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The Effect of Ultrasound Treatment on the Structural, Physical and Emulsifying Properties of Dairy Proteins

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Abstract

The effect of ultrasound treatment on the structural, physical and emulsifying properties of three dairy proteins: sodium caseinate (NaCas), whey protein isolate (WPI) and milk protein isolate (MPI) was investigated. The pH of untreated NaCas, WPI and MPI solutions was 7.1, 6.8 and 6.7, respectively. Protein solutions at different concentrations (0.1 – 5 wt. %) were treated by ultrasound radiation for 2 min at a frequency of 20 kHz and with a power intensity of ~34W.cm⁻². The structural and physical properties of the untreated and ultrasound treated proteins were studied in terms of changes in protein size, molecular structure and hydrodynamic radius using dynamic light scattering (DLS), SDS-PAGE and intrinsic viscosity, respectively. The emulsifying properties of the ultrasound treated proteins were compared to the untreated proteins and to a low molecular weight surfactant, Tween 80. Ultrasound treatment reduced the micelle size and hydrodynamic volume of the proteins as measured by DLS and intrinsic viscosity, while SDS-PAGE showed that there was no measurable reduction in molecular weight. 10% Rapeseed oil-in-water emulsions prepared with untreated NaCas and WPI had submicron sized droplets (~120 nm) at all concentrations, while the emulsions produced with untreated MPI and Tween 80 had micron sized droplets (> 1 µm) at the lower concentrations studied. Unexpectedly, the emulsions produced with ultrasound treated NaCas and WPI had the same submicron droplet sizes as the untreated proteins at all concentrations, despite the observed reduction in micelle size and reduction of intrinsic viscosity (i.e. increase in hydrophobicity) of the sonicated proteins. These results suggest that ultrasound treatment did not affect the rate at which the sonicated proteins were adsorbed at the oil-water interface, since no significant changes in interfacial tension were measured between the untreated and sonicated NaCas and WPI. Emulsions prepared with sonicated MPI at concentrations ≤ 1 wt. % had smaller droplet sizes than the emulsions produced with untreated MPI at the same concentrations. This effect was consistent with the observed decrease in interfacial tension for ultrasound treated MPI, which will facilitate droplet break-up during emulsification.

Keywords: Sodium caseinate, Milk protein isolate, Whey protein isolate, Ultrasound, Protein size, Intrinsic viscosity, Emulsion.

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1. Introduction

Proteins are highly functional molecules that are widely used in the pharmaceutical and food industries, having a wide range of applications. Proteins are of particular interest in food systems in terms of their emulsifying properties, due to their abilities to adsorb at oil-water interfaces and to form interfacial films (Foegeding & Davis, 2011; Lam & Nickerson, 2013). The surface activity of proteins is due to their amphiphilic nature, owing to the presence of both hydrophilic and hydrophobic groups in their molecular structure (Beverung, Radke, and Blanch, 1999). Due to their bulky structure, proteins diffuse slowly to the interface, by comparison to low molecular weight emulsifiers, such as Tween 80 (McClements, 2005). Once at the interface, proteins undergo conformational changes (surface denaturation) and rearrange themselves in order to position their hydrophobic amino acids within the oil phase and hydrophilic amino acids within the aqueous phase (McClements, 2004; Walstra & van Vliet, 2003), the effect of which reduces the interfacial tension and the overall free energy of the system (McClements, 2004). One particular advantage of proteins is that protein-protein interactions at the interface, lead to the formation of strong viscoelastic films that are more resistant to coalescence and provide either electrostatic or steric stabilisation (Lam & Nickerson, 2013; McClements, 2004). Therefore, it is of great interest for the food industry, to investigate methodologies that are capable to enhance the emulsifying properties of proteins.

In recent years, low frequency high energy ultrasound (US) (i.e. frequency ≤ 100 kHz, power intensity 10–100 W.cm$^{-2}$) has been used in the food industry to modify the functional properties of proteins. The effect of ultrasound on the physicochemical properties of the treated molecules is related to cavitation (rapid formation and collapse of gas bubbles), which is generated by highly localized changes in pressure (up to 50 MPa) and heat (up to 5000 °C),
occurring during very short periods of time (O’Donnell, Tiwari, Bourke, & Cullen, 2010). High shear forces and turbulence resulting from these cavitations, also contribute to the observed effects of ultrasound (Güzey, Gülseren, Bruce, & Weiss, 2006).

The application of ultrasound to proteins has been related to effects on the structural and functional properties of whey protein concentrates (Arzeni et al., 2012; Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011; Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014), soybean proteins (Arzeni et al., 2012; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009; Karki et al., 2010), and egg white proteins (Arzeni et al., 2012; Krise, 2011). Arzeni et al., (2012) studied the influence of ultrasound on the structural properties of whey protein concentrate (WPC), soy protein isolate (SPI) and egg white protein (EWP). They observed a significant reduction of the protein size for WPI and SPI. Guzey & Weiss, (2001) investigated the effect of high-intensity ultrasonic processing on the surface activity of bovine serum albumin (BSA) and WPI. It was reported that ultrasound treatment improves significantly the emulsifying properties of BSA and WPI. However, there are contradictory reports on the effect of ultrasound on the molecular weight of proteins. For example, ultrasound treatment of 20 and 40 kHz for 30 min resulted in a significant decrease in molecular weight for WPC, WPI (Jambrak et al., 2014) and α-lactalbumin (Jambrak, Mason, Lelas, & Krešić, 2010). Whereas, sonication at 20 kHz for 30 min with varying power intensities was reported to have no significant effect on the molecular weight of SPI (Hu et al., 2013; Karki et al., 2010). In addition, no significant changes in molecular weight were reported for EWP treated with ultrasound at 55 kHz for 12 min (Krise, 2011). Therefore, it is necessary to further investigate the effects of ultrasound on the structural and functional properties of food proteins.

