90 % protein, 0,8 % lactose, 0,5 % fat, 5 % water, 2,5 % ash. WPI powders were mechanically processed in a patented device for tribomechanical micronization according to the procedure previously described (Krešić at al., 2008; Rimac Brnčić at al., 2010).

Surface hydrophobicity

The surface hydrophobicity of proteins was determined using the 1-anilinonaphthalene-8-sulphonate (ANS, Sigma Chemical Co.) assay according to the method of Kato & Nakai (1983). Five concentrations of WPI in phosphate buffer pH=7 (0.0001 %, 0.005 %, 0.01 %, 0.0150 %, 0.2 %) were prepared. The relative fluorescence intensity of the ANS-protein isolates (native or TMA treated) was measured at a room temperature using a Perkin Elmer LS-50 Luminescence Spectrophotometer (Rodgau, Germany). The wavelengths of excitation and emission were 390 and 480 nm, respectively. Quantification of ANS binding was carried out fluorimetrically by addition of 15 mL of ANS solution (8 mM) to 3mL of untreated and TM treated WPI solution. Surface hydrophobicity was expressed as the slope of the plot of fluorescence intensity values as a function of protein concentration. The data were expressed as a percentage of the value for the untreated sample.

Emulsifying properties

Protein dispersions and suspensions were analysed by the turbidometric technique for emulsion activity index (EAI) and emulsion stability index (ESI) (Webb at al., 2002). Emulsions were prepared with 3 % protein dispersions (w/v) using 10 mL of sunflower oil (Zvijezda d.o.o., Zagreb, Croatia), by mixing for 90 sec in a blender (Philips, model HR 2304). The absorbance of the diluted emulsions was measured by spectrophotometer (Helios-β, Pye Unicam Ltd, Cambridge, UK) at 500 nm in 1 cm path length cuvettes. The absorbance was read initially, while turbidity and EAI were calculated using the following equation:

$$T = 2.303A/r \qquad /1/$$

Where: T = turbidity, A = absorbance at 500 nm and r = path length of cuvette (m).

The emulsion activity index (EAI) was then calculated as

$$EAI = 2T\left(\frac{A \times r}{C \times \phi \times 10000}\right) \qquad /2/$$

Where: φ = oil volume fraction of the emulsion, C = the weight of protein per unit volume (g /mL) of the protein aqueous phase before emulsion formation.

Emulsion stability: The emulsions were held at 4 °C for 24 h and reanalyzed for emulsion activity as described previously. An emulsion stability index (ISE) was calculated using the following equation:

$$ISE = \left(\frac{T \times \Delta t}{\Delta T}\right) \qquad /3/$$

Where: T = turbidity value at 0 h, ΔT = change in turbidity during 24 h period, Δt = time interval (24 h).

Conductivity measurements

Conductivity of 10 % (w/w) whey protein dispersions was measured in triplicate at 20 °C using a SevenGo pro™ (Metler Toledo) conductometer.

Statistical analysis

Experimental data were analyzed by variance analysis (ANOVA) to determine the influence of pH and tribomechanical treatment on the emulsion activity and stability. Differences between samples at the 1 % ($p<0.01$) value were considered significant. All statistical analyses were performed using a software program Statistics for Windows.