Sodium caseinate (NaCas) is a functional ingredient widely used in the food industry. This protein is used as an emulsifier in a wide range of food applications, including coffee
creamers, infant formulas, soups and processed meat (O’Connell, Grinberg, & de Kruif, 2003). NaCas is a composite mixture of four protein fractions: αs1-, αs2-, β- and κ-caseins (Srinivasan et al., 2002). In solution, these caseins are prone to form spherical colloidal associations, or micelles, due to regions of high hydrophobicity and the charge distribution arising from the amino acid sequence, phosphorylation and glycosylation (O’Regan, Ennis, & Mulvihill, 2009). The internal structure of the casein micelle is constituted of the calcium sensitive protein fractions (αs1-, and αs2-), which are held together by cohesive hydrophobic interactions and calcium-phosphoserine crosslinks. The micelle is stabilised by κ-casein which is predominately found at the micelle surface due to its highly hydrophilic C-terminal protruding into the aqueous phase. β-casein exists in a temperature dependant equilibrium between the aqueous phase and the micelle (Dalgleish, 2011; O’Connell & Flynn, 2007).

Whey protein isolate (WPI) is a nutritional ingredient used in the food industry because of its desirable functional properties, such as emulsification, gelation and foaming (Arzeni et al., 2012). The main protein fractions in WPI are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serum albumin (BSA). Whey proteins have globular conformations. β-lg contains five cysteine residues, four of which occur as intra-molecular disulfide cross-links and one as a free thiol group (SH). α-lac is a calcium metalloprotein that has four intra-molecular disulphide cross-links. The binding of calcium is essential for proper folding and disulphide bond formation of α-lactalbumin (O’Regan et al., 2009). BSA is stabilised to a great extent by its 17 cysteine disulphide bonds (Nakamura et al., 1997).

Milk protein isolate (MPI) is a mixture of micellar casein (~80%) and whey (~20%) (Fox, 2008). The casein in MPI has a micellar structure similar to the native form found in milk, and the whey proteins are present in the globular native form (O’Regan et al., 2009).

In the present work, analyses were carried out on commercially available dairy proteins widely used in the food industry, in order to assess the industrial relevance of
ultrasound treatment on composite mixtures of food protein systems. The objective of this research was to understand the effects of ultrasound treatment on the structural and physical properties of three dairy proteins: sodium caseinate (NaCas), whey protein isolate (WPI) and milk protein isolate (MPI). Changes in the structural and physical properties of the proteins were measured in terms of protein size, molecular structure and intrinsic viscosity. Moreover, we investigated whether the proteins treated by ultrasound have the ability to increase the stability of oil-in-water emulsions against coalescence. Oil-in-water emulsions were prepared with either untreated or ultrasound treated NaCas, WPI and MPI at different concentrations and compared between them and to a low molecular weight emulsifier, Tween 80.

2. Materials and Methods

2.1. Materials

Acid casein (Kerrynor™ A290), whey protein isolate (W994) and milk protein isolate (Ultranor™ 9075) were all kindly provided by Kerry Ingredients (Listowel, Ireland). The composition of the three dairy proteins is provided in Table 1. Tween 80 and sodium azide were purchased from Sigma Aldrich (UK). The oil used in this study was commercially available rapeseed oil. The water used in all experiments was passed through a double distillation unit (Aquatron A4000D). All materials were used with no further purification or modification of their properties.

2.2. Methods

2.2.1. Preparation of untreated protein solutions

Sodium Caseinate (NaCas) was prepared from acid casein using the method outlined by O’Connell and Flynn (O’Connell & Flynn, 2007). NaCas, WPI and MPI were dispersed in
water to obtain solutions at concentrations within the range of 0.1 – 5 wt. %. All proteins were completely soluble at this range of concentrations. Sodium azide (0.02 wt. %) was added to the solutions as an anti-microbial agent.

2.2.2. Ultrasound treatment of protein solutions

An ultrasonic processor (Viber Cell 750, Sonics, USA) with a 12 mm diameter probe in stainless steel was used to sonicate NaCas, WPI and MPI solutions at concentrations of 0.1 to 5 wt. %. 50 ml of protein solution were sonicated in 100 ml glass beakers, which were placed in an ice bath to reduce heat gain. The protein solutions were sonicated for up to 2 min with a frequency of 20 kHz and maximum amplitude of 95% (ultrasonic wave of 108 µm). This power setting yielded an ultrasonic intensity of ~34 W.cm$^{-2}$, which was determined calorimetrically by measuring the temperature rise of the sample as a function of treatment time, under adiabatic conditions. The acoustic power, $P$ (W), was calculated as follows (Margulis & Margulis, 2003):

$$P = m \cdot c_p \left( \frac{dT}{dt} \right)$$  \hspace{1cm} (1)

where $m$ is the mass of ultrasound treated solution (g), $c_p$ is the specific heat of the material (J/gK) and $dT/dt$ is the rate of temperature change with respect to time, starting at $t = 0$.

The temperature of the protein solutions was measured before and after ultrasound treatment by means of a digital thermometer (TGST3, Sensor-Tech Ltd., Ireland), with an accuracy of ± 0.1 °C. After sonication treatment, the temperature of all protein solutions raised to approximately ~45 °C.
2.2.3. Characterisation of untreated and ultrasound treated proteins

2.2.3.1. pH measurements

The pH of the protein solutions was measured before and after ultrasound treatment. pH measurements were made by using a pH meter (SevenEasy, Mettler Toledo, UK). This instrument was calibrated with standard solutions of known pH. The pH values are reported as the average and the standard deviation of three replicates.

2.2.3.2. Microstructure characterisation

The size of untreated and ultrasound treated proteins was measured by dynamic light scattering using a Zetasizer Nano Series (Malvern Instruments, UK). Protein micelle size values are reported as Z-average ($D_z$), that is expressed as the intensity based harmonic mean (2,3) ($D_z = \Sigma S_i / \Sigma(S_i/D_i)$), where $S_i$ is the scattering intensity from a given particle $i$ and $D_i$ is the diameter of the particle $i$. The width of the protein size distribution was expressed in terms of span ($Span = D_{v0.9} - D_{v0.1}/D_{v0.5}$), where $D_{v0.9}$, $D_{v0.1}$, and $D_{v0.5}$ are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively. Small span values indicate a narrow protein size distribution. The micelle size and span values are reported as the average and the standard deviation of three replicates.

2.2.3.3. Microstructure visualisation

Cryo Scanned Electron Microscopy (Cryo-SEM, Philips XL30 FEG ESSEM) was used to visualise the microstructure of untreated and ultrasound treated proteins. One drop of protein solution was frozen to -198 °C in liquid nitrogen. Samples were then fractured at -180 °C and
etched for 5 min at -90 °C inside a cryo preparation chamber. Afterwards, samples were coated with gold and scanned at -160 °C.

### 2.2.3.4. Molecular structure characterisation

The molecular structure of untreated and ultrasound treated proteins was determined by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), using a Mini-Protean 3 Electrophoresis System (Bio-Rad, UK). 100 µL of protein solution at 1 wt. % concentration were added to 1 mL of native sample buffer (Bio-Rad, UK) in 2 mL microtubes and sealed. A 10 µL aliquot was taken from each sample and loaded onto a Tris-acrylamide gel (Bio-Rad, UK; 4-20% Mini Protean TGX Gel, 10 wells). A protein standard (Bio-Rad, UK; Precision Plus Protein® All Blue Standards) was used to determine the molecular weight of the samples. Gel electrophoresis was carried out initially at 55 V (I > 20 mA) for 10 min, then at 155 V (I > 55 mA) for 45 min in a running buffer (Bio-Rad, UK; 10x Tris/Glycine/SDS Buffer). The gels were removed from the gel cassette and stained with Coomassie Bio-safe stain (Bio-Rad, UK) for 1 hr and de-stained with distilled water overnight.

### 2.2.3.5. Intrinsic viscosity measurements

The intrinsic viscosity of untreated and ultrasound treated proteins was determined by a double extrapolation to an infinite dilution method, as described by Morris et al., (1981), using the models of Huggins and Kraemer, as follows:

Huggins (Huggins, 1942): \( \frac{\eta_{sp}}{c} = [\eta] + k[\eta]^2c \)
Kraemer (Kraemer, 1938): 
\[ \ln \frac{\eta_{sp}}{c} = [\eta] + k_K [\eta]^2 c \]  
(3)

where \( \eta_{sp} \) is the specific viscosity (viscosity of the solvent, \( \eta_0 \) / viscosity of the solution, \( \eta \)), \( c \) the protein concentration (w/v%), \( [\eta] \) the intrinsic viscosity (dL/g), \( k_H \) the Huggins constant. \( \eta_{rel} \) is the relative viscosity (viscosity of the solution, \( \eta \) / viscosity of the solvent, \( \eta_0 \)), \( k_K \) is the Kraemer constant.

The concentration ranges used for the determination of the intrinsic viscosity of NaCas, WPI and MPI were 0.25 – 0.45 wt. %, 1 – 2.5 wt. % and 0.5 – 2 wt. %, respectively. The validity of the regression procedure is confined within a discrete range of \( \eta_{rel} \), 1.2 < \( \eta_{rel} \) < 2. The upper limit is due to the hydrodynamic interaction between protein molecules, and the lower limit is due to inaccuracy in the determination of very low viscosity fluids. A value of \( \eta_{rel} \) approaching to 1 indicates the lower limit (Morris et al., 1981).

The viscosity of the protein solutions was measured at 20 °C using a Kinexus rheometer (Malvern Instruments, UK) equipped with a double gap geometry (25 mm diameter, 40 mm height). As reported by Morris et al. (1981), in order to derive the intrinsic viscosity by extrapolation to infinite dilution, there must be linearity between shear stress and shear rate, which indicates a Newtonian behaviour region on the range of shear rate used in the measurements. The Newtonian plateau region of the NaCas, WPI and MPI solutions at the range of concentrations used, was found within a shear rate range of 25 - 1000 s\(^{-1}\) (data not shown). Thus, the values of viscosity of the protein solutions and that of the solvent (distilled water) were selected from the flow curves data at a constant shear rate of 250 s\(^{-1}\) (within the Newtonian region), which were subsequently used to determine the specific viscosity, \( \eta_{sp} \), the relative viscosity, \( \eta_{rel} \), and the intrinsic viscosity, \([\eta]\). At least three replicates of each measurement were made.
2.2.4. Preparation of oil-in-water emulsions

10 wt. % of oil phase (rapeseed oil) was added to the continuous aqueous phase containing either untreated or sonicated proteins or Tween 80 at different concentrations, ranging from 0.1 to 5 wt. %. This mixture was emulsified first at 8000 rpm for 2 min using a high shear mixer (SL2T, Silverson, UK) to form an oil-in-water pre-emulsion. Afterwards, oil-in-water submicron emulsions were prepared by further emulsifying the pre-emulsion using a high-pressure valve homogeniser (Panda NS 1001L-2K, GEA Niro Soavi, UK) at 125 MPa for 2 passes. The emulsions were prepared at 20 °C in a controlled temperature laboratory.