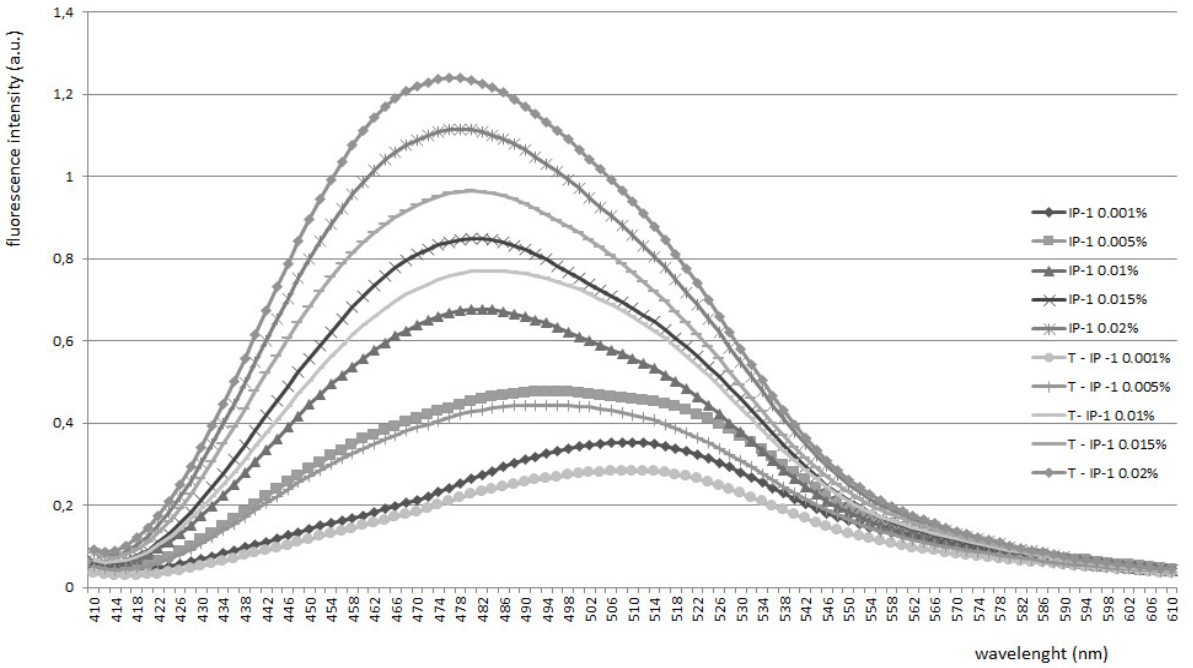

Figure 1. Emission spectra of whey protein isolate solutions affected by tribomechanical micronization (IP-1 - non-treated WPI BiPRO; T-IP-1 - tribomechanically treated WPI BiPRO)

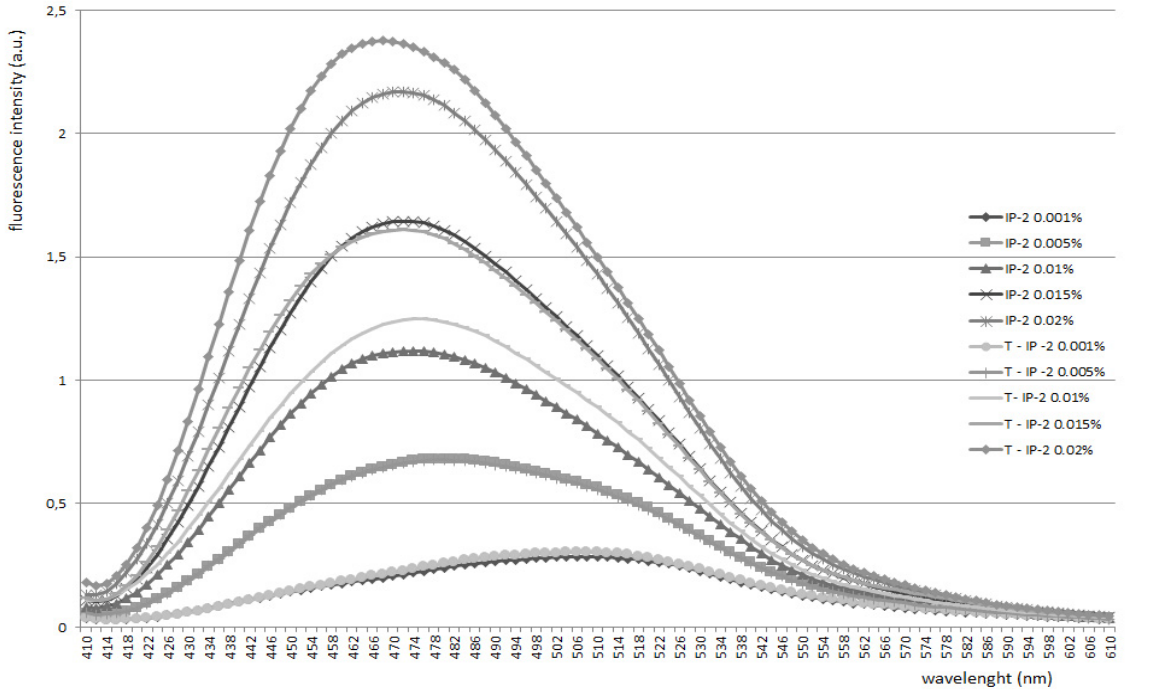

Figure 2. Emission spectra of whey protein solutions affected by tribomechanical micronization (IP-2 - non-treated WPI RT-90; T-IP-1 tribomechanically treated WPI RT-90)

Table 1. Hydrophobicity index (S_0)

Sample	Hydrophobicity index (S_0)
IP – 1	43.723
T – IP -1	53.253
IP -2	97.294
T – IP -2	103.200

Results and discussion

Both functional and nutritional properties of whey proteins are changed under the influence of molecular changes. Literature data have shown how protein functionality and assessment domain could be evaluated by surface hydrophobicity determination (Kato and Nakai, 1980). Protein hydrophobicity could be measured with various experimental (chromatographic; spectrophotometric and by binding ability measurement of different ligands - carbohydrates, fats, SDS) and computational methods (Q - average protein hydrophobicity and NPI - Net Polarity Index). However, evaluation of average protein hydrophobicity by means of side chain of individual amino acids hydrophobicity gives no accurate information of conformation type of investigated protein. For these reasons, it is recommended to determine hydrophobicity by experimental methods depending on intrinsic (chemical composition, amino acid sequence and localisation of particular amino acid residues) and extrinsic factors. Surface hydrophobicity values measured using fluorescent probe ANS showed so called aromatic hydrophobicity which reflects localisation of non-polar hydrophobic aromatic amino acid residues (tyrosine, phenylalanine, tryptophan). ANS fluorescence is very weak in aqueous solutions, but is enhanced when bound to ß-lactoglobulin (Li at al, 2005). The results obtained in this research from hydrophobicity measurements are presented in Fig. 1 and 2 as well as in Table 1. Hydrophobicity index was calculated under dependence of whey protein concentration (0.02 %) on fluorescence intensity. In their native form, whey proteins have a compact and rigid structure stabilized by intramolecular, namely hydrophobic, hydrogen, electrostatic and disulphide bonds. TM treatment of whey proteins caused an increase in hydrophobicity values as a consequence of exposing hydrophobic amino acids residues previously buried in the interior of globules.

Fluorescence intensity was magnified proportionally due to increase of solid matter content. The highest whey protein isolates concentration (0.02 %) resulted in the highest fluorescence (Figures 1 and 2). TM treatment has changed the surface hydrophobicity (S_0 value) of BiPRO WPI sample and it was higher (53.253) in comparison with S_0 value of the untreated control (43.723) sample at the same concentration. Surface hydrophobicity of untreated RT WPI sample (97.294) was lower compared to TM treated one (103.200) (Table 1).

Electrical conductivity measurements could be used to detect compositional variations and structural changes in food products as well as to monitor emulsion stability (Guerin at al, 2004). Many factors, such as chemical composition, concentration of charged compounds, pH and temperature, can have a significant effect on conductivity (Therdthai and Zhou, 2001). A higher mineral content has a positive effect on electrical conductivity (Mabrook and Petty, 2003). During whey protein concentrate production most of the minerals pass to the permeate, but still some of those (mainly Ca, Na, K, P) remain in retentate (Noël et al., 2008). From the results presented in Figure 3 it is obvious that electrical conductivity of whey protein dispersions ranged from 1092 to 1563 μS/cm. Generally, RT WPI samples showed lower conductivity compared to BiPRO WPI samples. This conductivity differences can be explained by different fat content of examined WPI (RT WPI samples contain more fat than BiPRO WPI).

Namely, fat hinders the conduction of electricity by occupying volume and by impeding the mobility of ions and on the other hand electro-conductivity of TM treated WPI were higher for both samples, respectively.