2.2.5. Characterisation of oil-in-water emulsions.

2.2.5.1. Droplet size measurements

The droplet size of the emulsions was measured by using static light scattering (Hydro 2000SM, Mastersizer 2000, Malvern Instruments, UK) immediately after emulsification. Emulsion droplet size values are reported as the volume-surface mean diameter \(\bar{d}_{3,2} = \frac{\Sigma n_i d_i^3}{\Sigma n_i d_i^2}\), where \(n_i\) is the number of droplets of diameter \(d_i\). The stability of the emulsions was assessed by droplet size measurements over 28 days. The emulsions were stored under refrigerated conditions (4 °C) throughout the duration of the stability study. The droplet size values and the error bars are reported as the average and the standard deviation, respectively, of three replicates.
2.2.5.2. Interfacial tension measurements

The interfacial tension between the aqueous phase (pure water, protein solutions and low molecular weight surfactant solutions) and oil phase (rapeseed oil) was measured using a tensiometer K100 (Krüss, Germany) with the Wilhelmy plate method. The Wilhelmy plate is made of platinum, of a length, width and thickness of 19.9 mm, 10 mm and 0.2 mm, respectively. The Wilhelmy plate was immersed in 20 g of aqueous phase to a depth of 3 mm with a surface detection speed of 15 mm/min. The surface detection is the speed of the vessel drive used for the detection of the liquid surface. Once the surface has been detected by the microbalance in the tensiometer the vessel moves at the chosen surface detection speed to the position specified by the immersion depth (3 mm). Subsequently, an interface between the aqueous phase and oil phase was created by carefully pipetting 50 g of the oil phase over the aqueous phase. The test was conducted over 3,600 s and the temperature was maintained at 20 °C throughout the duration of the test. The interfacial tension values and the error bars are reported as the average and the standard deviation, respectively, of three replicates.

2.3. Statistical analysis

One way analysis of variance (ANOVA) with a 95% confidence interval was used to assess the significance of the results obtained. The ANOVA data with P < 0.05 were considered statistically significant.
3. Results and Discussion

3.1. Effect of ultrasound treatment on the structural and physical properties of NaCas, WPI and MPI.

The effect of time of ultrasound treatment on the size and pH of NaCas, WPI and MPI was initially investigated. Proteins solutions at concentration of 0.1 wt. % were sonicated for 15, 30, 60, and 120 s, with a frequency of 20 kHz and maximum amplitude of 95%. Protein size and pH measurements as a function of sonication time, for untreated and sonicated NaCas, WPI and MPI are shown in Table 2. As can be seen from results in Table 2, there is a significant reduction (P < 0.05) in the size of all proteins with the increase in the sonication time. The results also indicate that after 1 min of ultrasound treatment there is no further reduction in protein size for NaCas, WPI and MPI. This decrease in protein size is suggested to be due to the disruption of the untreated protein micelles caused by changes in electrostatic and hydrophobic interactions, induced by the high shear forces originating from ultrasonic cavitations (O’Brien, 2007). It can also be seen (cf. Table 2), that the pH of all the protein solutions decreased significantly (P < 0.05) as the time of ultrasound treatment increased. Furthermore, after 1 min of sonication the pH of all the proteins solutions was not further decreased. The reduction in the pH of the proteins can be due to the exposure of acidic amino acid residues (Sakurai et al., 2009) which were contained within the aggregated structure of the proteins micelles prior to sonication.

The stability over time in protein size and width of the protein size distribution (span) of ultrasound treated NaCas, WPI and MPI were also investigated. Proteins solutions at concentration of 0.1 wt. % were sonicated for 2 min at 20 kHz and ~34 W.cm⁻², since after 1 minute of sonication there was no further decrease in the size of protein (cf. Table 2). The micelle size of the ultrasound treated proteins was measured immediately after sonication and
after 1 and 7 days, in order to assess the stability of micelle size. Protein size measurements and span values obtained by dynamic light scattering for untreated and sonicated NaCas, WPI and MPI are shown in Table 3.

As can be seen from Table 3, the ultrasound treatment produced a significant reduction (P < 0.05) in the size of NaCas and narrowed the protein size distribution. However, on day 7 after ultrasound treatment an increase in size of NaCas can be observed and the width of the size distribution slightly increases. Thus, the ultrasound treatment applied to NaCas induced an effective micelle size reduction of 32% on day 7. A similar behaviour can be seen for WPI (Table 3), which results showed a significant size reduction (P < 0.05) and narrowing of the protein size distribution after ultrasound treatment, and on day 7 a slight increase in the width of the distribution and an increase in size, representing an effective micelle size reduction of 50%. In the case of MPI, results in Table 3 showed that ultrasound treatment caused a significant decrease in size (P < 0.05) and narrowed the protein size distribution. It can also be seen that on day 7, the width of the protein size distribution was slightly narrower and the protein micelle size slightly decreased further, representing an effective size reduction of 75%. Our results are in agreement with those of Jambrak et al., (2014), which showed a significant reduction in WPI micelle size after an ultrasound treatment of 15 min at 20 kHz and ~48 W.cm$^{-2}$. Yanjun et al., (2014) also observed a decrease in particle size for MPC treated by ultrasound at 12.5 W and 50% amplitude for 2 min. The reason for the observed decrease in size for NaCas and WPI is suggested to relate to a structural disruption in the untreated protein micelles associated with the cleavage of hydrophobic interactions in the molecule, likely induced by the high shear forces and turbulence resulting from cavitation. The subsequent size increase observed in NaCas and WPI on day 7 after sonication is thought to be due to a reorganisation of the proteins into smaller sub-associates due to non-covalent molecular interactions such as electrostatic and
hydrophobic interactions. In the case of MPI, the observed reduction in micelle size is presumably due to ultrasonic cavitation effects, which break up the aggregates of proteins and reduce their size. In order to test these hypotheses, cryo-SEM micrographs were captured of untreated and 7 days after ultrasound treatment of NaCas, MPI and WPI solutions at 1 wt. % for all proteins tested (Fig. 1).

As can be seen in Fig. 1, the untreated aggregates of NaCas in solution (Fig 1a) appear to be distributed within a densely packed network and to have a polydisperse protein size; whereas the NaCas treated by ultrasound (Fig. 1b) appear to be distributed into discrete entities, having a smaller and a slightly more uniform size in comparison to the untreated aggregates of NaCas. The structure of untreated WPI in solution (Fig. 1c) appears to have a highly polydisperse size distribution, which micelles also appear to be distributed within a packed network; whilst for the sonicated WPI (Fig. 1d) a clear reduction in the size can be seen, where the size distribution is monodispersed. Also, the sonicated WPI micelles appear to be more evenly distributed and separated from each another, in comparison to their untreated counterparts. In the case of untreated MPI in solution (Fig. 1e), we can distinguish discrete protein micelles of large and polydisperse size; whereas the MPI micelles treated by ultrasound (Fig. 1f) appear to have a smaller size and a monodisperse size distribution. These findings are consistent with the previously observed reduction in micelle size of sonicated NaCas, WPI and MPI (cf. Table 3), and validate our hypothesis that ultrasound treatment causes the disruption of the protein micelles, which then reorganise themselves into smaller sub-micelles.

The molecular structure of untreated and ultrasound treated proteins NaCas, MPI and WPI was subsequently investigated. Proteins solutions at concentration of 0.1 wt. % were sonicated for 2 min at 20 kHz and ~34 W.cm$^{-2}$, as after 1 minute of sonication there was no
further decrease in the size of protein (cf. Table 2). Electrophoretic profiles obtained by SDS-PAGE for untreated and sonicated NaCas, WPI and MPI are shown in Fig. 2. As can be seen from results in Fig. 2, no difference in protein fractions between the untreated and ultrasound treated NaCas, WPI and MPI was observed. These results are in agreement with those reported by Gülseren et al., (2007) who showed no differences in molecular weight between untreated and sonicated bovine serum albumin (BSA), which treatment was carried out at 20 kHz, ~20W.cm\(^{-2}\) for 15 min. Yanjun et al., (2014) also observed that ultrasound treatment (12.5 W at 50% amplitude for 2 min) induced no changes in the molecular weight of milk protein concentrate (MPC) solutions. On the other hand, Jambrak et al., (2014) observed a reduction in the molecular weight of WPI and WPC treated by ultrasound (20 kHz, ~48W.cm\(^{-2}\) and 15 min). The difference between our results and those of Jambrak et al., (2014) may have resulted from the different ultrasonic intensity and time of treatment applied to WPI. They used an ultrasound treatment of 15 min and their ultrasound probe provided 35% more ultrasonic intensity to WPI, which might have caused higher shear stress and turbulence effects in their WPI solutions and resulted in the split of the molecular structure of the protein.

The intrinsic viscosity was obtained from the fitting of the Huggins and Kraemer equations to the experimental viscosity data, for the untreated and ultrasound treated NaCas, WPI and MPI in solution at different concentrations, as shown in Fig. 3. The values of intrinsic viscosity and Huggins and Kraemer constants for each of the studied proteins are listed in Table 4.

Intrinsic viscosity, \([\eta]\), measurements provide information about the molecular properties of biopolymers in solution. More specifically, \([\eta]\) reflects the ability of a solvent to hydrate proteins and provides information about the molecular hydrodynamic volume, which is related to the chain conformation of the proteins in solution (Behrouzian, Razavi, &
Karazhiyan, 2014). By comparing the obtained values of intrinsic viscosity between the untreated and sonicated dairy proteins (cf. Table 4), we can see that ultrasound treatment induced a significant reduction (P < 0.05) in the intrinsic viscosity of NaCas, WPI and MPI in solution, and thus a significant reduction in the hydrodynamic volume occupied by the proteins and the solvent they entrapped. These results are also consistent with the reduction in associate size measured by dynamic light scattering (cf. Table 3) and observed on the cryo-SEM micrographs (cf. Fig. 1). Lefebvre, (1982) reported intrinsic viscosity values of 0.234 dL/g and 0.514 dL/g for $\alpha_s$-casein and BSA, respectively. These values are lower than the results obtained in this work for untreated NaCas, WPI and MPI (cf. Table 4). These differences may arise due to the complexity of the untreated NaCas, WPI and MPI solutions, which are composed of a mixture of proteins rather than single $\alpha_s$-casein or BSA used by Lefebvre, (1982). Another possibility is the type of solvent used, which in the work of Lefebvre, (1982) was 6M guanidine hydrochloride, whilst in our work the untreated proteins were diluted in distilled water.

As reported by Tanner & Rha, (1980), the intrinsic viscosity of a protein solution can give a measure of the degree of hydrophobicity of the protein. Indeed, the viscosity of a protein depends on its conformation and thus on its level of hydration, which are a result of the amount of hydrophobic side chains that are buried in the interior of the protein micelles in solution. Khan et al., (2012) also reported that a decrease in intrinsic viscosity led to the dehydration of amphiphilic biopolymer micelles, increased the hydrophobicity of the biopolymer and hence reduced the energy required for the adsorption of amphiphilic biopolymers at the oil-water interface. Therefore, the reduction in intrinsic viscosity of the proteins induced by the ultrasound treatment (cf. Table 4), indicates an increase in the degree
of hydrophobicity of all the proteins, the effect of which is slightly more significant for MPI (0.041), followed by NaCas (P < 0.043) and WPI (P < 0.044).

The Huggins and Kraemer coefficients are adequate to assess the quality of a solvent. Values for the Huggins coefficient (k_H) within a range of 0.25 to 0.5 are attributed to a good solvation, whilst values above 0.5 - 1.0 are related to poor solvents (Delpech & Oliveira, 2005). Similarly, negative values for the Kraemer coefficient (k_K) indicate good solvents and positive values indicate a poor solvation (Delpech & Oliveira, 2005). As can be seen from results in Table 4, the values obtained for the Huggins (k_H) and Kraemer (k_K) constants are both negative, which indicate a good solvation considering k_K, but an unusual behaviour in the case of k_H. However, negative values of k_H have also been reported in literature for biopolymers with amphiphilic properties, such as bovine serum albumin dissolved in water (Curvale, Masuelli, & Padilla, 2008), and polydimethylsiloxane–polyurea copolymers dissolved in isopropyl alcohol (Yilgor, Ward, Yilgor, & Atilla, 2006). It is also generally accepted, for hydrocolloids, that the relation of k_H + k_K = 0.5 would indicate the adequacy of the experimental results. However, the results presented in Table 4 do not yield this value. This effect is thought to be due to the amphiphilic character of the proteins (in comparison to non amphiphilic polysaccharides) which yields negative values of k_H and k_K. Similar results have been reported in literature for other amphiphilic biopolymers (Curvale et al., 2008; Delpech & Oliveira, 2005; Yilgor et al., 2006).