Tribomechanically treated samples had higher electrical conductivity. Modification of whey proteins structure by TM processing might lead to changes in whey protein tertiary structure (these

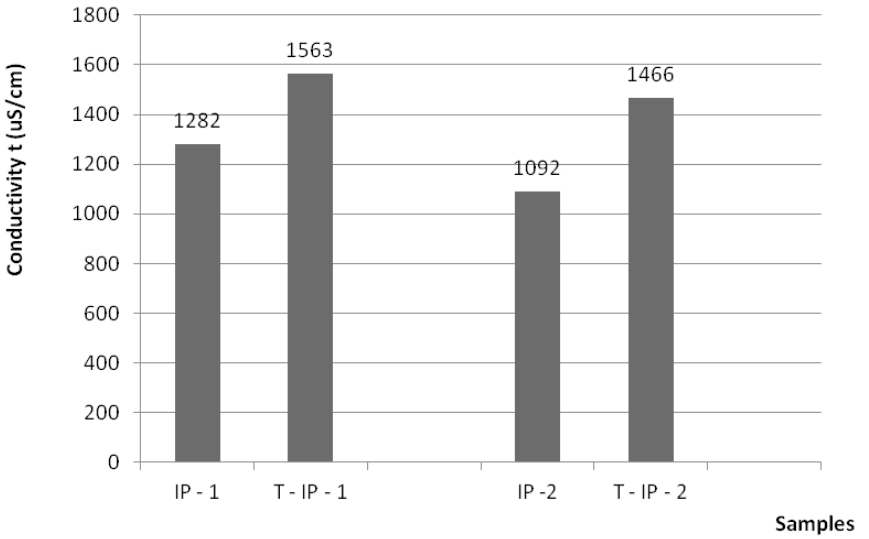

Figure 3. Electrical conductivity of WPI dispersions

Table 2. Emulsion activity index of whey protein isolates stabilized emulsions at various pH values (m^2/g)

Sample	pH 3	pH 5	pH 7
IP – 1	124.55	114.42	136.73
T – IP – 1	142.13	117.91	155.30
IP – 2	92.36	56.21	102.92
T – IP – 2	109.17	67.26	119.89

include hydrophobic interactions and electrostatic interactions) as well as quaternary structure increasing their energy potential. These results confirm that tribomechanical treatment could induce changes of WPI structure.

Whey proteins are the main source of globular proteins used in food industry because of their emulsifying properties due to their surface activity properties reflecting in interfacial tension decrease.

Whey proteins solubilised in aqueous phase and homogenized with oil phase, intent to be adsorbed rapidly on to the newly created surfaces to prevent bridging flocculation and coalescence of droplets. According to Dybowska (2008) emulsion properties are influenced by the protein type, the ratio of protein to dispersed phase, the extent to which the protein alters the dynamic interfacial rheology, the characteristics of the continuous phase, such as protein size, pH and ionic strength, and also by droplet size distributions of dispersed phase as well.

Emulsifying properties of whey protein isolates were examined at three selected pH values: one below the isoelectric point (pH 3) one at isoelectric point (pH≈5) and one above isoelectric point (pH 7).

The EAI (emulsion activity index) is related to the ability of the protein to be adsorbed at the surface area of fat globules and its capacity to unfold/spread over the oil-water interface, stabilizing the new created area (Mangino, 1994). Pearce and Kinsella (1978) defined emulsion activity index as units of area of interface stabilized per unit weight of protein.

Whey protein isolates emulsifying abilities examination have shown how model systems prepared with TM treated whey proteins get significantly higher EAI in comparison with native whey protein isolates under each investigated pH values (Table 2); ($p<0.01$). Mentioned fact leads to larger stabilizing surface area for such treated proteins. The lowest

Table 3. Emulsion stability index (h) of whey protein stabilized emulsions at various pH values

Sample	pH 3	pH 5	pH 7
IP – 1	53.86	21.76	64.66
T – IP – 1	59.98	22.68	74.59
IP – 2	46.54	21.64	49.31
T – IP – 2	54.26	21.69	57.31

EAI was obtained at pH 5, close to the protein isoelectric point because the proteins are susceptible to aggregation. IP-2 had lower EAI (56.21 m^2/g) than IP-1 (114.42 m^2/g).