3.2. Comparison of the emulsifying properties of untreated and ultrasound treated NaCas, WPI and MPI protein

A series of oil-in-water emulsions were produced with 10 wt. % rapeseed oil and an aqueous continuous phase containing either untreated or ultrasound treated (2 min at 20 kHz,
~34 W.cm\(^{-2}\)) NaCas, WPI and MPI, or a low molecular weight surfactant, Tween 80 at different concentrations (0.1 - 5 wt. %). The emulsions were passed through a high-pressure valve homogenizer at 125 MPa for 2 passes. Emulsion droplet size measurements obtained by static light scattering are shown in Fig. 4. The emulsion droplet size was measured immediately after emulsification.

As can be seen from Fig. 4a-b, the emulsions prepared with untreated and ultrasound treated NaCas and WPI had the same droplet sizes for all the concentrations used, and resulted in similar droplet sizes as those obtained with Tween 80. This behaviour is unusual, considering the significant micelle size reduction (increase in surface area-to-volume ratio) observed for sonicated NaCas and WPI (cf. Table 3), for which it would have been expected to result in a faster adsorption of the proteins at the water-in-oil interface, as reported by Damodaran & Razumovsky, (2008), and thus lead to a higher reduction in the interfacial tension and to smaller emulsion droplet sizes. Furthermore, the significant increase in the hydrophobicity of the sonicated NaCas and WPI with the decrease in intrinsic viscosity (cf. Table 4; Khan, Bibi, Pervaiz, Mahmood, & Siddiq, 2012; Tanner & Rha, 1980) would also be expected to lead to a faster adsorption of the proteins to the oil-water interface, thus reducing interfacial tension and facilitating droplet break-up. However, it appears that the rate of adsorption to the interface of sonicated NaCas and WPI remains unchanged despite the smaller micelle sizes and higher hydrophobicity obtained, in comparison with untreated NaCas and WPI. Results in Fig. 4a-b also showed that droplet sizes decreased significantly (P < 0.05) with the increase in NaCas and WPI concentration, which is in agreement with the results obtained by Srinivasan et al., (2002) for emulsions formed with NaCas, and those measured by Tcholakova et al., (2006) for emulsions containing whey protein concentrate (WPC). The submicron emulsion droplet sizes obtained for both, untreated NaCas and WPI are in agreement with droplet sizes obtained by Dybowska (2011), in the order of ~120 nm for
emulsions containing WPC (3% wt.), and with those measured by Lee & Norton (2013), in
the order of ~170 nm for emulsions containing NaCas (3% wt.).

In the case of MPI, results in Fig. 4c showed that at concentrations ≤ 1 wt. % the
emulsions prepared with ultrasound treated MPI resulted in significantly (P < 0.05) smaller
droplet sizes than those formed with untreated MPI. However, above 1 wt. % concentration,
the emulsions prepared with untreated and sonicated MPI, as well as with Tween 80 exhibited
similar droplet sizes. The droplet sizes obtained for untreated MPI are in agreement with the
results reported by Euston & Hirst (1999), where micron sized droplets were obtained with
MPC at concentrations ≤ 1 wt. %. The reason for the observed reduction in emulsion droplet
size obtained with ultrasound treated MPI at concentrations ≤ 1 wt. % is suggested to be
related in part to the increase in surface area-to-volume ratio of sonicated MPI (due to their
smaller micelle size, cf. Table 3). This effect would result in a faster adsorption of the proteins
at the water-in-oil interface (Damodaran & Razumovsky, 2008), the effect of which would
decrease significantly the interfacial tension and facilitate droplet break-up during
emulsification. Moreover, this droplet size reduction is also suggested to be due to the slightly
more significant increase in the hydrophobicity of sonicated MPI, in comparison with
ultrasound treated NaCas and WPI (cf. Table 4, decrease in intrinsic viscosity). This effect
would contribute to a faster adsorption of sonicated MPI to the interface (Khan et al., 2012;
Tanner & Rha, 1980), reduce further the interfacial tension and lead to the production of
smaller emulsion droplet sizes. Yanjun et al., (2014) also observed that the emulsifying
properties of milk protein concentrate (MPC) were improved by an ultrasound treatment of 2
min at 12.5 W and 50% amplitude.

It can also be seen (Fig. 4) that the obtained emulsion droplet sizes are comparable to
the size of untreated proteins (cf. Table 3). However, it must be considered that the protein
size data displayed in Table 3 represents aggregates, and not the individual protein fractions
composing the micelles. In fact, in solution, proteins form aggregates (micelles) due to electrostatic and hydrophobic interactions (O’Connell et al., 2003). But, in the presence of a hydrophobic dispersed phase (i.e. rapeseed oil), the individual protein fractions detach from the bulk micelles and adsorb to the oil-water interface (Beverung et al., 1999; O’Connell & Flynn, 2007). As an example, the size of NaCas discrete molecules has been reported to be ~8 nm (O’Connell & Flynn, 2007; O’Connell et al., 2003), which makes it possible to form the submicron droplets presented in this work.