Growing demand for new formulated foods creates necessity for developing new technologies that could provide consumers with high quality stable products. Emulsion stability refers to the ability of an emulsion to resist changes in its properties over time. The rate at which an emulsion breaks down and mechanism by which this process occurs, depends on its composition and microstructure, as well as on the environmental conditions (temperature, agitation, storage conditions). Emulsion stability is expressed as emulsion stability index (Table 3). The stability of the emulsions made from TM treated WPI were significantly higher than those prepared from the untreated one ($p<0.01$).

Better emulsifying properties of TM treated whey proteins could be explained by their reduced molecular size (Rimac Brnčić, 2006). Proteins chains with lower molar mass reach to intermediate stage faster and are considerably flexible with superior ability of reorientation on phase boundaries and thereby improved emulsifying properties. In their research, Christiansen et al. (2004) showed that limited proteolysis enhances the emulsifying properties. Whey concentrate protein hydrolysates generate more stable emulsions compared with untreated whey protein concentrate as a result of its structure which enables increased number of hydrophobic and electrostatic interactions. However, extensive hydrolysis, due to the production of many short-chain peptides, has been found to be detrimental to the emulsifying and stabilising properties of proteins (Agboola and Dalgleish, 1996; Foegeding at al, 2002). Degree of hydrolysis above 20 % affects negatively on emulsifying properties of whey proteins. The highest ESI were observed for emulsions prepared with TM treated WPI BiPRO (74.59 h) at pH 7 probably due to an increase in repulsion by the electrostatic charge of the proteins. The net charge at pH 5 (near to the isoelectric point of whey proteins) is minimized, and the repulsion between the fat globules emulsified by the proteins is reduced resulting in lower emulsion stability.

On the basis of results presented in Tables 1, 2 and 3, it is apparent how emulsifying properties of WP are influenced by TM treatment and considering that the protein adsorption and orientation at the O/W interface is likely influenced by its hydrophobicity.

Examined samples with higher electrical conductivity also had higher values of emulsion stability index. These results confirm that tribomechanical treatment could induce changes of structure and functional properties of whey proteins.

Conclusion

Modifications of protein structure by TM treatment that enhance surface hydrophobicity exhibited good relation with surface functional properties such as emulsifying properties. This study shows that stability of whey protein stabilized emulsions depends on pH value. Emulsions were relatively stable at low and high pH, but were highly unstable around the isoelectric point of proteins. Emulsions prepared with tribomechanically treated WPI were more stable than those prepared with untreated whey proteins.

Emulgirajuća svojstva tribomehanički obrađenih proteina sirutke

Sažetak

Proteini sirutke upotrebljavaju se u velikom broju prehrambenih proizvoda zbog njihove visoke nutritivne vrijednosti i jedinstvenih funkcionalnih svojstava. Funkcionalna svojstva proteina sirutke ovise ne samo o podrijetlu sirutke, sezonski ovisnih količina proteinskih i neproteinskih komponenti, već i o uvjetima procesa koji su uključeni u njihovu izolaciju, pročišćavanje i modifikaciju (temperatura, pH, tlak, kemijski dodaci). U ovom radu, tribomehanička mikronizacija (TM) je korištena kao potencijalna tehnika za unapređenje pojedinih funkcionalnih svojstava proteina sirutke. Analizirana su dva različita komercijalna izolata proteina sirutke u prahu (WPI). Površinska hidrofobnost i emulgirajuća svojstva (sposobnost stvaranja emulzija i stabilnost emulzije) određena su prije i poslije TM obrade. Dobiveni rezultati su pokazali povećanje površinske hidrofobnosti WPI nakon TM obrade što ukazuje na činjenicu da TM može izazvati konformacijske promjene proteina i povećati izloženost prethodno skrivenih hidrofobnih područja. Emulzije pripremljene s tribomehanički obrađenim WPI pokazale su veću aktivnost i bolju stabilnost emulzija. Dobiveni rezultati ukazuju da TM može biti učinkovita tehnika za poboljšanje funkcionalnih svojstava WPI.

Ključne riječi: proteini sirutke, emulgirajuća svojstva, tribomehanička mikronizacija

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