The results observed in emulsion droplet sizes (Fig. 4), which were shown to be dependent on the type of emulsifier, can be explained by considering the interfacial tension of the studied systems. Fig. 5 presents the interfacial tension between water and oil, obtained for untreated and sonicated NaCas, WPI, MPI, as well as for Tween 80 at 0.1 wt. % concentration. In order to assess the presence of interfacial impurities of the systems, the interfacial tension between pure water and rapeseed oil was measured. As can be seen from Fig. 5, the interfacial tension of all systems decreased with time. In view of these results, it is our opinion that the decrease in interfacial tension with time is due to a great extent on the nature of the oil used, and to a lesser extent on the type of emulsifier. As reported by Gaonkar (1989; 1991), the interfacial tension of commercial vegetable oils against water decreases with time due to the adsorption of surface active impurities, in the oils, at the interface. It was also reported (Gaonkar, 1989; Gaonkar 1991) that after purification of the vegetable oils, the time dependency of the interfacial tension is no longer observed.

As can be seen in Fig. 5a-b, no significant differences (P > 0.05) in the obtained values of interfacial tension between the untreated and ultrasound treated NaCas and WPI were observed. These results are consistent with the emulsion droplet sizes seen in Fig. 4a-b at 0.1 wt. % concentration, and add evidence to our hypothesis that the rate of protein adsorption at the oil-water interface is the same for the untreated and ultrasound treated NaCas and WPI.
Results in Fig. 5a-b also showed that lower interfacial values were obtained for Tween 80 than those obtained for untreated and sonicated NaCas and WPI. This effect is likely due to the smaller size and molecular weight of this emulsifier as compared with the bulkier structure of NaCas and WPI. It can also be seen (Fig. 5c) that the interfacial tension values obtained for ultrasound treated MPI were significantly lower (P < 0.05) than those obtained for untreated MPI, and slightly lower than those obtained with Tween 80. This result is consistent with the obtained emulsion droplet sizes presented in Fig. 4c, and confirms our hypothesis that the micelles of sonicated MPI adsorb faster to the oil-water interface, due to the higher surface area-to-volume ratio (cf. Table 3, smaller micelle size) and higher hydrophobicity of these proteins (cf. Table 4, lower intrinsic viscosity), which reduced significantly the interfacial tension, enhanced oil droplet break-up during emulsification and produced smaller droplet sizes.

The stability of the oil-in-water emulsions prepared with untreated and ultrasound treated NaCas, WPI and MPI was investigated during a 28 day period. Emulsions prepared with Tween 80 were also assessed for comparative purposes. Fig. 6 shows the evolution of droplet size \(d_{3,2}\) as a function of time for emulsions prepared with untreated and sonicated NaCas, MPI and WPI, as well as with Tween 80 at 1 wt. % concentration.

As can be seen from Fig. 6a-b, the emulsions prepared with untreated and sonicated NaCas and WPI, as well as with Tween 80 were all stable against coalescence for 28 days. This stability behaviour observed for untreated and ultrasound treated NaCas and WPI was the same for all the concentrations used in this work (data not shown). In all cases, no oil layer was observed on the upper part of the emulsions over 28 days. In the case of MPI, results in Fig. 6c showed that the emulsions prepared with untreated MPI exhibited coalescence at 1 wt. % concentration, as seen by the increase in droplet size over time. Coalescence was also observed for emulsions prepared with untreated MPI at 0.1 and 0.5 wt. % concentrations, but the
emulsions prepared with untreated MPI at a concentration higher than 1 wt. % were stable for 28 days (data not shown). A layer of oil was observed at the top of the emulsions which exhibited coalescence. However, it can also be seen (cf. Fig. 6c) that the emulsions prepared with ultrasound treated MPI at 1 wt. % concentration were resistant against coalescence over 28 days and had the same stability as the emulsions prepared with Tween 80. This behaviour observed for sonicated MPI was the same for all the concentrations used in this work (data not shown). This improved stability of the emulsions prepared with sonicated MPI in comparison with untreated MPI is thought to be related to the reduction in micelle size (i.e. increase in surface are-to-volume ratio, cf. Table 3) and to the increase in hydrophobicity (i.e. decrease in the intrinsic viscosity, cf. Table 4) of sonicated MPI as aforementioned. The effect of which results in a faster adsorption of sonicated MPI to the oil-water interface, higher reduction in interfacial tension and thus to smaller droplet sizes.


This study showed that ultrasound treatment (20 kHz, 34 W.cm$^{-2}$ for 2 min) of NaCas, WPI and MPI caused a significant (P < 0.05) reduction in the micelle size and hydrodynamic volume of the proteins. This effect was attributed to the high shear forces resulting from ultrasonic cavitations. However, no differences in molecular weight were observed between untreated and ultrasound treated NaCas, WPI and MPI.

Unexpectedly, the emulsions prepared with ultrasound treated NaCas and WPI had the same submicron droplet sizes as those obtained with their untreated counterparts, and were stable at the same concentrations. These results suggested that ultrasound treatment did not affect significantly the rate at which protein adsorption occurs at the interface, since no significant (P > 0.05) changes in interfacial tension were observed between the untreated and
sonicated NaCas and WPI. In contrast, the emulsions prepared with sonicated MPI at concentrations ≤ 1 wt. % had smaller droplet sizes than those obtained with untreated MPI at the same concentrations. This effect was explained by the significant reduction in micelle size (i.e. an increase in surface area-to-volume ratio) and increase in hydrophobicity (reflected by the decrease in intrinsic viscosity) of ultrasound treated MPI. These effects led to a faster adsorption of the protein to the oil-water interface, significantly reduced the interfacial tension and thus facilitated droplet break-up during emulsification. In addition, the emulsions prepared with ultrasound treated MPI were stable against coalescence for 28 days at all the concentrations tested, whereas the emulsions produced with untreated MPI showed coalescence 7 days after emulsification at concentrations ≤ 1 wt. %.

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References


Güzey, D., & Weiss, J. (2001). High-intensity ultrasonic processing improves emulsifying properties of proteins. Colloidal and Interfacial Food Science Laboratory, Department of Food Science and Technology, The University of Tennessee.


Fig. 1. SDS-PAGE electrophoretic profiles of protein solutions: (a) Molecular weight standard (10 kDa – 250 kDa), (b) Untreated NaCas, (c) Ultrasound treated NaCas, (d) Untreated MPI, (e) Ultrasound treated MPI, (f) Untreated WPI and (g) Ultrasound treated WPI.

Fig. 2. Cryo-SEM micrographs of protein solutions: (a) 5% Untreated NaCas solution, (b) 5% Ultrasound treated NaCas solution, (c) 1% Untreated WPI solution, (d) 1% Ultrasound treated WPI, (e) 1% Untreated MPI solution and (f) 1% Ultrasound treated MPI. Scale bar is 2 µm in all cases.

Fig 3. Fitting of the Huggins (closed circles) and Kraemer (open circles) equations to the viscosity data of the studied protein solutions: (a) Untreated NaCas, (b) Ultrasound treated NaCas, (c) Untreated WPI, (d) Ultrasound treated WPI, (e) Untreated MPI and (f) Ultrasound treated MPI.

Fig. 4. Average droplet size as a function of concentrations of: (a) Untreated NaCas, sonicated NaCas and Tween 80, (b) Untreated WPI, sonicated WPI and Tween 80, and (c) Untreated MPI, sonicated MPI and Tween 80.

Fig. 5. Interfacial tension between water and pure vegetable oil as a function of emulsifier type: (a) Untreated NaCas, sonicated NaCas and Tween 80, (b) Untreated WPI, sonicated WPI and Tween 80 and (c) Untreated MPI, sonicated MPI and Tween 80. The concentration for all emulsifiers was 0.1 wt. %.

Fig. 6. Effect of emulsifier type on droplet size as a function of time for O/W emulsions stabilised by: (a) Untreated NaCas, sonicated NaCas and Tween 80, (b) Untreated WPI, sonicated WPI and Tween 80 and (c) Untreated MPI, sonicated MPI and Tween 80. The concentration for all emulsifiers was 1 wt. %.
Table 1. Composition of acid casein, whey protein isolate (WPI) and milk protein isolate (MPI).

<table>
<thead>
<tr>
<th></th>
<th>Acid Casein</th>
<th>Whey Protein Isolate</th>
<th>Milk Protein Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (wt. %)</td>
<td>86</td>
<td>91</td>
<td>86</td>
</tr>
<tr>
<td>Moisture (wt. %)</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fat (wt. %)</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Lactose (wt. %)</td>
<td>0.1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Calcium (wt. %)</td>
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<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Sodium (wt. %)</td>
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<td>0.08</td>
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<tr>
<td>Potassium (wt. %)</td>
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<td>0.35</td>
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<tr>
<td>Phosphorus (wt. %)</td>
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<tr>
<td>Magnesium (wt. %)</td>
<td>0.01</td>
<td>0.02</td>
<td>0.08</td>
</tr>
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Table 2. Effect of sonication time on pH and protein size ($D_z$) of NaCas, WPI and MPI solutions at a concentration of 0.1 wt. %

<table>
<thead>
<tr>
<th>D$_z$ (nm)</th>
<th>pH (-)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s)</td>
<td>NaCas</td>
<td>WPI</td>
<td>MPI</td>
</tr>
<tr>
<td>0</td>
<td>245 ± 12</td>
<td>433 ± 11</td>
<td>956 ± 48</td>
</tr>
<tr>
<td>15</td>
<td>164 ± 6</td>
<td>291 ± 7</td>
<td>338 ± 5</td>
</tr>
<tr>
<td>30</td>
<td>113 ± 5</td>
<td>152 ± 15</td>
<td>299 ± 15</td>
</tr>
<tr>
<td>60</td>
<td>60 ± 5</td>
<td>75 ± 11</td>
<td>247 ± 12</td>
</tr>
<tr>
<td>120</td>
<td>58 ± 4</td>
<td>72 ± 9</td>
<td>256 ± 6</td>
</tr>
</tbody>
</table>
Table 3. Average protein size ($D_z$) and span of untreated and ultrasound treated NaCas, MPI and WPI at a concentration of 0.1 wt. %.

<table>
<thead>
<tr>
<th>Protein type</th>
<th>$D_z$ (nm)</th>
<th>Span (nm)</th>
<th>$D_z$ (nm)</th>
<th>Span (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
<td>Day 7</td>
<td>Day 0</td>
</tr>
<tr>
<td>NaCas</td>
<td>245 ± 12</td>
<td>10.45 ± 0.31</td>
<td>58 ± 4</td>
<td>145 ± 2</td>
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<tr>
<td>WPI</td>
<td>433 ± 11</td>
<td>1.93 ± 0.24</td>
<td>72 ± 9</td>
<td>189 ± 8</td>
</tr>
<tr>
<td>MPI</td>
<td>956 ± 48</td>
<td>3.84 ± 0.43</td>
<td>256 ± 6</td>
<td>250 ± 14</td>
</tr>
</tbody>
</table>

Table 4. Intrinsic viscosity ([η]), Huggins ($k_H$) and Kraemer ($k_K$) constants obtained for untreated and ultrasound treated NaCas, MPI and WPI solutions.

<table>
<thead>
<tr>
<th>Protein in solution</th>
<th>[η] Untreated (dL/g)</th>
<th>$k_H$ Untreated</th>
<th>$k_K$ Untreated</th>
<th>[η] Ultrasound (dL/g)</th>
<th>$k_H$ Ultrasound</th>
<th>$k_K$ Ultrasound</th>
</tr>
</thead>
</table>
Highlights:

- Effect of ultrasound (US) on physical properties of dairy proteins was assessed.
- High power ultrasound (30W.cm$^{-2}$, 20kHz) reduced micelle size of all dairy proteins.
- SDS-PAGE confirmed US had no effect on the molecular weight of all dairy proteins.
- US treated dairy proteins led to similar droplet sizes as their untreated counterparts.
- US treated milk protein isolate produced more stable W/O emulsions than untreated MPI.